STUDIES of CATALYSIS of the HYDROLYSIS of

PHENYL GLYCINATE and

SOME RELATED ESTERS

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Lim Eng Lee

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Abstract

This thesis reports the results and conclusions from the first kinetic studies undertaken of the hydrolysis of phenyl ester of an α-amino acid (glycine), previous studies having been limited to 4-nitrophenyl esters. Some data on 4-methoxyphenyl glycinate are also reported, together with some new data on 4-nitrophenyl esters required for comparison purposes.

Studies have concentrated on catalysts previously reported as effective in the hydrolysis of 4-nitrophenyl esters of amino acids, the aim being to determine if results for a phenyl ester with a relatively poor leaving group might help in determining mechanisms involved in catalysis.

(i) HCO_3^-. Unlike the case for 4-nitrophenyl esters, phenyl glycinate hydrolysis is catalysed in bicarbonate solutions not by CO_2 as previously suggested but by HCO_3^-, the kinetic form is given by

$$\text{Rate} = k[\text{neutral ester}][HCO_3^-]$$

Kinetically equivalent forms involving CO_2 and CO_3^{2-} terms have been excluded. Comparison of the catalytic effect of HCO_3^- with that of imidazole and that of HPO_4^{2-} excludes the possibilities of general base and nucleophilic catalysis by HCO_3^- and a mechanism involving initial nucleophilic addition of amine to HCO_3^- followed by intramolecular nucleophilic substitution is suggested as most likely.

(ii) 4-nitrobenzaldehyde. Phenyl and 4-methoxyphenyl glycinate show the same kinetic form as 4-nitrophenyl esters but the catalytic effects are much smaller and consistent only with rate-determining attack on ester carbonyl. This supports the concept of rate-determining decomposition of a carbinolamine species, which is also consistent with an observed small effect of temperature on the overall catalytic rate constant.

(iii) Imidazole. Unlike the case for 4-nitrophenyl

esters, the kinetics are dominated for both neutral and protonated phenyl glycinate species by a term second order in imidazole, which is consistent with general base catalysis of nucleophilic substitution by imidazole in the formation of an acylimidazole intermediate. A term first order in imidazole makes a minor contribution and probably represents general base catalysis as indicated through comparison of the magnitude of the catalytic effect of imidazole with that of HPO_4^{2-}, which is also reported on for the first time in this thesis.

(iv) N-ethylmorpholine. An unusual non-linear dependence of the observed first order rate constants on catalyst concentration is shown to be consistent with complexing of N-ethylmorpholine and ester prior to reaction. Equilibrium constants for complex formation and rate constants for decomposition are evaluated. However, the effects of model compounds as tests for medium effects of the amine and its cation throw some doubt on the validity of this interpretation. In particular, dioxane, as a model for N-ethylmorpholine, has an inhibitory effect on the hydrolysis of unprecedented magnitude.

(v) Hydroxide. Alkaline hydrolysis of phenyl glycinate has been studied so as to obtain rate constants for both the neutral and cationic ester species. The latter is about 300 times more reactive.

Studies on 4-nitrophenyl esters have also been advanced in tandem with those of the phenyl and 4-methoxyphenyl esters. The kinetic form for catalysis in bicarbonate solutions has been confirmed as involving CO_2 and not HCO_3^-, as is consistent with carbamate formation, but whether formation or decomposition of carbamate is rate-determining is still uncertain. The small effect of temperature on reaction rate together with the lack of base catalysis in the reaction support rate-limiting decom-

position of carbamate, but the overall rate is close to that predicted by interpolation from other studies for carbamate formation. The possibility of hydrolysis at the zwitterion stage is considered. New data are also reported for 4-nitrobenzaldehyde catalysis for comparison with those of the phenyl and 4-methoxyphenyl esters.

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****

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Section 1 General introduction to catalysis; Literature survey.

1:1 Introduction.

Many studies have been made to account for nucleophilic substitution reactions of carboxylic acid esters. They are discussed in detail in reviews by Bender [1], Johnson [2] and Patai [3]. Bruice and Benkovic [4] extended their review to bioorganic applications. Fife [5], Lazduski [6] and Coleman [7] in recent times (1971 onwards) have discussed the role of enzymatic processes in ester hydrolysis.

The following sections cover the more important aspects of nucleophilic substitution mechanisms for the hydrolysis of esters including some α-amino acid esters. Examples used to illustrate certain principles have been chosen with the particular area of the present study in mind. Section 1:2 deals with catalysis by bases of the hydrolysis of carboxylic acid esters, including intramolecular catalysis. Section 1:3 briefly covers alkaline hydrolysis which involves direct substitution by hydroxide ion.

1:2 The role of bases as catalyst of the hydrolysis of carboxylic acid esters.

The important categories of catalysis in this survey include general base catalysis, nucleophilic catalysis and intramolecular catalysis.

At fixed pH, increases in concentration of bases (e.g. as a buffer component) may lead to increases in rates of hydro-

lysis of carboxylic acid esters and related reactions. Such catalysis may represent a true base role of the catalyst (general base catalysis) or it may represent a nucleophilic role (nucleophilic catalysis). These distinct types of catalysis are discussed in the following two sections along with experimental evidence in their support. Cases involving intramolecular catalysis, which is pertinent to the present study are discussed in Section I:2.3.

I:2.1 General base catalysis.

In general base catalysis of the hydrolysis of carboxylic acid esters, the base abstracts a proton from a water molecule in the transition state (shown in brackets in Diagram I.1), thus assisting addition by increasing the nucleophilic power of the oxygen in the water.

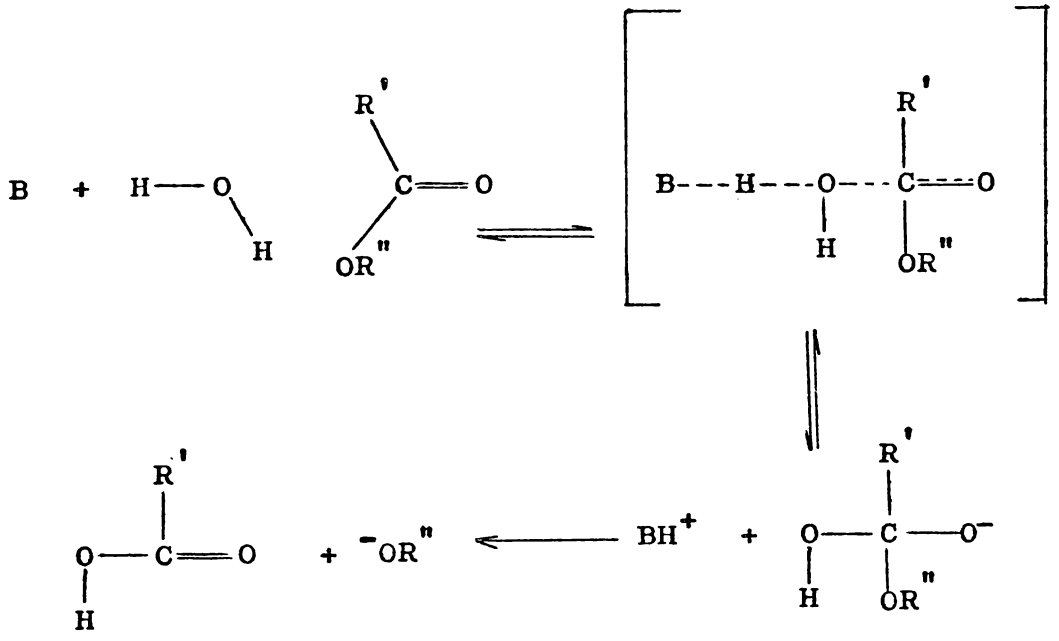


Diagram I.1 General base catalysed hydrolysis of an ester.

In terms of leaving group ability, reversion of the tetra-

hedral intermediate to reactants is furthermore much less likely than in the case of unassisted water attack (Diagram 1.2).

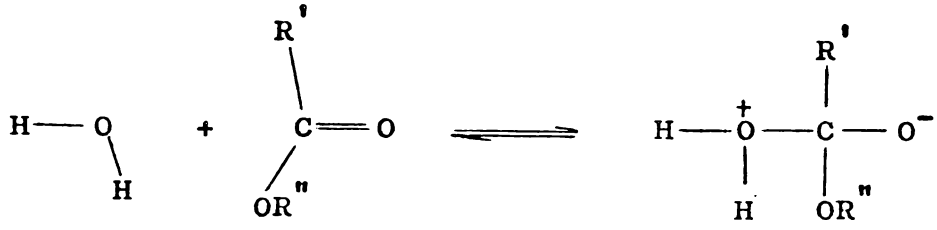


Diagram 1.2 Unassisted water attack on ester.

In the absence of base catalysts (and acid catalysts which are not considered here), esters must hydrolyse through slow water attack or through direct reaction with hydroxide ion. The latter in the neutral pH region is present in too low a concentration to be effective for most esters and consequently general base catalysis plays an important role in ester hydrolysis particularly in this pH region.

The hydrolysis of esters activated by electron withdrawing groups in the acyl portion, such as ethyl dichloroacetate and ethyl glycinate hydrochloride, is catalysed by many organic and inorganic bases by general base mechanisms[44]. Evidence for general base rather than nucleophilic catalysis by bases such as HPO_4^{2-}, imidazole and other amines [44] came from an observed two- to threefold rate decrease in deuterium oxide as solvent as compared with water, irrespective of the base used. Coupled with the fact that uncatalysed hydrolysis is also slower in deuterium oxide, this shows that $H^+(D^+)$ is being removed by base from a water molecule in the rate-determining step of the reaction, i.e. as in the mechanism shown in Diagram 1.1. Such an effect is not consistent with

nucleophilic catalysis.

In the same study [44], imidazole and HPO_4^{2-}, which are almost equal in base strength were found to be almost equally effective as (general base) catalysts for the hydrolysis of ethyl dichloroacetate whereas as nucleophilic catalysts for hydrolysis of alcohol activated esters, imidazole is of the order of 10^3 times more effective than HPO_4^{2-} as seen for instance in the catalysed hydrolysis of 4-nitrophenyl acetate [47, 48] (Table 1.1).

Table 1.1 Ratios of catalytic rate constants for imidazole, HPO_4^{2-} and OH^- in carbonyl displacement reactions.

ESTER	k_{OH^-}/k_{Im}	$k_{Im}/k_{HPO_4^{2-}}$	Mechanism
Ethyl dichloroacetate [44]	6.5×10^5	1.9	G.B.
Ethyl acetate [46, 47]	9×10^5	0.3	G.B.
4-nitrophenyl acetate [47, 48]	16	4.7×10^3	N.
Phenyl acetate [47]	1.4×10^2	-	N.
Acetic anhydride [47]	7.2	8.6×10^2	N.

Relative catalytic effects of imidazole and HPO_4^{2-} provide an indication of catalytic role. Similarly the ratio of rate constants for OH^- and imidazole (see Table 1.1) provide

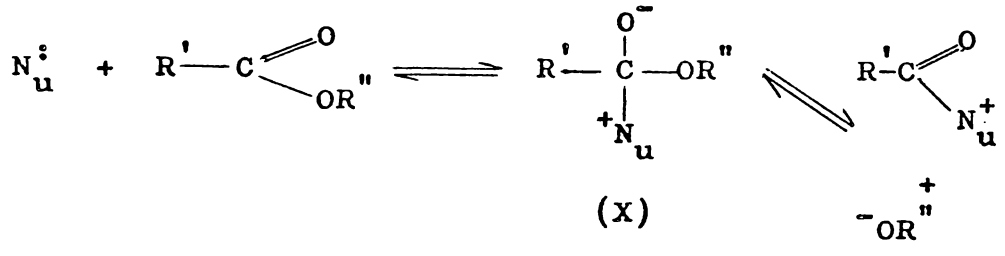
another guide as to the catalytic role of imidazole, a large ratio implying a general base catalytic effect.

The steric demand placed on a base in a general base catalysed water molecule attack on the carbonyl centre (Diagram 1.1) is smaller than the steric demand when the base in a nucleophilic catalyst role must directly attack the carbonyl centre. To illustrate this requirement, the results of pyridine and 2,6-dimethylpyridine catalysed hydrolysis of acetic anhydride were reported [51, 52]. The latter base, 2,6-dimethylpyridine (pK_a 6.7 [112]) was thought to catalyse the hydrolysis via a general base mechanism because pyridine even though a much weaker base (pK_a 5.25 [112]) was a better catalyst as is consistent with its nucleophilic role. So it appears that 2,6-dimethylpyridine prevents a direct nucleophilic attack on the carbonyl centre. The base catalysed hydrolysis of acetic anhydride has been studied quite extensively [18-22, 47, 49, 50, 123] and in most cases involving sterically non-bulky catalysts, evidence for nucleophilic mechanisms was reported.

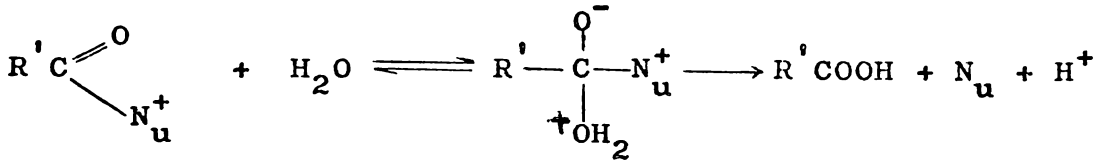
Another different type of situation in which general base catalysis has more recently been recognised as being of critical importance, is in trapping of short-lived zwitterion intermediates produced on addition of nucleophiles e.g. amines to carbonyl groups of both aldehydes and esters[86, 87, 110]. Such proton transfer is rate-determining in the reactions of amines with phenyl acetates [110]. This type of general base catalysis is discussed in more detail in Section 7 where such a catalytic role is suggested for imidazole in the hydrolysis of phenyl glycinate.

1:2.2 Nucleophilic catalysis.

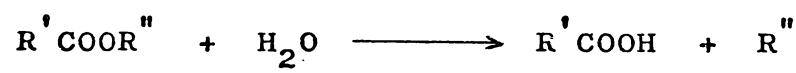
The general principle of nucleophilic catalysis in ester hydrolysis is outlined in Equations 1.1 and 1.2. The overall reaction , the sum of 1.1 and 1.2 is given in Equation 1.3.



(X) 1.1



(X) 1.2



1.3

The mechanism requires that the nucleophile (N_u) adds to the carbonyl centre to give a reactive acyl-intermediate X (Equations 1.1 and 1.2), which is so much more susceptible to water attack than the original ester itself, that the overall two-stage reaction is faster than the reaction in absence of N_u; i.e. N_u is a catalyst.

Electron withdrawing substituents in the alcohol portion increase the susceptibility of the carbonyl group to attack by the nucleophile concerned and furthermore, with the alcoholate ion then a relatively good leaving group, the initially formed tetrahedral intermediate (see Equation 1.1) partitions sufficiently favourably towards product for the new acyl-intermediate X to be readily formed. With a poor leaving group

as in say alkyl ester, partitioning of such a tetrahedral intermediate favours reversion to reactants and nucleophilic catalysis is less effective than general base catalysis for which the partitioning problem is overcome (see Section 1:2.1) when H_2O is effectively replaced, via base catalysis by the poor leaving group OH^- .

The base catalysed hydrolysis of 4-nitrophenyl acetate has been studied extensively [10-12, 14, 17, 23-26, 35-37] and illustrates the importance of activation in the alcohol portion of the ester (4-nitrophenolate ion as a leaving group).

The most thoroughly investigated reaction is the imidazole catalysed hydrolysis of 4-nitrophenyl acetate [10-12, 23-26], the mechanism for which is shown in Diagram 1.3.

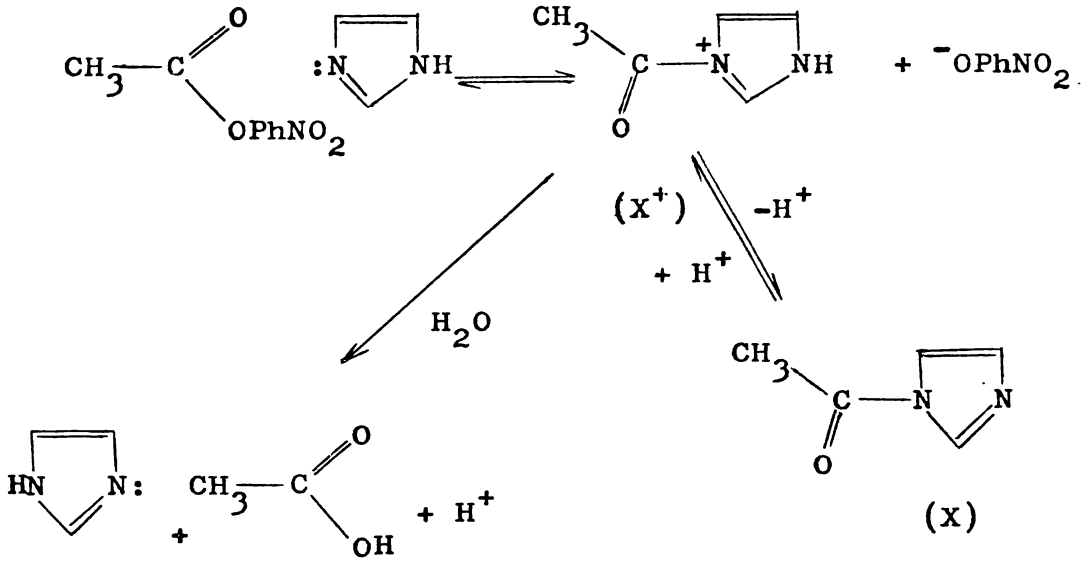


Diagram 1.3 Mechanism for imidazole catalysed hydrolysis of 4-nitrophenyl acetate.

The characteristic absorption of the acyl-intermediate is

observed spectrophotometrically at 245 nm. The intermediate X^+ hydrolyses relatively slowly and the overall reaction involves rate-determining decomposition of X^+. Other amines such as pyridine, picoline, trimethylamine and aniline likewise been shown to give nucleophilic catalysis via acyl intermediates [18-22].

Reports on imidazole catalysed hydrolysis of the esters of amino acids are relatively few. In the imidazole catalysed hydrolysis of 4-nitrophenyl-N-benzyloxy-carbonyl glycinate [38] and 4-nitrophenyl glycinate [39], the formation and decomposition of the acyl-intermediate was suggested as a possibility, but such an intermediate was detected spectrophotometrically only in the former case.

Butler and Gold [50] suggested that in the acetate catalysed hydrolysis of 4-nitrophenyl acetate, the reaction proceeds through the acetic anhydride intermediate (X_A),which can not be isolated because of its reactivity in water. The mechanism is given in Diagram 1.4.

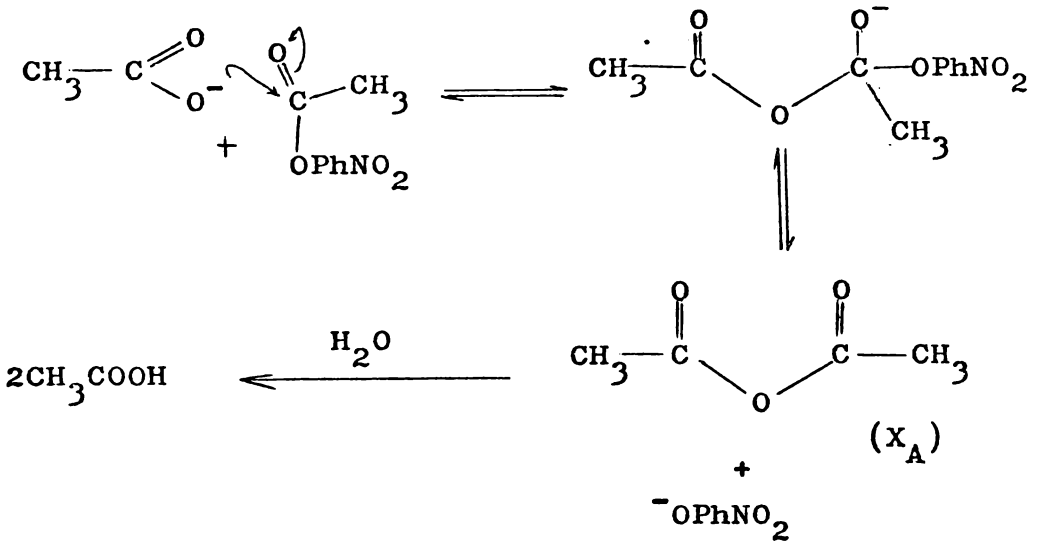
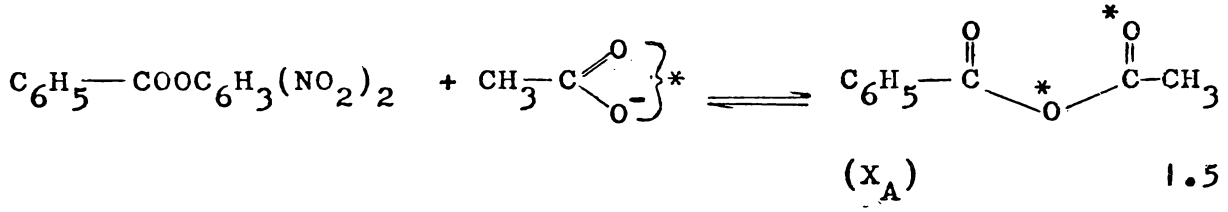
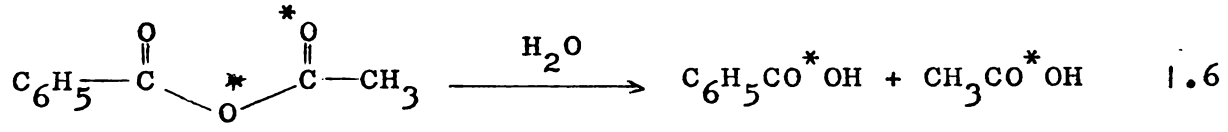


Diagram 1.4 The acetate-catalysed hydrolysis of 4-nitrophenyl acetate.

The formation of the acetic anhydride during the catalysed process though not detectable spectrophotometrically was demonstrated by trapping with added aniline which reacted rapidly to form acetanilide which was isolated [50, 123]. In a parallel study, Bender and Neveu [124] by the use of ^{18}O-labelled acetate as a catalyst in the hydrolysis of 2,4-dinitrophenyl benzoate showed that the products of this reaction resulted from an acid anhydride intermediate, as indicated in Equations 1.5 and 1.6. The presence of the ^{18}O label in the benzoic acid



(X_A) 1.5



(X_A) 1.6

product clearly indicates a nucleophilic role for the acetate catalyst.

Pyridine catalysed reactions of acyl anhydrides have been studied quite extensively also [18-22, 47, 48, 49, 51, 52, 123]. The reactions were believed to proceed with nucleophilic catalysis except for sterically hindered bases (such as 2,6-dimethylpyridine [51, 52]) for which general base catalysis, a process with less steric demand, was suggested as discussed in Section 1:2.1.

The ratio of catalytic reactivities, $k_{Im}/k_{HPO_4^{2-}}$ and k_{OH^-}/k_{Im} have been used successfully as indicators of the role played by the bases concerned. In the imidazole and HPO_4^{2-}

catalysed hydrolysis of 4-nitrophenyl acetate [47, 48], an alcohol activated ester , the former base is about 4000 times more reactive than the latter even though their base strengths are almost equal in magnitude (Table 1.1). In contrast, for the catalysed hydrolysis of ethyl dichloroacetate [44], an acyl-activated ester, both bases have almost similar catalytic reactivity. The comparison of the reactivities of OH^- and imidazole is also useful as discussed in Section 1:2.1 but is used to a lesser extent.

1:2.3 Intramolecular catalysis by neighbouring group effects.

Intramolecular reactions where the reacting groups can take up appropriate geometry are often found to show a huge acceleration relative to the intermolecular analogues. This area has been reviewed in detail [4, 40, 53, 129].

Neighbouring group effects in hydrolysis reactions include the intramolecular forms of the two types of catalysis by bases already discussed.

(a) Intramolecular general base catalysis.

The principle of general base catalysis has been discussed in Section 1:2.1.

The importance of general base catalysis in intramolecular reactions specifically is reviewed by Kirby and Fersht [53]. An example to illustrate the principle of intramolecular general base catalysis is given in Diagram 1.5 where the neighbouring carboxylate group in aspirin acts as a general base to assist

the attack of water on the ester carbonyl group by abstracting a proton.

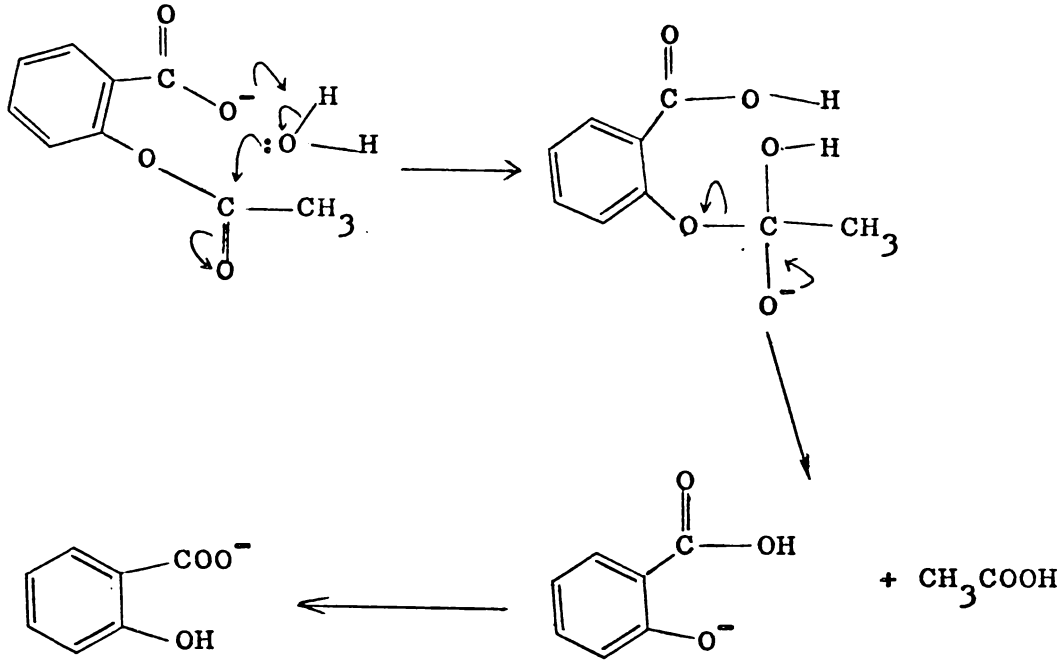
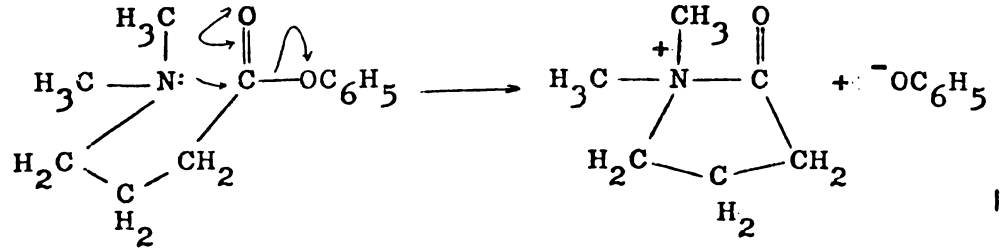


Diagram 1.5 Intramolecular general base catalysis of the hydrolysis of acetyl salicylate.

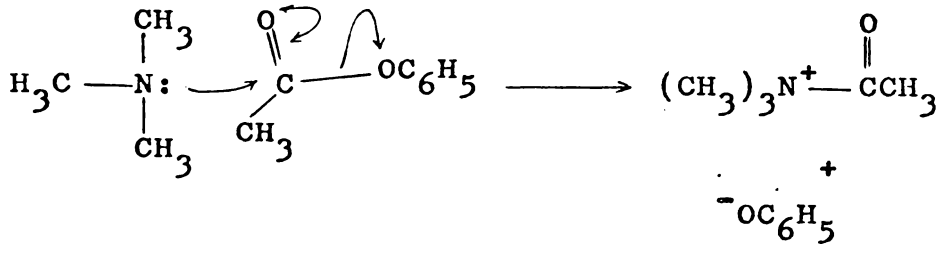
(b) Intramolecular nucleophilic catalysis.

The principle is the same as stated for intermolecular nucleophilic catalysis in Section 1:2.2 but the catalysis is very much more effective in the intramolecular case. For example, in the hydrolysis of phenyl γ-(N,N-dimethylamimo) butyrate [22] (Equation 1.7),



1.7

a unimolecular rate constant of 10 min^{-1} at 20°C was reported [22] but in an analogous intermolecular situation according to Equation 1.8,



1.8

a bimolecular rate constant of 8 x 10^{-3} 1 mol^{-1} min^{-1} at 20°C was reported [22]. The ratio of the rate constants is found to be about 1250 mol l^{-1} and this often referred to as the "effective concentration" for an intramolecular process [22]. Since an effective concentration is not attainable under any circumstances for intermolecular processes, the rapid rate of reaction is ascribed to favourable steric factors and local concentration effects [5, 53-59, 60].

The imidazole group is of particular interest in intramolecular catalysis because of its presence in the histidine residue of many enzymes responsible for hydrolysis of acyl compounds [61-63]. The solvolysis of 4-nitrophenyl γ-4(imidazolyl) butyrate [64-66] reveals intramolecular nucleophilic catalysis by imidazole as shown in the following mechanism (Diagram 1.6).

Intramolecular nucleophilic catalysis is involved in certain cases of covalent catalysis. The principle of covalent catalysis is that the catalyst adds to substrate and by doing so introduces a new functional group which promotes the required bond-breaking (or bond-formation) either by electronic effects or by neighbouring group participation, the latter

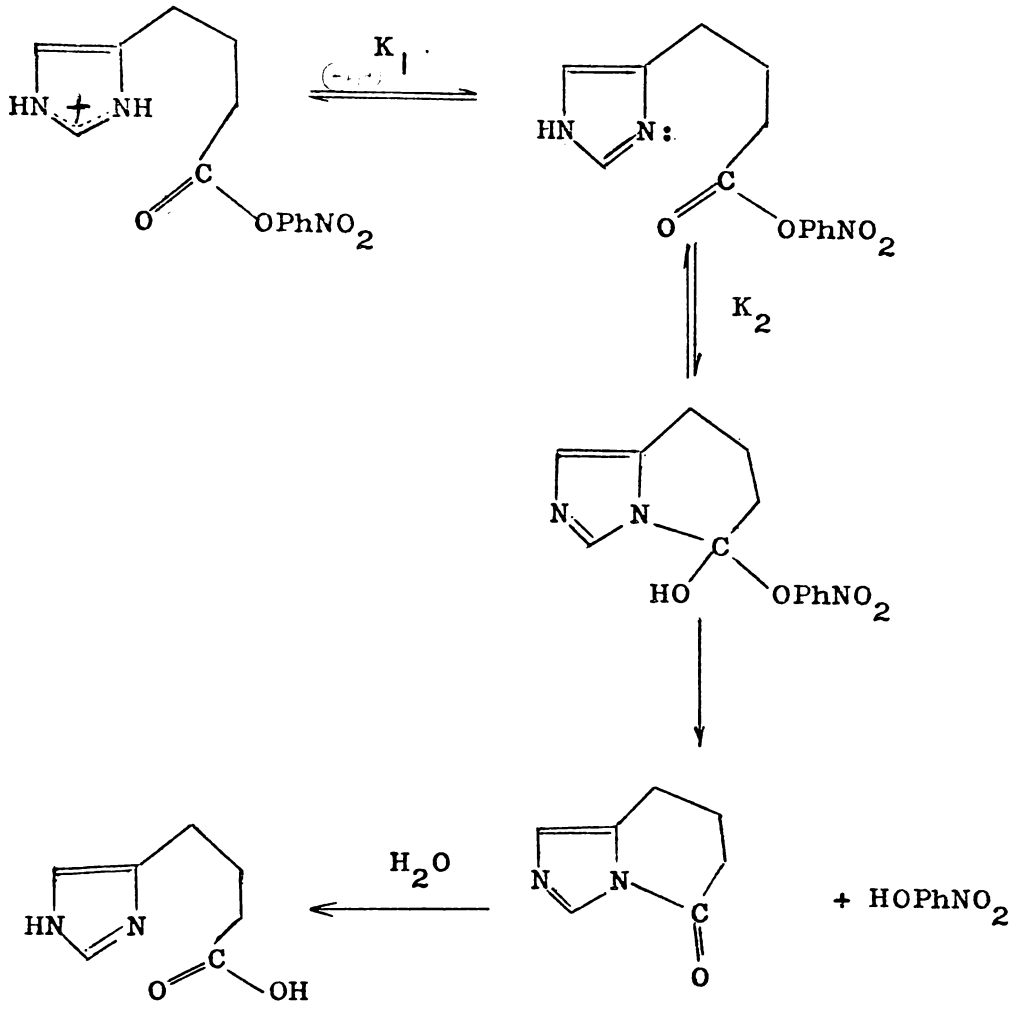


Diagram 1.6 Mechanism for the solvolysis of 4-nitrophenyl γ-4(imidazolyl) butyrate.

being involved in the case of intramolecular catalysis.

There are a number of examples of covalent catalysis in which intramolecular nucleophilic catalysis is involved following addition of a nucleophile to a carbonyl group. For instance, the hydrolysis of ortho-formylbenzoates is accelerated in the presence of morpholine [67]. The mechanism proposed [67] to account for the catalysis (Diagram 1.7) is

supported by the spectrophotometric detection of an appreciable concentration of intermediate.

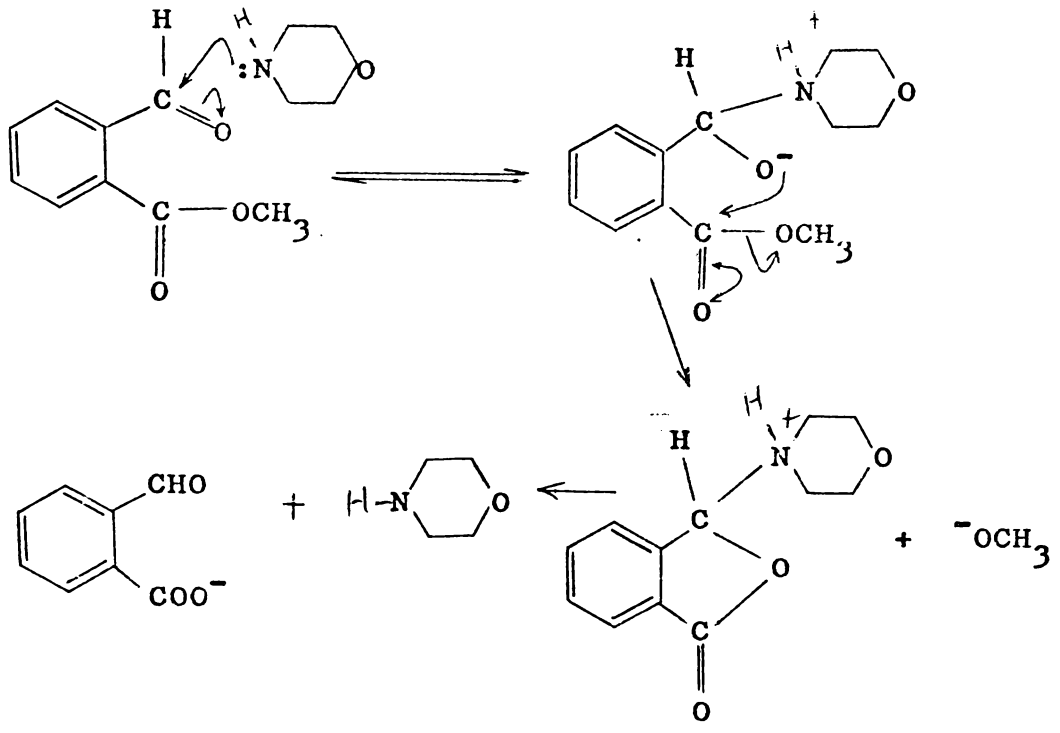


Diagram 1.7 Mechanism for the hydrolysis of methyl ortho-formyl benzoate.

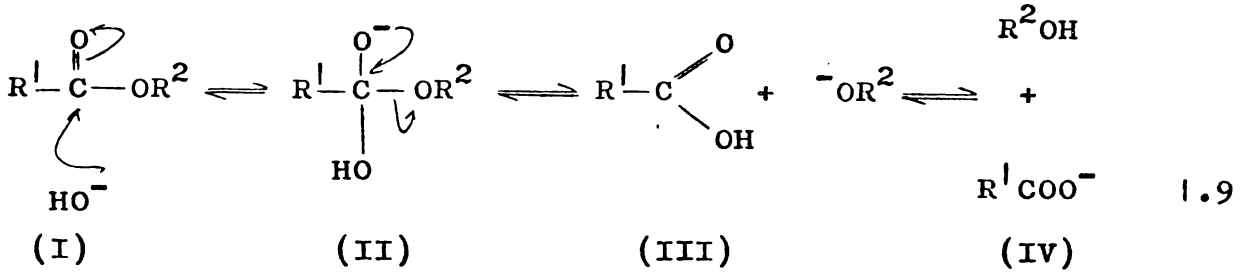
The suggested mechanism is that the catalyst adds to the aldehyde carbonyl to form a reactive anionic carbinolamine intermediate which gives intramolecular nucleophilic attack on the ester carbonyl forming a tetrahedral intermediate. The latter eliminates methoxide to form the spectrophotometrically detected intermediate which hydrolyses to product acid regenerating the morpholine catalyst.

Further examples are discussed in Section 2 of this thesis, where intramolecular nucleophilic catalysis in carbamate and carbinolamine intermediates formed from amino acid esters with

CO_2 and with aldehyde respectively is discussed in relation to catalysis of ester hydrolysis.

1:3 Alkaline hydrolysis of esters and some amino acid esters.

Equation 1.9 describes the alkaline hydrolysis of esters.



(I) (II) (III) (IV) 1.9

The ester (I) forms a tetrahedral intermediate (II) when hydroxide attacks the ester carbonyl. The tetrahedral intermediate then breaks down to (III) which transfers a proton to alkoxide ion giving the final pruducts.

Evidence of the existence of the tetrahedral intermediate is shown in the concurrent ^{18}O exchange with $H_2^{18}O$ during the hydrolysis of ester [2]. This provides evidence for a two-step process in ester hydrolysis [1, 2, 3]. Results of concurrent ^{18}O exchange during the hydrolysis of ester can be measured in terms of the factor k_h/k_e, where k_h is the hydrolytic rate constant and k_e, the exchange rate constant (Diagram 1.8).

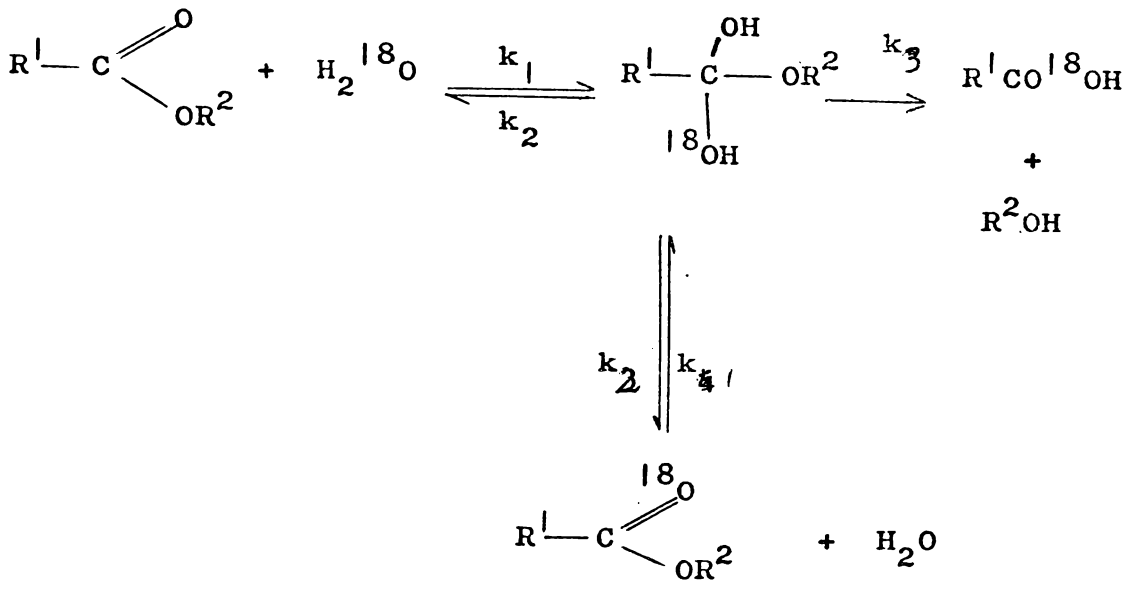


Diagram 1.8 Concurrent ^{18}O exchange during the hydrolysis of ester [2]

Equation 1.10 can be derived on the basis of Diagram 1.8 [2]. The factor k_h/k_e, is expected to be larger for esters

$$k_h/k_e = 2k_3/k_2 \qquad 1.10$$

with better leaving groups(as measured by the basicity). This result was born out in alkaline hydrolysis of a series of carboxylic acid esters (Bender and Heck [68]). Bunton et al [69] observed ^{18}O exchange in alkaline hydrolysis of alkyl esters and supported the two-step nature of ester hydrolysis.

Alkaline hydrolysis of alkyl esters of amino acids has been reviewed by Hay and Morris [130]. The amino acid esters are unique in that at appropriate pH values contributions to the overall reaction rate may be made by both the more reactive protonated ester (EH^+, Equation 1.11) and by the less

reactive neutral ester (E).

$$EH^+ \rightleftharpoons E + H^+ \qquad 1.11$$

In their review, Hay and Morris [130] showed that the protonated esters hydrolyse many times faster than the neutral counterparts and the enhanced rates of hydrolysis of the protonated amino acid esters were attributed to electrostatic and inductive effects.

Protonation of the amino group could also give rise to a completely different mechanism because protonation will allow an intramolecular general base assisted mechanism (Diagram 1.9). Analogous specific-base general-acid mechanisms have

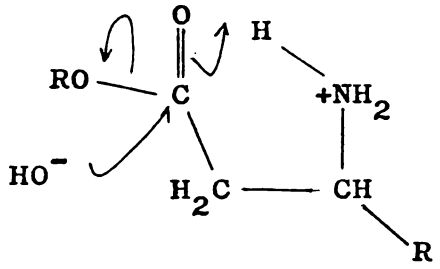


Diagram 1.9 Specific base general acid mechanism.

been considered in other systems for intramolecular catalysis of ester hydrolysis by amine function, see for instance: Schmir and Bruice [72], Agren et al [73], Hansen and Flormark [74, 75], Schatzle et al [66, 76] and Larsson [78]. Bruice and Benkovic [22] have suggested this possibility for γ-amino acid esters but to our knowledge this possibility has not been suggested for α-amino acid esters.

Relative rates of hydrolysis of neutral and protonated forms of phenyl esters of amino acids have not been reported prior to the present study.

Section 2 Previous studies of CO_2-catalysis and aldehyde catalysis of the hydrolysis of amino acid esters.

2:1 CO_2 catalysed hydrolysis of amino acid esters.

Wieland and Jaenicke [100] reported for the first time catalysis of the hydrolysis of phenyl glycinate (at pH 7.5) in bicarbonate solutions. They suggested that carbon dioxide was involved via reaction with the neutral species (Diagram 2.1) to give a carbamate ion (II) which allows catalysis via the intramolecular nucleophilic attack indicated, the catalyst, CO_2, and amino acid finally being reformed.

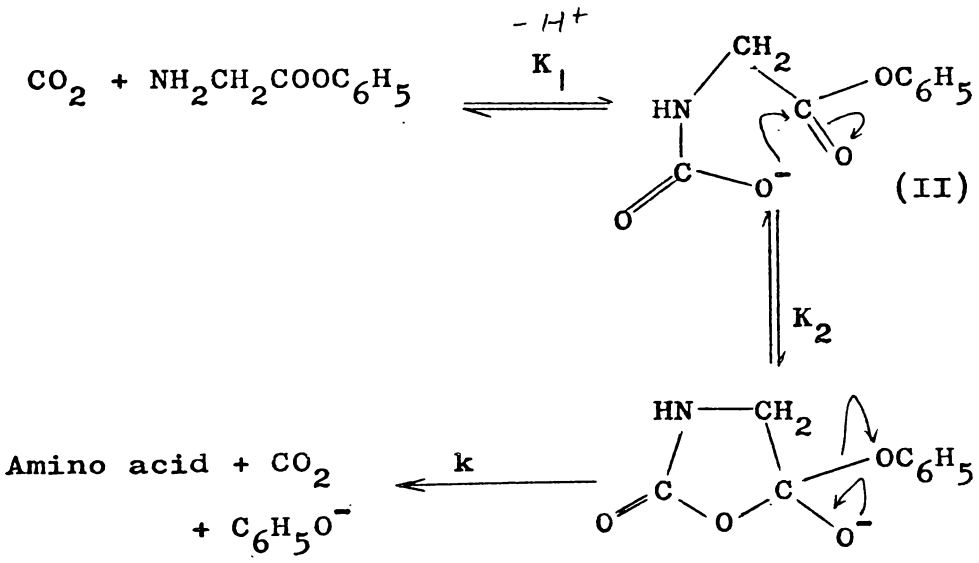


Diagram 2.1 Mechanism proposed by Wieland and Jaenicke for the catalysed hydrolysis of phenyl glycinate in bicarbonate solutions at pH 7.5 [100].

No study of the effect of variation of pH on catalysis of phenyl glycinate hydrolysis in bicarbonate solutions was made.

Such a study might have established with certainty whether CO_2 was indeed responsible for catalysis or whether one of the carbonate species HCO_3^- or CO_3^{2-} might be catalytically active.

However, CO_2 itself is indeed responsible for such catalysis in the case of the hydrolysis of 4-nitrophenyl esters of amino acids (leucine, glycine and phenylalanine) in bicarbonate solutions, as shown kinetically by a pH variation study [39]. Other carbonate species HCO_3^- and CO_3^{2-} are not involved, the kinetic form over a range of pH in which both the fraction of ester present as the neutral form (E) and the fraction of total carbonate present as CO_2 change sharply, being consistently in agreement with the kinetic form;

$$\text{Rate} = k[E][CO_2] \qquad 2.1$$

This kinetic form was shown to be consistent with a reaction scheme of the form [39],

$$E + CO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Carbamate} \xrightarrow{k_2} \text{Products} \qquad 2.2$$

where k in Equation 2.1 is given by $k_1k_2/(k_{-1} + k_2)$, the kinetic form being followed irrespective of whether formation or decomposition of intermediate is rate-determining. This scheme (Equation 2.2) is in accord with the suggested mechanism for phenyl glycinate hydrolysis, i.e. Diagram 2.1 applies but with 4-nitrophenolate as the leaving group.

It was suggested [39] that the relative rates of CO_2-catalysed hydrolysis of the 4-nitrophenyl esters of glycine, leucine and phenylalanine were consistent with rate-determining formation of carbamate rather than decomposition, the glycine ester being the most reactive as is consistent with minimal

steric hindrance by the ester α-substituent of reaction of the α-amino group with CO_2.

Initial research for the understanding of carbamate formation, Equation 2.3, were model studies of peptides (glycine and higher peptides [95, 96]) which is closely related to the amino group of haemoglobin.

$$RR^1NH + CO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RR^1\overset{+}{H}N\ COO^- \overset{K}{\underset{H_2O}{\rightleftharpoons}} RR^1NCOO^- + H_3O^+ \qquad 2.3$$

The kinetics and mechanism for formation was described in detail according to Equation 2.3 [96, 97, 120]. The decomposition of carbamate was studied also [98, 99, 120].

2:2 Aromatic aldehyde catalysed hydrolysis of some α-amino acid esters.

The formation of carbinolamines from amino acids and aldehydes has been extensively studied [40, 78-90].

The first suggestion of a critical role of a carbinolamine formed between an aldehyde and an ester of an α-amino acid came in relation to the benzaldehyde catalysed hydrolysis of 4-nitrophenyl leucinate[91]. The carbinolamine formed by nucleophilic addition of the amino group of the ester to the carbonyl group of benzaldehyde could undergo intramolecular nucleophilic attack of the hydroxy group as shown in Diagram 2.2 and such a process was proposed to account for the large catalytic effect of benzaldehyde on the hydrolysis.

Evidence for rate-determining carbinolamine formation in the

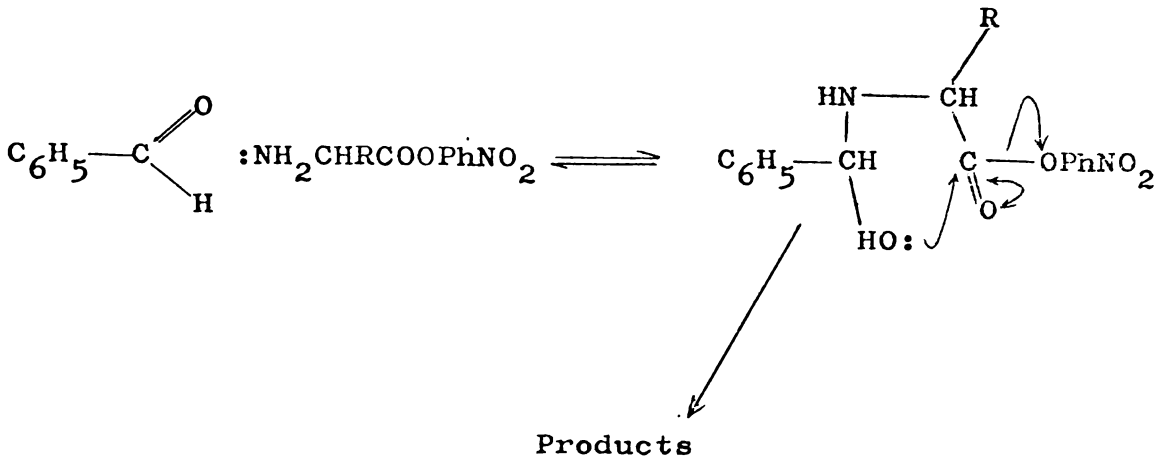


Diagram 2.2 The formation and decomposition of carbinolamine in the benzaldehyde catalysed hydrolysis of 4-nitrophenyl leucinate according to Capon and Capon ($R = CH_2CH(CH_3)_2$).

catalysed hydrolysis of 4-nitrophenyl leucinate comes from the results of Capon and Capon [91]. Firstly, it was found that benzaldehyde had no detectable catalytic activity in the hydrolysis of 4-nitrophenyl acetate even though the concentration of benzaldehyde used was 10 times greater than that used for the corresponding leucine ester. Secondly, it was found that both pyridine -2-carboxyaldehyde and pyridine-4-carboxyaldehyde were much more effective than benzaldehyde as catalysts. This enhanced catalytic effect is not due to intermolecular nucleophilic catalysis by pyridine nitrogen because pyridine on its own had no detectable catalytic effect on the hydrolysis of the leucine ester. Nor is it due to intramolecular neighbouring group participation by the pyridine nitrogen, likely for pyridine-2-carboxyaldehyde (Diagram 2.3) but geometrically impossible for pyridine-4-carboxyaldehyde which is nevertheless equally effective as a catalyst.

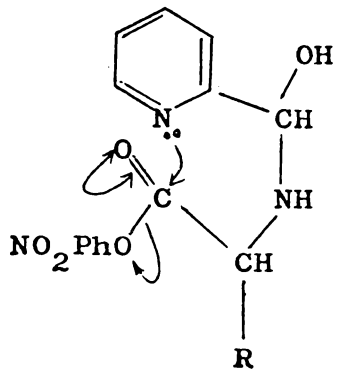


Diagram 2.3 A possible intramolecular neighbouring group effect on the decomposition of carbinolamine.

The failure of pyridine-2-carboxyaldehyde to be a much more effective catalyst than pyridine-4-carboxyaldehyde suggested that formation of carbinolamine might be rate-determining with carbinolamine decomposition, whether according to Diagram 2.3 or in the case of pyridine-4-carboxyaldehyde according to Diagram 2.4, being fast.

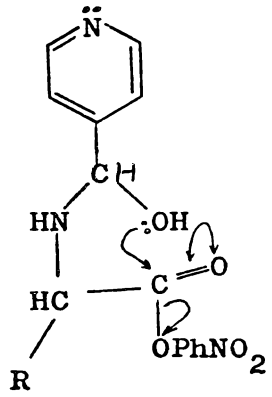


Diagram 2.4 The decomposition of carbinolamine by intramolecular nucleophilic catalysis.

Hay and Main [39, 92] carried out detailed studies on the

catalysed hydrolysis of 4-nitrophenyl esters of leucine, glycine and phenylalanine in the presence of benzaldehyde and derivatives. They observed [39] that in the 4-nitrobenzaldehyde catalysed reactions of 4-nitrophenyl esters of leucine and glycine, the latter hydrolyses about 3 times faster than the former at pH 7.6. This rate enhancement is not consistent with rate-determining formation of carbinolamine as suggested by the Capons [91] because the rate of formation of the carbinolamine would be expected on steric grounds to favour the glycine ester (R= H, Diagram 2.2) over the leucine ester (R = $CH_2CH(CH_3)_2$, Diagram 2.2). They suggested [39] that the rate-determining decomposition may apply instead and the overall rate enhancement can be accounted for by considerations of the carbinolamine species (Diagrams 2.5 and 2.6)

It is to be expected that the most likely conformation for the glycine carbinolamine, is one where the reacting groups, which are the bulky groups, are furthest apart(Diagram 2.5) and

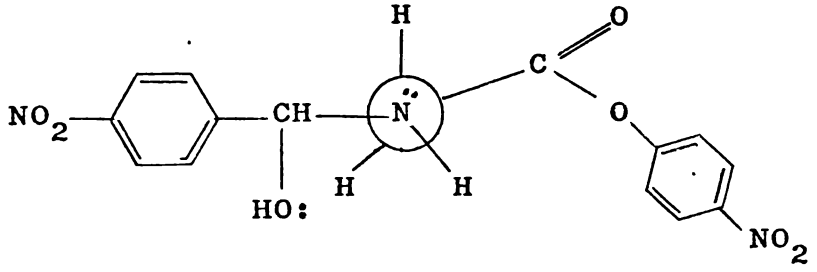


Diagram 2.5 The favoured conformer for the glycine carbinolamine.

hence a low rate of decomposition is expected.

For the leucine ester (in which the group $-CH_2CH(CH_3)_2$ replaces H in glycine), steric effects are such that there will be two favoured conformers, one of which (Diagram 2.6)

has the reacting groups in close proximity for intramolecular nucleophilic catalysis, thus allowing a faster rate of decomposition.

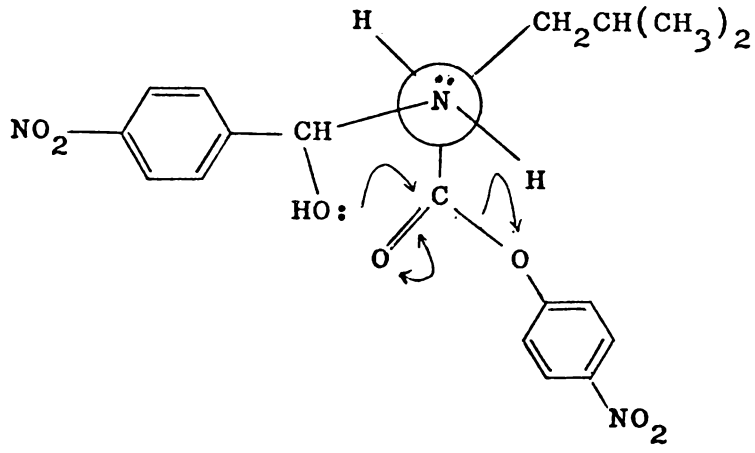


Diagram 2.6 One favoured conformer for the leucine carbinolamine.

The results of recent studies by Lee [94] on the catalysed hydrolysis of 4-nitrophenyl esters of glycine, leucine, valine and proline by benzaldehyde, 2-hydroxybenzaldehyde and 4-hydroxybenzaldehyde gave no further insight into what the rate-determining stage might be. Various intermediates possibly involved in catalysis were considered in more detail than in the earlier studies as shown in Diagram 2.7 [94].

Briefly (Diagram 2.7), the zwitterionic carbinolamine, C_Z, presumably sometimes exists long enough to lose a proton to form the anionic carbinolamine C_A, which is a highly basic species that would be readily protonated to a neutral carbinolamine C. Lee [94] suggested that either of the carbinolamine species, C_A and C, might be implicated in the mechanism for hydrolysis. The latter, C, could be expected to decompose to an imine intermediate (Diagram 2.8) by OH^- transfer to the

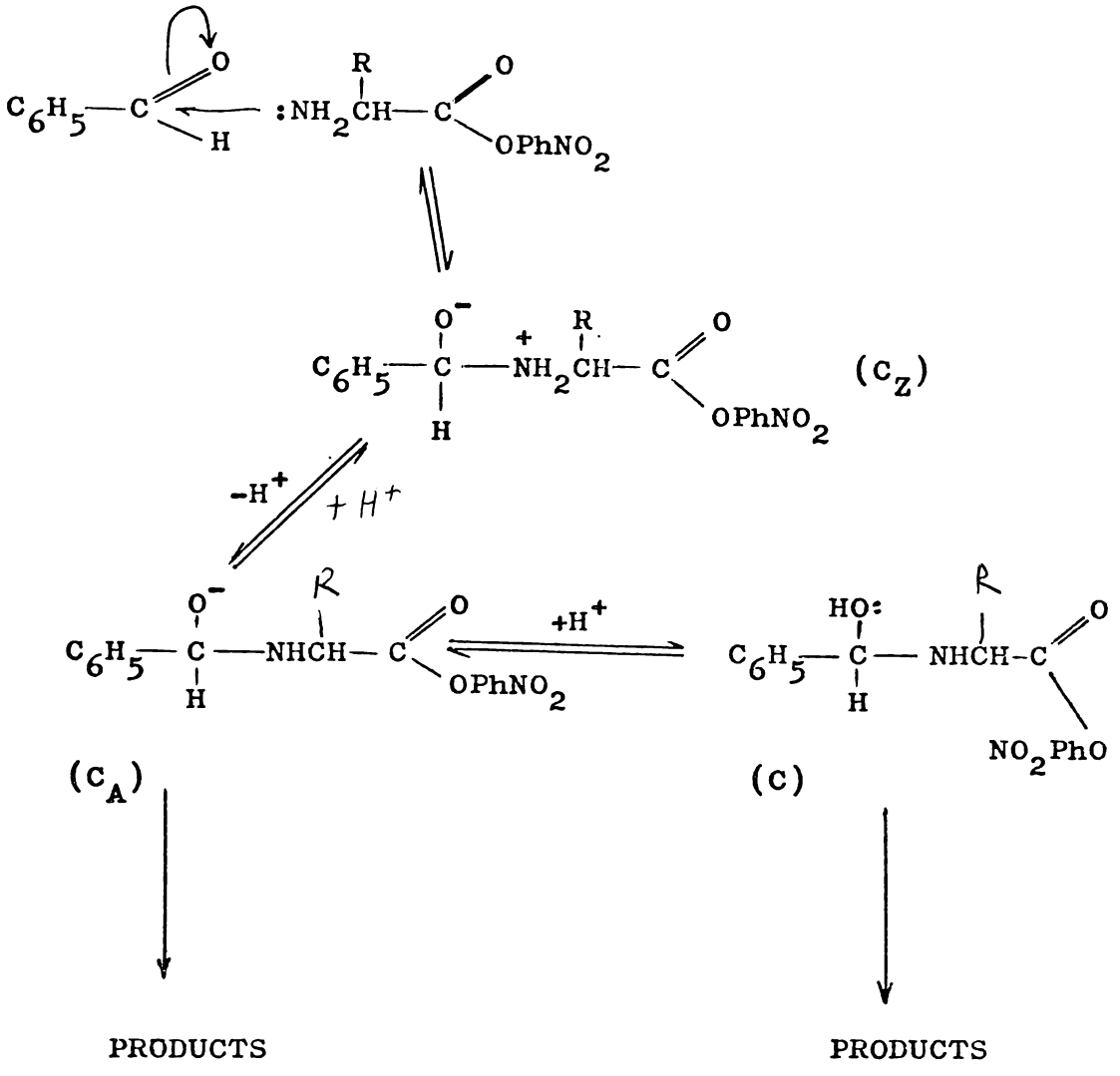


Diagram 2.7 Possible intermediate species involved in benzaldehyde catalysed hydrolysis of 4-nitrophenyl esters of amino acids (Lee [94]).

ester carbonyl centre, thereby ensuring loss of 4-nitrophenolate ion from the tetrahedral intermediate formed. Alternatively, the inherently more reactive species, C_A, could also cyclize intramolecularly to form a tetrahedral intermediate (Diagram 2.9), the partitioning of which would favour the release of 4-nitrophenolate ion over the basic carbinolamine anion. This means that, once formed, the tetrahedral inter-

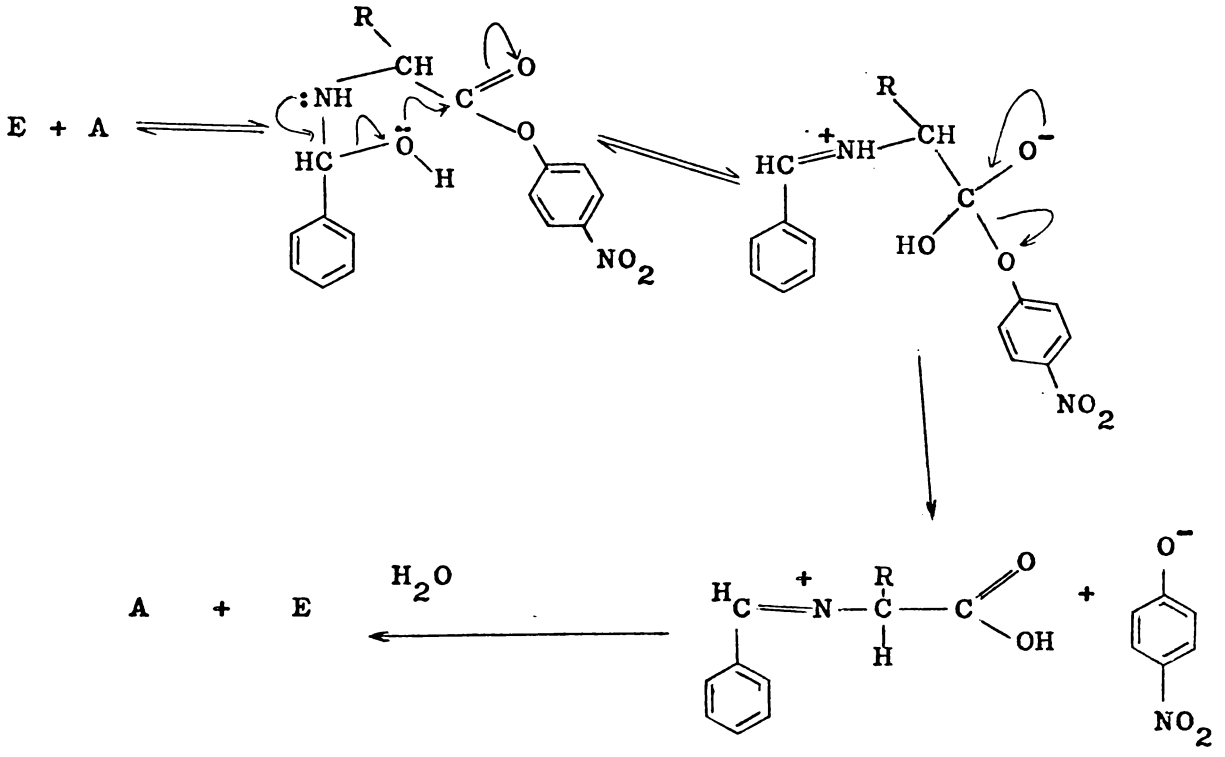


Diagram 2.8 Suggested mechanism for benzaldehyde catalysed hydrolysis of 4-nitrophenyl ester of amino acids involving neutral carbinolamine (Lee [94]).

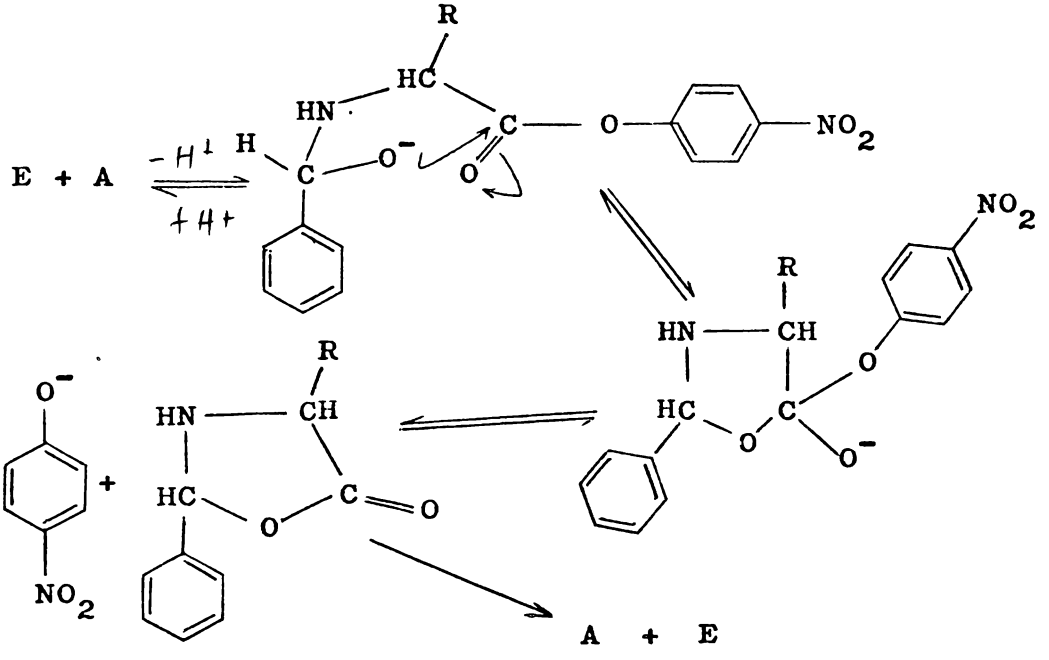


Diagram 2.9 Benzaldehyde catalysed hydrolysis of amino acid esters involving carbinolamine anion (Lee [94]).

mediate will decompose to products. The unanswered question is, will the carbinolamine anion exist long enough or in sufficient concentration at about neutral pH to cyclize according to the mechanism in Diagram 2.9? If not, then the route (Diagram 2.8) via neutral carbinolamine probably applies.

The unexpectedly large catalytic effect of salicylaldehyde anion, which in spite of electronic effects unfavourable to carbinolamine formation is more reactive than the neutral salicylaldehyde species, was interpreted by Lee [94] as possibly involving trapping of the short-lived zwitterion C_Z to give C_A via intramolecular proton abstraction by the phenoxide function at the 2-position (Diagram 2.10) such an effect not being possible for the neutral species.

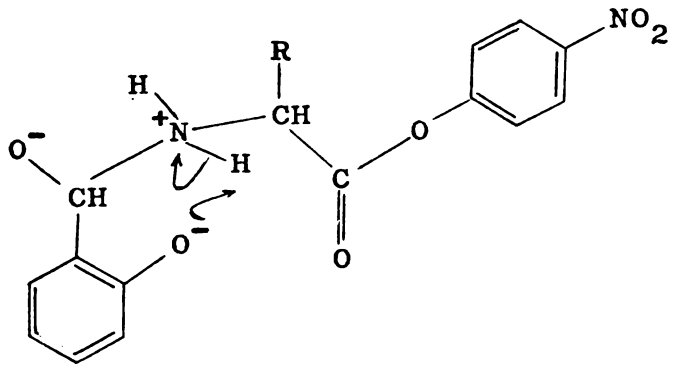


Diagram 2.10 Intramolecular proton abstraction in the salicylaldehyde anion catalysed hydrolysis of 4-nitrophenyl leucinate (Lee [94]).

There are no reports in the literature of studies of hydrolysis of phenyl and 4-methoxyphenyl esters of amino acids in the presence of aromatic aldehydes. Studies have previously been limited only to 4-nitrophenyl esters.

2:3 Aims of present study.

2:3.1 With phenyl glycinate.

Previous studies of the hydrolysis of aryl esters of amino acids have been restricted to 4-nitrophenyl esters with the exception of hydrolysis of phenyl glycinate in bicarbonate solutions. The general aim of the present work was to extend our knowledge of catalysis of phenyl esters of amino acids by compounds which were previously known to catalyse 4-nitrophenyl ester hydrolysis (bicarbonate solutions, aromatic aldehydes and imidazole) and to study also catalysis by bases, a little studied area for aryl esters of amino acids. We hoped that comparison of results for phenyl and 4-nitrophenyl esters might throw light on mechanisms operating in both cases since differences in leaving group ability in other systems have provided information valuable in elucidating mechanisms.

Using phenyl glycinate as reactant ester more precise aims within specified areas were as follows:

(a) Covalent catalysis.

(i) Bicarbonate solutions. We wished to establish whether the kinetic form for catalysed hydrolysis was the same as or different from that applying for 4-nitrophenyl esters since this was not determined by the work of Wieland [100].

(ii) Aromatic aldehydes. We wished to establish whether the kinetic form is the same as for 4-nitrophenyl esters and to make a comparison of catalytic efficiency for different leaving group abilities.

(b) Base catalysis. We hoped that by studying catalysis by a base over a pH range in which the ester protonates (pK_a = 7.15), it might be possible to establish individual

kinetic forms and rate constants for both the neutral and the protonated species. Both species are activated towards hydrolysis by electron withdrawing groups in the acyl portion, such acyl activation being, however, much greater for the protonated ester. Previous studies have shown that acyl activation of alkyl esters promotes general base catalysis, whereas activation in the alcohol portion (good - leaving group ability) promotes nucleophilic catalysis. The roles of bases in catalysis were therefore of interest, neutral and protonated forms of amino acid aryl esters both being activated in both acyl and alcohol portions.

(i) Imidazole and N-ethylmorpholine .

Imidazole acts as a nucleophilic catalyst in some cases, a general base catalyst in others, so it seemed an ideal base to study the nature of base catalysis for phenyl glycinate. Imidazole had previously been shown to catalyse the hydrolysis of 4-nitrophenyl esters of amino acids, with a term first order in imidazole in the kinetic form. In the event, we found (see Section 7) that the kinetics of imidazole catalysis are dominated by a term second order, not first order, in imidazole, which implies a nucleophilic catalyst role as discussed in the thesis. We therefore carried out a study with another base, N-ethylmorpholine, which we reasoned would be less likely because of steric effects to assume a nucleophilic role in catalysis and which might provide the basis for comparison of general base catalysis for the neutral vs the protonated ester. As it turned out, this amine also gave abnormal kinetic behavior.

(ii) Phosphate.

The phosphate dianion, HPO_4^{2-}, is known to have a very similar catalytic effect to imidazole as a general base (their pK values are nearly the same), but is often of the

order of 1000 times less reactive as a nucleophilic catalyst. We therefore studied catalysis by phosphate solutions with the aim of comparing catalytic effect of HPO_4^{2-} with imidazole to see if this might help in assigning catalysis by the latter to a general base or a nucleophilic role.

(iii) OH^-. Alkaline hydrolysis has been studied for alkyl esters of amino acids and the relative rates of nucleophilic substitution by hydroxide of the neutral and protonated ester species have been determined. No such study has been made for an aryl ester, so we planned to do this with phenyl glycinate for comparison purposes.

2:3.2 With 4-Nitrophenyl Esters.

Earlier studies on both CO_2 and aromatic aldehyde catalysis of hydrolysis have led to suggestions and contradictions concerning the rate-determining step, for whether carbamate or carbinolamine formation or decomposition is rate-determining.

We therefore compared catalysis for the glycine and valine esters, the latter not being studied previously, taking the view that the α-substituent in the valine case ($-CH(CH_3)_2$) might sufficiently hinder carbinolamine or carbamate formation and/or promote carbinolamine or carbamate decomposition, so that the question of whether formation or decomposition was rate-determining might be answered.

We also planned to make some initial studies on the temperature effect on the catalysed hydrolyses. For certain reactions previously studied in which there is rate-determining decomposition of intermediate formed in a pre-equilibrium, the rate is very little increased with temperature, as the

increase in rate constant is partially compensated by a decrease in equilibrium constant. We were interested, therefore, in the magnitude of temperature effect as a possible guide to rate-determining step.

Finally, since the possibility of an E1cB mechanism due to intramolecular reaction as base by carbamate formed from 4-nitrophenyl ester and CO_2 has not previously been considered, we planned to study ease of amino acid ester α-proton exchange in the presence of CO_2 using the ^{1}H-NMR technique with solutions in D_2O. Catalysis of proton exchange by CO_2 could be reasoned to point to the possibility of an E1cB mechanism in the 4-nitrophenyl ester case.

Section 3 Experimental Methods

3.1 Materials

Mono- and di- potassium hydrogen phosphate (May and Baker) were used without further purification.

The following were obtained from Sigma Chemical Company: N-carbobenzoxy glycine, ethyl phenylalaninate hydrochloride and the N-carbobenzoxy 4-nitrophenyl esters of glycine, valine and proline.

The following materials were purified. Imidazole (m.p. 90°) was recrystalized twice from absolute ethanol. 4-nitrobenzaldehyde (m.p. 106°) was recrystalized twice from dry methanol. N-ethylmorpholine (b.p. 139°) was fractionally distilled. Dry ethanol was obtained by the action of magnesium on ethanol followed by distillation[115].

Diphenyl sulphite and di-(4-methoxyphenyl)sulphite were prepared and used as starting materials for the synthesis of the corresponding esters of glycine using the methods given by Greenstein and Winitz [49] except that ethyl acetate and triethylamine were purified by fractional distillation before use in the preparations. The N-carbobenzoxy derivatives of phenyl glycinate (m.p. 68°-70°C)[49] and 4-methoxyphenyl glycinate (m.p. 70°-72°C)[49] were then prepared also according to Greenstein and Winitz [49]. To remove the N-carbobenzoxy group, the esters (0.1 g) were treated with hydrogen bromide in glacial acetic acid (10 ml, 45% sol^{n}) (BDH) until bubbling stopped. This usually takes 2-3 minutes. Upon adding about 100 ml of anhydrous diethyl ether, the crude products were obtained. They were purified by recrystalization from absolute ethanol. Dry ethanol and sodium dried diethyl ether were essential in the preparations of the more reactive ester hydrobromides to minimize losses due to hydrolysis.

The ester hydrobromide salts had the following melting points:

4-nitrophenyl glycinate	211°C	(212°C, [94])
4-nitrophenyl valinate	207°C	(207°C, [94])
4-nitrophenyl prolinate	197°C	(197°C, [94])
phenyl glycinate	208°C	
4-methoxyphenyl glycinate	213°C	

The esters were stored in the N-carbobenzoxy form and when required they were obtained by the method just described.

3:2 The preparation of buffer solution.

Phosphate buffer solutions, described by Perrin [13] were used for the bicarbonate and 4-nitrobenzaldehyde catalysed hydrolyses of phenyl glycinate, 4-methoxyphenyl glycinate, 4-nitrophenyl glycinate, 4-nitrophenyl valinate and 4-nitrophenyl prolinate. The ionic strength of these buffer solutions was 0.2 mol l^{-1}.

For the N-ethylmorpholine, imidazole and phosphate catalysed hydrolyses of phenyl glycinate, the catalysts are self-buffering. Calculated amounts of potassium chloride were added to maintain a constant ionic strength of 1 mol l^{-1} for these studies.

Buffer solutions were prepared using doubly-distilled water, freshly-boiled and cooled under a stream of oxygen free nitrogen to minimize the amount of residual carbon dioxide. This is required because carbon dioxide is an effective catalyst for the hydrolyses of the 4-nitrophenyl esters.

For the phosphate buffers, doubly-distilled and degassed water was added to dissolve the weighed amounts of phosphate salts in a nitrogen flushed volumetric flask. The prepared

solution was then flushed with nitrogen for 7 - 10 minutes and kept aside for immediate use.

For buffer solutions of N-ethylmorpholine and imidazole, the calculated amount of amine was dissolved in doubly-distilled, degassed water and the required amount of concentrated hydrochloric acid was added, the final pH reading being recorded after the volumetric flask containing the buffer was topped up with water and flushed with nitrogen.

Only freshly prepared buffer solutions were used. All pH readings were recorded with a Radiometer 26 pH-meter at 30°C with standard radiometer buffers: Pthalate (pH = 4.011) and Borax (pH = 9.138). The pH of the reaction solution was checked at the completion of each kinetic run.

3.3 Kinetic Methods.

Kinetic runs were carried out on a Unicam SP 1800 spectrophotometer equipped with a linear recorder and an externally-thermostatted cell-block (30°C). The appearance of the phenolate, 4-methoxyphenolate and 4-nitrophenolate ions were followed at 270 nm, 286 nm and 420nm (pH > 7.0), respectively. (For 4-nitrophenyl esters, free phenol at 320 nm was detected at pH < 7.0).

Standard solutions of potassium bicarbonate were prepared by dissolving the calculated amount of the salt in the appropriate buffer solution already prepared. However, standard solutions of 4-nitrobenzaldehyde were prepared by dissolving the calculated amount in dry methanol instead.

The freshly prepared catalyst solution was transfered by a 10 or 100 microlitre syringe. Manufacturer specifications for the syringes used are as follows:

(i) Readibility 0.5%

(ii) Accuracy 1%

(iii) Reproducibility 1%

The prepared solution were transfered by pipette into the spectrophotometric cells which were previously flushed with nitrogen. The cells were then placed into the cell-block of the spectrophotometer to equilibrate to temperature at 30°C for about 10 minutes. The ester (dissolved in dry methanol) was injected into the reaction cell by syringe and the cell was inverted and shaken to mix. The recorder was switched on after about 15 seconds delay for mixing. An infinity reading was taken for each run after a minimum of ten half-lives.

3:4 Determination of ionization constant of phenyl glycinate by pH-titration method.

A stoppered, thermostatted, double-walled beaker complete with magnetic stirrer was used in the experiment to evaluate the ionization constant of phenyl glycinate.

The stopper was perforated to hold the Radiometer 26 pH-meter electrodes, the burette (containing the titrant, standardized sodium hydroxide solution) and two capillaries for the in-flow and out-flow of nitrogen gas.

The double-walled beaker containing 50 ml of doubly-distilled and degassed water was equilibrated to 30°C by water heated in a thermostat bath and pumped through the double-walled beaker. Phenyl glycinate hydrobromide salt (0.01 mol l^{-1}) was then dissolved and the stopper was replaced, all this being performed under a steady stream of nitrogen to exclude carbon dioxide which could affect the pH.

The solution was magnetically stirred and after each addition of sodium hydroxide, the stirrer was stopped and when the

pH reading had settled it was then recorded.

Results for the pK_a determination are given in Section 4 of this thesis.

Section 4 The potassium bicarbonate catalysed hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate.

4:1.1 Results.

The hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate has been investigated. In this pH variation study, where there is a large excess of the carbonate species over the amino acid esters, all the observed rate constants, k_h, were found to be pseudo first order (Tables 4.1 and 4.2) in ester.

The plots of k_h vs the total concentration of the carbonate species $[C_T]$ at constant pH were linear, implying a first order dependence on at least one of the carbonate species (CO_3^{2-}, HCO_3^- and CO_2) present in the solution. The rates of the catalysed process are expressed in terms of the catalytic rate constant, k_c, defined in the following section. The values are gradients of the plots k_h vs $[C_T]$ at a particular pH and they are listed on the last column of Tables 4.1 and 4.2. Two representative plots of k_h vs $[C_T]$ are shown in Figures 4.1 and 4.2.

4:1.2 Analysis

The values of k_c listed in Tables 4.1 and 4.2 show variation with pH. This variation is a direct result of the varying proportions with pH of the neutral ester (E) and its conjugate acid (EH^+) and the carbonate species, expressed by Equations 4.1 and 4.2, respectively.

$$^{+}NH_3CH_2COOPh \xrightleftharpoons{pK\ (E) = 7.15} H^+ + NH_2CH_2COOPh \qquad 4.1$$

$$CO_2 + H_2O \xrightleftharpoons{pK=6.37} H^+ + HCO_3^- \xrightleftharpoons{pK=10.33} 2H^+ + CO_3^{2-} \qquad 4.2$$

The variation of k_c with pH was analysed and found to be consistent with a catalysed process involving only the neutral ester (E) and the bicarbonate ion (HCO_3^-). The kinetically equivalent pair EH^+ and CO_3^{2-} is considered unlikely as will be discussed later . The analysis showed that reactions involving other combinations of species in solution were not responsible for experimentally-detectable parallel reactions.

The analysis on which this is based is as follows. Assuming that only the bicarbonate ion (HCO_3^-) and the neutral ester (E) are participants, then, the theoretical rate for the catalysed process is expressed by Equation 4.3 and the experimental rate is expressed by Equation 4.4.

$$\text{Rate} = k_2[E][HCO_3^-] \qquad 4.3$$

$$\text{Rate} = k_c[E]_T[C_T] \qquad 4.4$$

Equating Equations 4.3 and 4.4, we have;

$$k_2 = k_c([E]_T/[E])x([C_T]/[HCO_3^-])$$
$$= k_c/f_E x f_{HCO_3^-} \qquad 4.5$$

where f_E is defined as the fraction of the ester present in the neutral form (E in Equation 4.1) and $f_{HCO_3^-}$ is the fraction of the carbonate species present as the bicarbonate ion (HCO_3^- in Equation 4.2).

Table 4.1 The potassium bicarbonate catalysed hydrolysis of phenyl glycinate.

Temperature 30°C

Error in k_h value ± 5%

pH	$10^3 [C_T]$ (mol l^{-1})	$10^4 k_h$ (s^{-1})	k_c (l mol^{-1} s^{-1})
7.37	0	6.1, 8	
	3	22.3	
	4.65	29.8	
	6	36.9	
	9	51.2	
	9.3	49.8	
	12	60.3	
	13.05	65.7	
	14	68.8	
	18.6	89.5	
	22.25	103	0.44 ± .04
6.91	0	3.1, 4	
	2.4	10.4	
	4.8	17.6	
	7.2	21.7	
	9.6	28.2	
	12	31.8	
	19	49.3	
	22	55.5	0.23 ± .03

continued

6.57	0	2.2, 2.8	
	2.2	5.9	
	3.96	6.5	
	4.4	7.9	
	6.6	9.85	
	7.92	12	
	8.8	11.8	
	11	16.1	
	11.9	14.8	
	17	20	
	23	25.3	$0.105 \pm .05$
6.14	0	1.5, 1.7	
	2.3	2	
	3.2	2.1	
	4.7	2.7	
	4.8	2.9	
	6.8	3.3	
	8	3.6	
	10	4.23	
	14	5.5	$0.027 \pm .002$

Table 4.2 The potassium bicarbonate catalysed hydrolysis of 4-methoxyphenyl glycinate.

Temperature 30°C

Error in k_h values ± 5%

pH	10^2 $[C_T]$ (mol l^{-1})	10^4 k_h (s^{-1})	k_c (l mol^{-1} s^{-1})
7.37	0	3.3	
	0.3	13.3	
	0.6	18.3	
	2.4	46.7	
	3.0	58.3	0.2 ± .03
6.91	0	2.8	
	0.3	8.7	
	0.6	11.0	
	2.4	27.5	
	3.0	32.2	0.09 ± .007
6.57	0	1.7	
	0.3	3.3	
	0.6	4.7	
	1.2	7.3	
	1.8	10.8	
	2.4	13.8	0.044 ± .005
6.14	0	1.92	
	0.3	2.83	
	0.6	3.2	
	1.2	4.0	
	1.8	4.7	
	2.2	5.0	
	2.5	5.6	0.012 ± .002

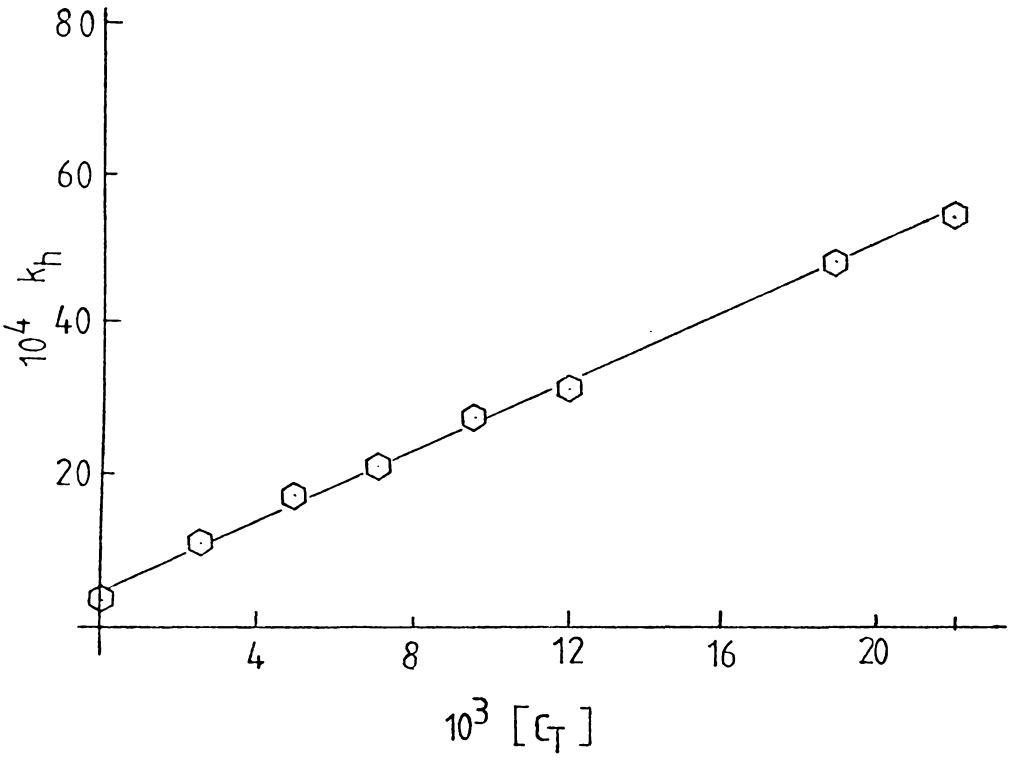


Figure 4.1 The plot of k_h vs [C_T].
Phenyl Glycinate (pH 6.91)

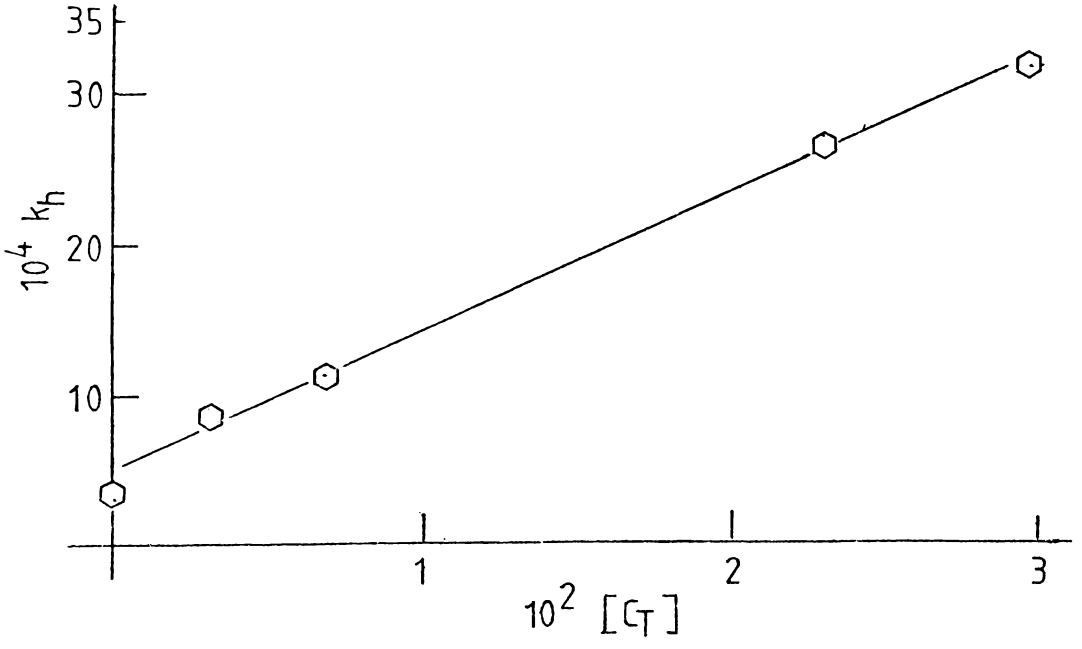


Figure 4.2 The plot of k_h vs [C_T].
4-methoxyphenyl Glycinate (pH 6.91)

Therefore, Equation 4.5 can be interpreted as such that if the catalysed process involves the species E and HCO_3^- only, the expression $k_c/f_E x f_{HCO_3^-}$ should be invariant with pH, since this expression equals the rate constant k_2 (Equation 4.5) defined for the specific reaction of E and HCO_3^- (Equation 4.3). Table 4.3 shows that the values of k_2 are indeed constant with pH within experimental error, thus providing support for the kinetic form of Equation 4.3.

Values of $f_{HCO_3^-}$ were calculated from a pK_a value of 6.366 [101] at 30°C. Values for f_E were calculated from a pK_E value of 7.15 ± .05 at 30°C. The pK_E was calculated from pH-titration results (Table 4.4).

4.2 Discussion

Equation 4.5 was based on the reaction of species E and HCO_3^-. The analysis does not distinguish between the two reactive pairs, E and HCO_3^- on the one hand and EH^+ and CO_3^{2-} on the other, these pairs being usually referred to as kinetically equivalent. The overall rate constant for the kinetically equivalent pair, EH^+ and CO_3^{2-}, is defined by an analogous expression to that of Equation 4.3. The analysis for k_2 is carried out the same way except that the fractions applied in the equation analogous to Equation 4.5 are f_{EH^+} and $f_{CO_3^{2-}}$ i.e.

$$k_2 = k_c/f_{EH^+} x f_{CO_3^{2-}} \qquad 4.6$$

The value of $f_{CO_3^{2-}}$ is calculated using a pK_a value of 10.33 [104].

The k_2 values calculated for the EH^+ and CO_3^{2-} pair (Table 4.5) appear to be independent of the pH of the solution. On

Table 4.3 The effect of pH on the potassium bicarbonate catalysed hydrolysis of (a) Phenyl Glycinate (b) 4-methoxyphenyl Glycinate.
Temperature 30°C

(a) Phenyl Glycinate: pK (Ester) = 7.15

pH	f_E	$f_{HCO_3^-}$	k_c ($l\ mol^{-1} s^{-1}$)	$k_2 = k_c/f_E x f_{HCO_3^-}$ ($l\ mol^{-1} s^{-1}$)
7.37	0.624	0.9098	0.44 ± .04	0.78 ± .07
6.91	0.365	0.778	0.23 ± .03	0.81 ± .08
6.57	0.208	0.615	0.105 ± .005	0.82 ± .04
6.14	0.089	0.373	0.027 ± .002	0.81 ± .06

(b) 4-methoxyphenyl Glycinate: pK (Ester) = 7.3

pH	f_E	$f_{HCO_3^-}$	k_c ($l\ mol^{-1} s^{-1}$)	$k_2 = k_c/f_E x f_{HCO_3^-}$ ($l\ mol^{-1} s^{-1}$)
7.37	0.54	0.9098	0.2 ± .03	0.41 ± .04
6.91	0.289	0.778	0.09 ± .007	0.40 ± .03
6.57	0.157	0.615	0.044 ± .005	0.46 ± .06
6.14	0.065	0.373	0.012 ± .002	0.49 ± .06

Table 4.4 Data for and results of pH titration for the determination of the ionization constant of Phenyl Glycinate.

Temperature 30°C

$[EHBr]_A$ mol l^{-1}	$[EHBr]_C$ mol l^{-1}	$[E]_n$ mol l^{-1}	pH	Σ	pK = pH + Σ
.00992	.0091	.0008	5.9	1.057	6.96
.00984	.0083	.0016	6.28	0.713	6.99
.00977	.0082	.0016	6.61	0.54	7.15
.00969	.0066	.0031	6.81	0.328	7.14
.00960	.0057	.0039	6.93	0.17	7.10
.00954	.0049	.0046	7.12	0.035	7.15
.00946	.0042	.0053	7.25	-0.104	7.15
.00940	.0034	.0060	7.4	-0.249	7.15
.00933	.0026	.0067	7.55	-0.412	7.14
.00926	.0019	.0074	7.71	-0.602	7.11
.00920	.0011	.0081	7.93	-0.867	7.06
.00912	.0004	.0088	8.45	-1.38	7.07

List of abbreviation:

$[EHBr]_A$ - Actual concentration of Phenyl Glycinate.HBr salt.

$[EHBr]_C$ - Corrected " " " " "

$[E]_n$ - Concentration of neutral Phenyl Glycinate

$$\Sigma = \text{Log } [EHBr]_C/[E]_n$$

Note: Since pH was measured at ionic strength greater than 0.1 mol l^{-1}, the units of K are activity $^{-1}$, the units of K are activity $^{-1}$ rather than mol l^{-1}.

Table 4.5 The effect of pH on potassium bicarbonate catalysed hydrolysis of phenyl glycinate, based on the kinetically equivalent pair EH^+ and CO_3^{2-}.

pH	f_{EH^+}	$f_{CO_3^{2-}}$	k_c ($l\ mol^{-1}\ s^{-1}$)	$k_2 = k_c/(f_{EH^+})(f_{CO_3^{2-}})$ ($l\ mol^{-1}\ s^{-1}$)
7.37	.40	9.9×10^{-4}	.44 ± .04	1100 ± 100
6.91	.66	2.96×10^{-4}	.23 ± .03	1200 ± 150
6.57	.81	1.05×10^{-4}	.10 ± .01	1200 ± 120
6.14	.92	2.40×10^{-5}	.03 ± .002	1200 ± 120

the basis of this result it is possible that the pair EH^+ and CO_3^{2-} may be involved in the catalysed reaction with a k_2 of about 1200 $l\ mol^{-1}\ s^{-1}$ (30°C). This represents an extremely rapid base catalysed hydrolysis of $H_3^+NCH_2COOPh$ where the leaving group, the phenolate ion, is not considered to be good as becomes apparent when we compare it with a catalytic rate constant of 32 $l\ mol^{-1}\ s^{-1}$ (30°C and pH 9.73) for the carbonate catalysed hydrolysis of the 2-nitrophenyl ester of $(CH_3)_3N^+—CH_2COOC_6H_4NO_2$ [104]. This ester has the acyl substituent $(CH_3)_3N^+$- which might be expected to have a similar acyl-activating effect to the H_3N^+- group in phenyl glycinate, but it also has 2-nitrophenolate ion as a leaving group which is a much better leaving group than the phenolate ion.

Although the actual reacting base, CO_3^{2-} or HCO_3^-, was not established in the study concerned [104], at pH 9.73 a significant fraction (0.2) of the carbonate species exists as the

strong base CO_3^{2-} and this was almost certainly the catalyst. The value of the rate constant in terms of CO_3^{2-} can therefore be recalculated by dividing the observed rate constant by 0.2 giving k_2 = 160 l mol^{-1} s^{-1} . This value is only about an eighth of that calculated (Table 4.5) for CO_3^{2-} catalysed hydrolysis of $H_3\overset{+}{N}CH_2COOPh$. It is not conceivable, however, for the CO_3^{2-} catalysed hydrolysis of $H_3\overset{+}{N}CH_2COOPh$ to be 8 times faster than the CO_3^{2-} catalysed hydrolysis of $(CH_3)_3\overset{+}{N}CH_2COO$-$C_6H_4NO_2$ on the basis of leaving group ability.

In fact, Table 4.6 shows that phenyl esters normally have rate constants of the order 10^2 times slower than the corresponding nitrophenyl esters, irrespective of the role of the catalyst.

Table 4.6 The relative rates of catalysed hydrolysis of Phenyl Acetate (PA), 4-methoxyphenyl Acetate (4MPA) and 4-nitrophenyl Acetate (4NPA).

Reference	Ester	Catalyst	Cat. Role	k (25°C) (l mol^{-1} min^{-1})
11	PA	Imidazole	Nucl.	0.96
10	PA	Imidazole	Nucl.	0.53
11	PA	Hydrazine	Nucl.	0.25
11	4MPA	Imidazole	Nucl.	0.32
10	4MPA	Imidazole	Nucl.	0.19
11	4MPA	Hydrazine	Nucl.	0.1
10	4NPA	Imidazole	Nucl.	34.6
10	4NPA	OH^-	Nucl.	570
10	PA	OH^-	Nucl.	76
10	4MPA	OH^-	Nucl.	63

Therefore, the high value of k_2 in Table 4.5 can not be accepted by analogy with the literature data in Table 4.6. It is evident therefore that catalysis by CO_3^{2-} of the protonated form of phenyl glycinate and 4-methoxyphenyl glycinate is not responsible for the observed overall rates. The acceptable alternative is that expressed by Equation 4.3, i.e., that bicarbonate ion catalysed the hydrolysis of the neutral phenyl glycinate and 4-methoxyphenyl glycinate. A further possibility which can in fact be excluded is that a kinetic form $[E][CO_2][OH^-]$ applies. This is best considered from the point of view of both 4-nitrophenyl and phenyl glycinate and discussion is delayed until Section 5.2.

In addition, Table 4.6 compares literature results for relative rates of hydrolysis of phenyl acetate and 4-methoxyphenyl acetate. The general rate enhancement of the order of 2 to 3 times in favour of phenyl acetate over 4-methoxyphenyl acetate compares well with the relative rates for the bicarbonate catalysed hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate, a factor which suggests that reaction involving the ester carbonyl group has a rate-determining effect on the catalysed reactions.

4:3 Possible mechanisms for the hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate catalysed by bicarbonate solutions.

4:3.1 Earlier proposals.

Wieland and Jaenicke [100] found that bicarbonate solutions catalysed the hydrolysis of phenyl glycinate

at pH 7.5. They suggested that it was carbon dioxide and not the bicarbonate ion that is involved as a catalyst and they proposed the formation of the carbamate intermediate, Diagram 4.1. However, in our study, the analysis of the kinetic data over a pH range (6.1 - 7.37) showed quite conclusively the contrary that there was no detectable evidence of carbon dioxide being involved in the reaction. In fact the hydrolysis involves E and HCO_3^- only, as discussed in Section 4:1.2 (Equation 4.5).

We used the results at pH 7.5 given by Wieland and Jaenicke [100] and on the basis of reaction of E and HCO_3^- roughly estimated for the rate constant k_2 (defined by Equation 4.5) a value of 0.9 $\pm$ 0.2 1 mol^{-1} s^{-1} (the actual temperature was not stated but 25°C was reported for other studies in the same paper). This value is similar in magnitude to our result in Table 4.3.

We are left to consider the role of the bicarbonate ion in the catalysed hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate.

Phenyl esters which are termed as being activated in the alcohol portion are more generally subject to nucleophilic catalysis rather than general base catalysis [20, 116, 117, 118], whereas alkyl esters activated in the acyl portion by electron withdrawing groups are subject to general base catalysis [44]. Phenyl glycinate is activated in both alcohol and acyl portions so it is not possible to predict which type of catalysis by bases is more likely to apply. We shall consider both general base and nucleophilic catalysis and other possible roles for HCO_3^- as a catalyst for the hydrolysis of phenyl and 4-methoxyphenyl glycinate.

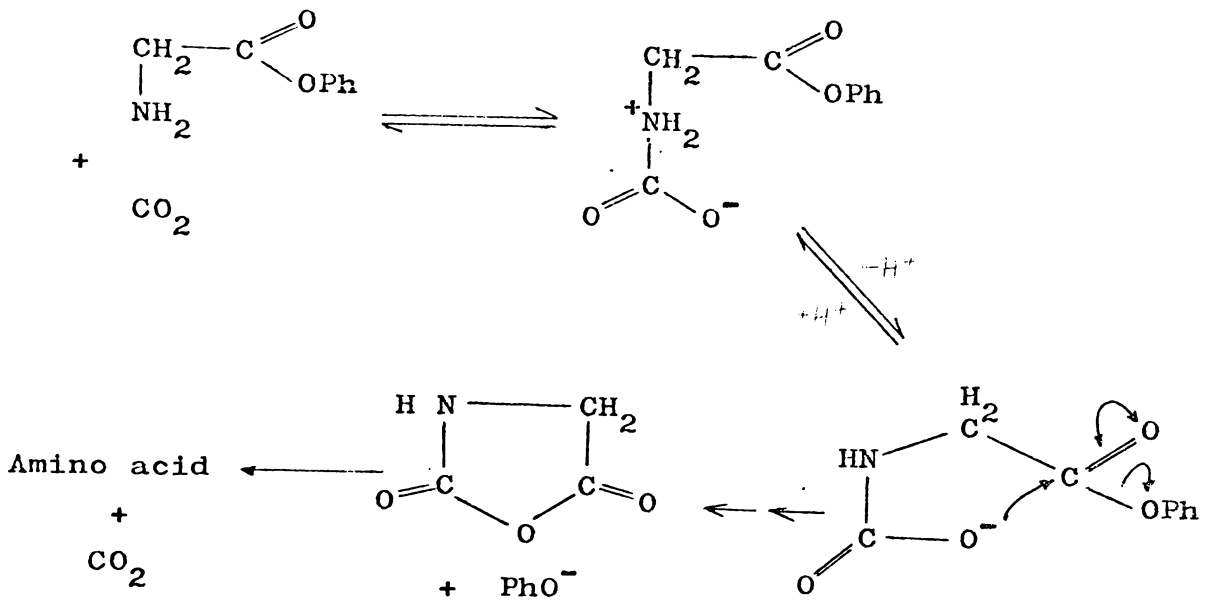


Diagram 4.1 Wieland's proposal for the mechanism of catalysed hydrolysis of phenyl glycinate in bicarbonate solutions.

4:3.2 General Base Catalysis..

Diagram 4.2 shows a general base mechanism where the bicarbonate ion attacks a water molecule first with the subsequent formation of a tetrahedral intermediate. This intermediate can decompose generating the phenolate ion.

Earlier studies [44] suggest that the greater degree of acyl activation, the greater an alkyl ester is subject to general base catalysis. If this extends to acyl-activated phenyl esters, we would expect that the protonated ester EH^+ would be more subject to general base catalysis by HCO_3^- than the neutral ester. However we found that the protonated ester is not subject to any measurable catalysis by HCO_3^- at all, suggesting that the catalysis for the neutral ester E is not

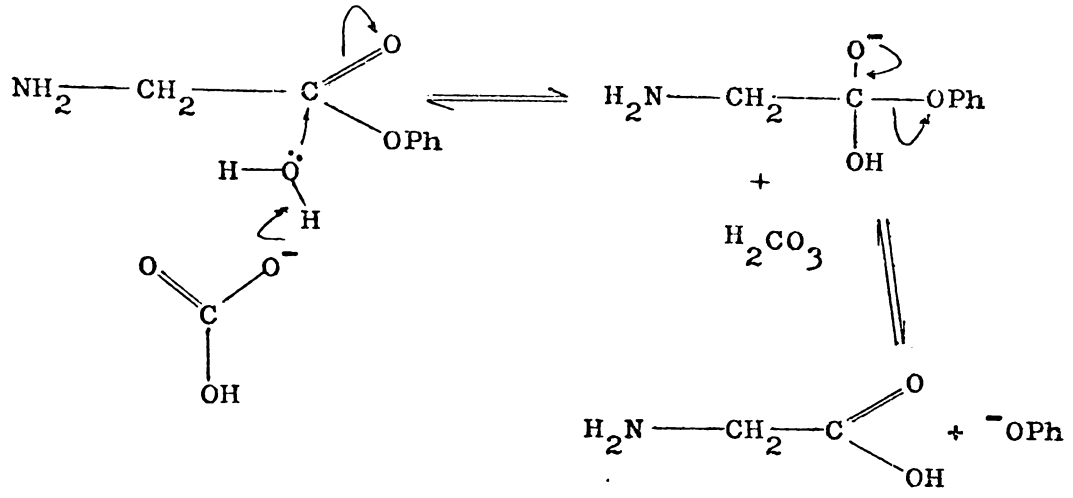


Diagram 4.2 General Base Mechanism.

general base.

A more compelling argument against general base catalysis comes from consideration of the relative reactivities of HPO_4^{2-}, imidazole and HCO_3^-. It is generally observed that HPO_4^{2-} and imidazole have nearly the same reactivity as general base catalysts [2, 44] whereas imidazole is generally some 10^3 times more effective than HPO_4^{2-} as a nucleophilic catalyst. Table 4.11 suggests that imidazole and HPO_4^{2-} catalysed the hydrolysis of phenyl glycinate with almost equal effect, as would be consistent with general base catalysis. From the same table we see that the bicarbonate ion (HCO_3^-) is about 550 times more effective as a catalyst than HPO_4^{2-}. This can hardly be consistent with a general base mechanism because HPO_4^{2-} is a stronger base than the bicarbonate ion by almost one pK unit. We have no correlation between the rates and base strength which would be expected by analogy with results for general base catalysis of other acyl-activated esters, e.g. chloroacetates (Jencks and Carriuolo [44]).

It is possible, therefore, to dismiss the catalytic role of HCO_3^- as a general base one and to go on to consideration of nucleophilic catalysis.

4:3.3 Nucleophilic catalysis.

Diagram 4.3 represents nucleophilic catalysis where the nucleophile directly attacks the electron deficient carbonyl centre to give the tetrahedral intermediate from which the phenolate ion is released.

As a nucleophile, imidazole would be expected to be a much better catalyst than the less basic bicarbonate ion. Results in Table 4.11 show the contrary. The fact that bicarbonate is some 350 times more effective than imidazole must indicate some form of special assistance given to the bicarbonate reaction. If nucleophilic catalysis is involved, such an unexpectedly high reactivity could be due to acid catalysis in the decomposition of the tetrahedral intermediate, allowing phenol loss rather than phenolate ion loss. The pK_a of phenol is about 10 and in the region between pH 6.1 and pH 7.4 (where the pH variation was studied), the release of phenol is obviously favoured. A modified mechanism illustrating this possibility is shown in Diagram 4.4. However, such acid catalysis would be equally likely in the case of HPO_4^{2-} catalysis and yet no unexpectedly high catalytic efficiency is observed for this species. It seems unlikely then, that the modified mechanism for HCO_3^- given in Diagram 4.4 provides the explanation for the species high catalytic activity.

A second factor is that if nucleophilic catalysis is important for the neutral ester, E, it should be even more so for the protonated ester, EH^+, with a more powerful electron

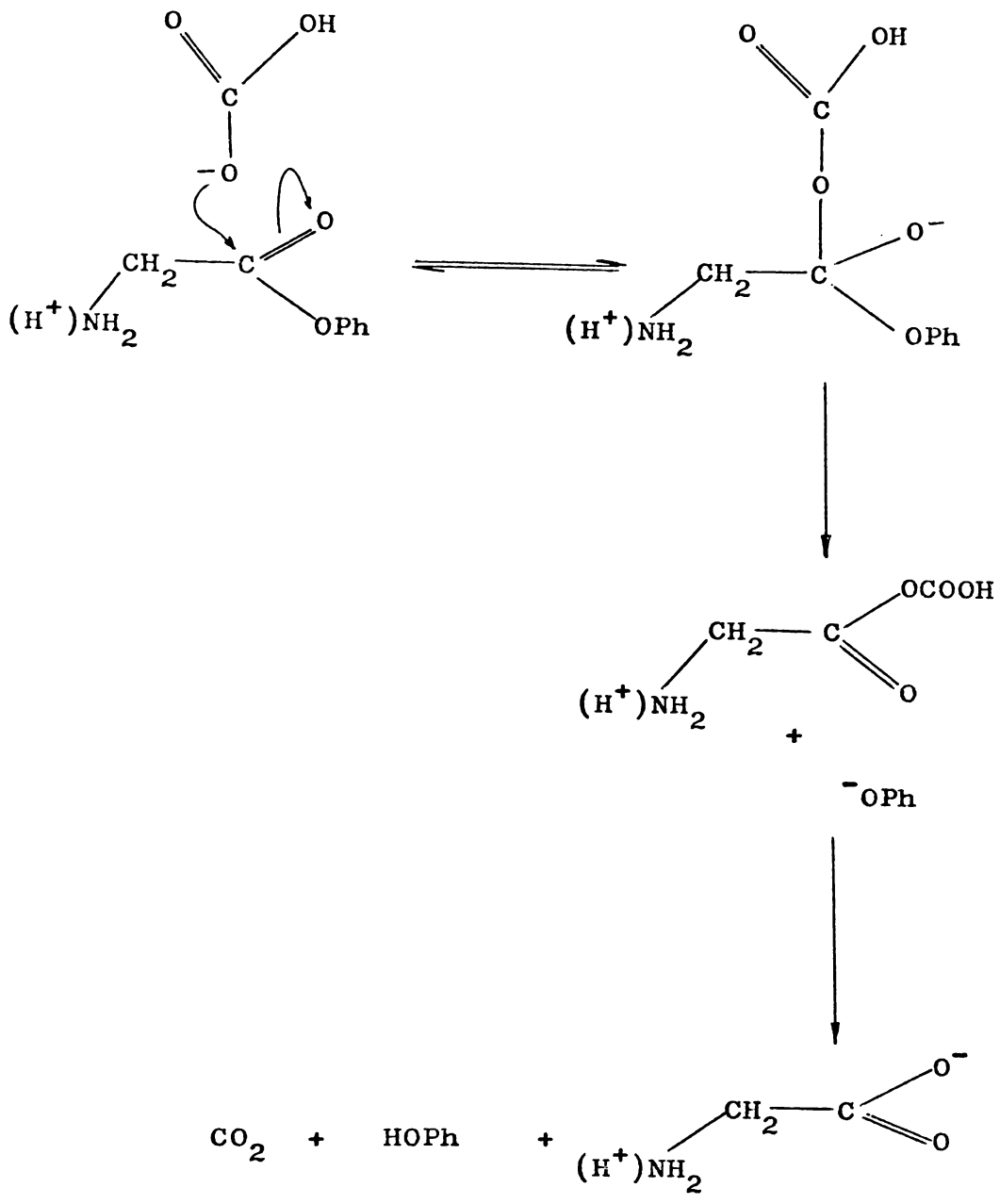


Diagram 4.3 Nucleophilic mechanism.

withdrawing group and an electrostatic factor in its favour. Indeed, protonated esters are known to hydrolyse in basic solution at rates two orders of magnitude or more larger than

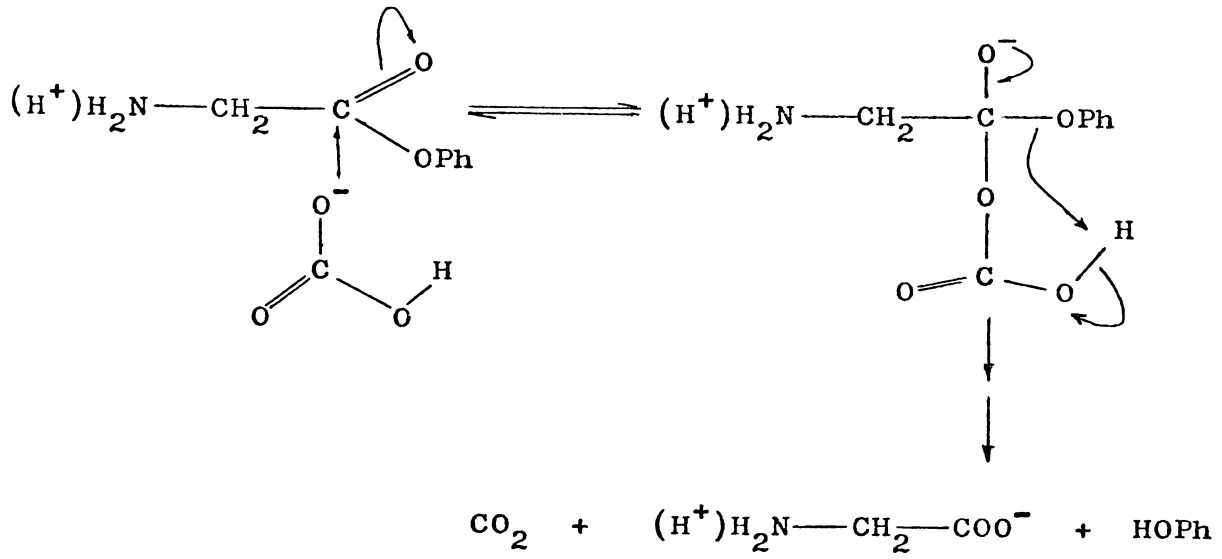


Diagram 4.4 Nucleophilic (General Acid Assisted) Mechanism.

those of the corresponding neutral ester [71] and this reaction involves nucleophilic attack by OH^- at the ester carbonyl. We found however no evidence for HCO_3^- catalysis of EH^+ under conditions in which this should be readily apparent if present.

Since neither general base catalysis nor nucleophilic catalysis provide an explanation for the reactivity of HCO_3^- we are left to consider whether the amino group of the ester might be involved in some special way.

4:3.4 Other possible mechanisms

By analogy with CO_2 catalysis, we consider addition by the ester amino group to the carbonyl group of bicarbonate ion (Diagram 4.5). The equilibrium constant, K_1,

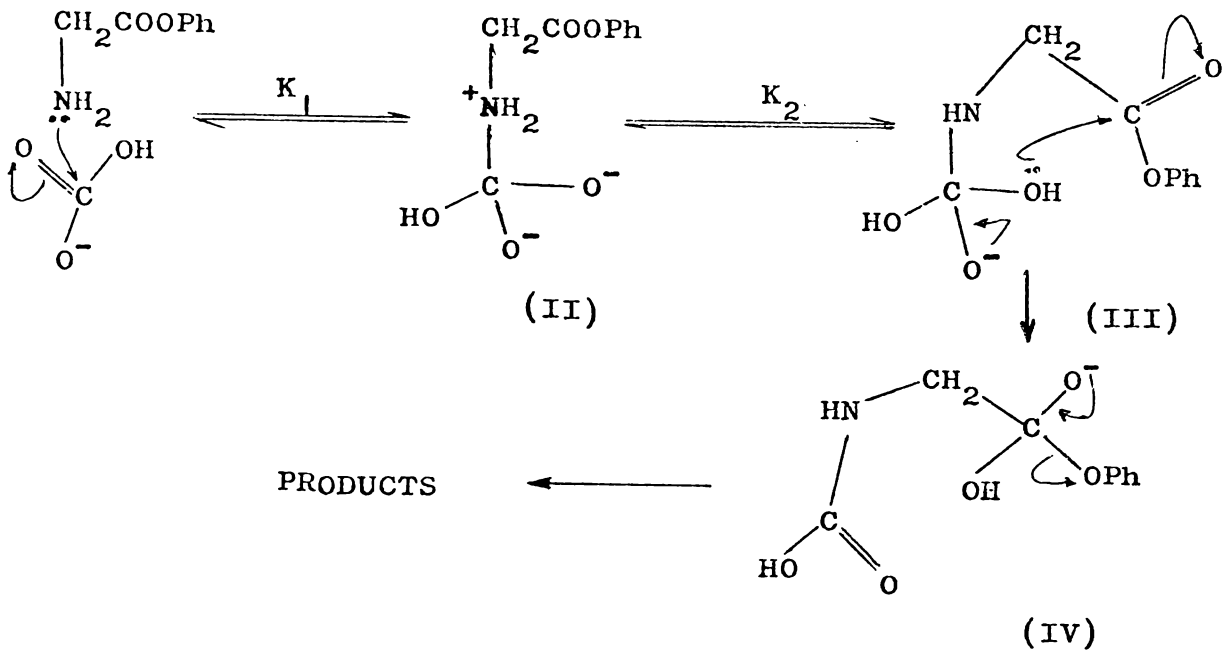


Diagram 4.5 General Acid Assisted Addition Mechanism.

would be expected to be very low because the nucleophile $-\ddot{N}H_2$ is attacking the (negatively charged) bicarbonate ion. However, once the addition compound (II) is formed, it could be rapidly trapped by proton transfer to one of the strongly basic $-O^-$ groups to form (III). The species (III) can then break down to a carbamic acid (IV) by OH^- transfer to the ester carbonyl instead of the solution, thereby ensuring hydrolysis once (IV) is formed.

An alternative mechanism also involves the participation of the amino group (Diagram 4.6). This could be regarded as nucleophilic catalysis assisted by proton transfer in the first step, which will increase the susceptibility of the carbonyl group to nucleophilic attack and commitantly increase the nucleophilic power of the nucleophile. Strongly against this mechanism, however, is the unfavourable requirement of achieving the correct geometry of the system (7-membered ring transition state) before reaction can occur.

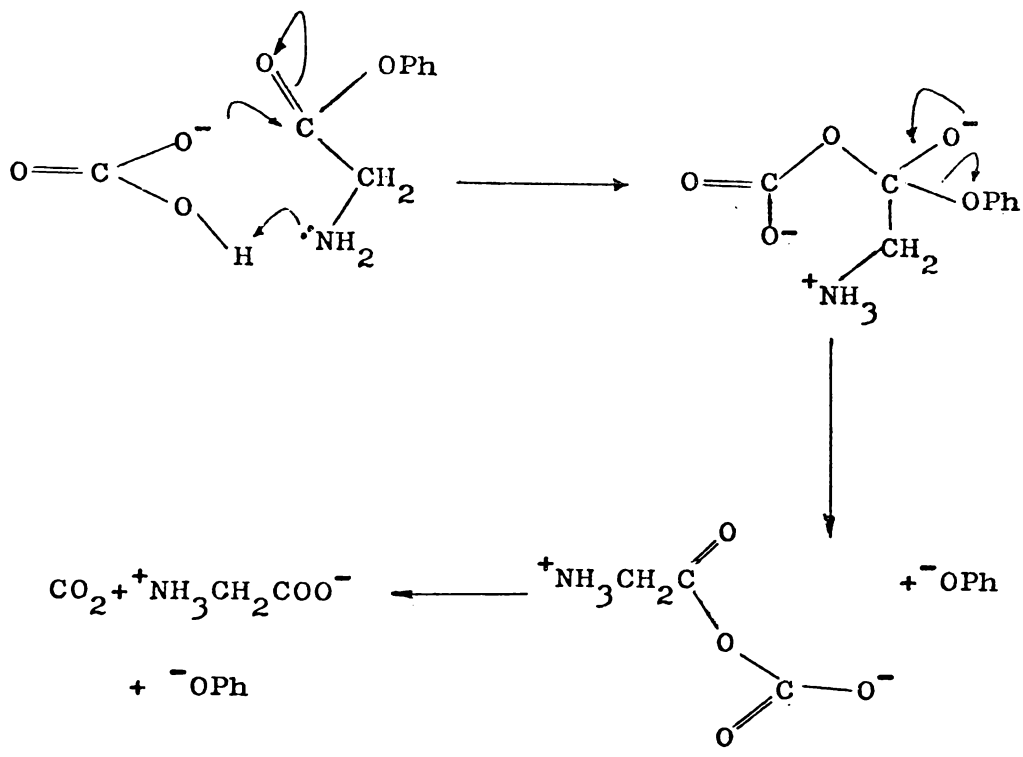


Diagram 4.6 Nucleophilic (Proton Transfer Assisted) Mechanism.

In conclusion, then, we are forced to the conclusion that bicarbonate catalysis of the hydrolysis of the neutral species of phenyl and also 4-methoxyphenyl glycinate involves some special type of catalysis involving the free amino group of the ester. Both mechanisms 4.5 and 4.6 have their drawbacks. In favour of mechanism 4.5, however, is the now known critical role of proton transfer, which may be rate determining, in trapping unstable zwitterion intermediate species (cf. (II) in Diagram 4.5) which otherwise rapidly revert to reactants. This factor is important in nucleophilic catalysis by amines of ester hydrolysis (Jencks and Satterthwait [110]) and is considered further in Section 7 where we discuss the imidazole catalysed hydrolysis of phenyl glycinate.

The 4-nitrophenyl esters of some amino acids undergo hydrolysis in bicarbonate solutions via CO_2 catalysis probably by a

mechanism as proposed by Hay and Main [39, 92]. (Diagram 2.6; a detailed discussion of evidence to support the mechanism is reviewed in Section 2 of this thesis).

We shall now discuss the reasons why carbon dioxide does not catalyse the hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate. If carbon dioxide were to catalyse the hydrolysis of either of these esters, then the formation of a carbamate intermediate is required (Diagram 4.7). The criterion for the ease of formation of a carbamate intermediate is the nucleophilicity of the free amino group, $-NH_2$, of the esters. Since the esters phenyl, 4-methoxyphenyl and 4-nitrophenyl glycinate have similar pK values (7.1 - 7.3), the formation of a carbamate with CO_2 presumably must occur for the phenyl and 4-methoxyphenyl glycinates if it occurs for the less basic 4-nitrophenyl glycinate. Therefore we can assume Diagram

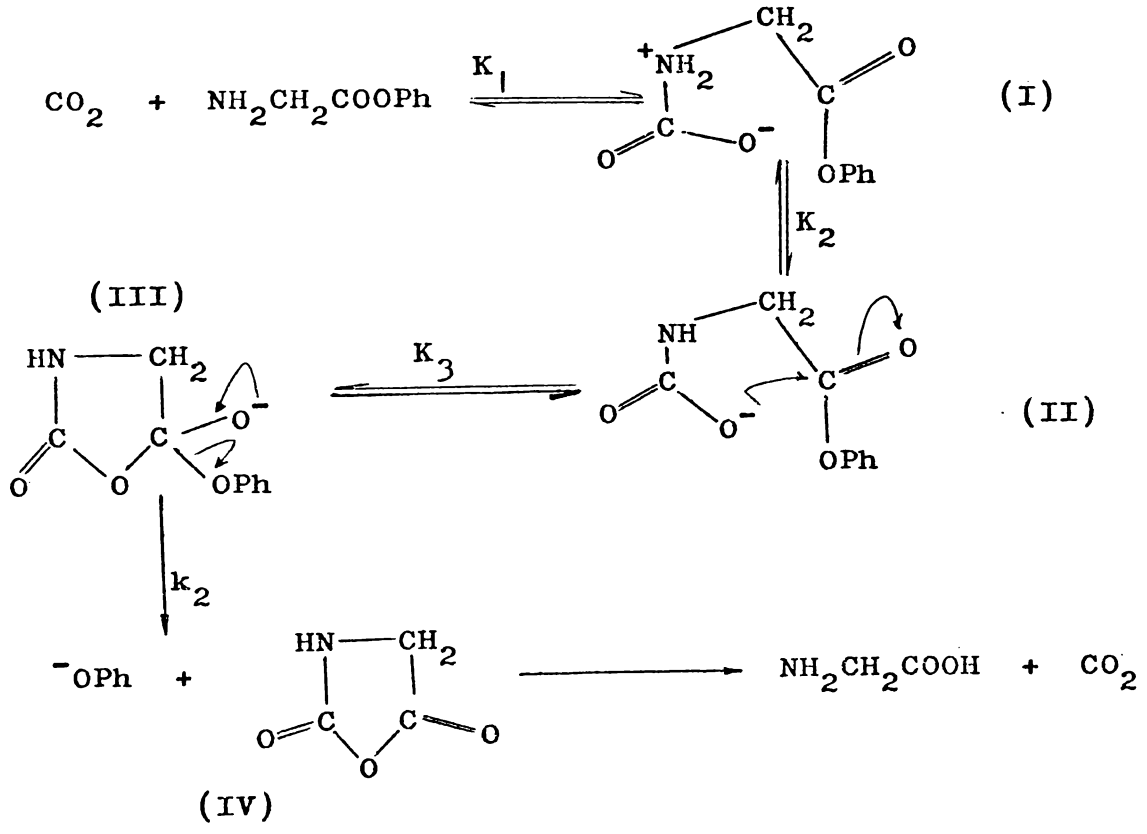


Diagram 4.7 Hypothetical mechanism for bicarbonate ion catalysed hydrolysis of Phenyl Glycinates.

4.7 to describe an available carbamate mechanism for phenyl glycinate (or 4-methoxyphenyl glycinate). However, in our kinetic analysis, we found that CO_2 does not in fact catalyse the hydrolysis at a detectable rate and the question to be answered is why the scheme in Diagram 4.7 is viable for the 4-nitrophenyl esters but not for the phenyl or 4-methoxyphenyl glycinates. An important concept to consider in this respect is the partitioning of the tetrahedral intermediate in carbonyl addition reactions. This concept has been reviewed by Jencks [40]. The concept relates the rate of reaction in forward or reverse directions from the tetrahedral intermediate to the relative leaving group ability of the group bonded to the tetrahedral carbon.

From Diagram 4.7, it is to be expected that all species up to the tetrahedral intermediate (III) should be accessible irrespective of the substituent in the phenolate leaving group. It is the partitioning of (III) that we believe decides whether hydrolysis proceeds or not. In the 4-nitrophenyl ester hydrolyses [39, 92], the carbamate function (pK_a of the conjugate acid is about 6 [95]) and the 4-nitrophenolate ion (pK_a of the conjugate acid is about 7.1 [112]) would be expected to be similar in their leaving group abilities. Therefore, even though the partition possibly favours the reverse direction to the carbamic acid, it allows part to proceed through the forward direction to a Leuch Anhydride (IV) and ultimately to to ester hydrolysis.

Returning to the phenyl (and 4-methoxyphenyl) glycinate, a value of 6 for the pK_a of the conjugate acid of the carbamate function still applies. However, the phenolate ion (or 4-methoxyphenolate ion) where the pK_a of the conjugate acid is about 10 [112], is now a much poorer leaving group than 4-nitrophenolate ion. We believe that the partitioning of

(III) favours the reverse direction so much that little ester hydrolysis results through this pathway. It is left to the competing reaction involving HCO_3^- to provide the major and detectable route to ester hydrolysis.

In terms of the mechanism favoured for HCO_3^- catalysis (Diagram 4.5) it is worth noting that the question of partitioning of the tetrahedral intermediate is not important. The intermediate is attained through OH^- transfer and the competition between OH^- and PhO^- as leaving group will favour the latter so much that the reaction would be essentially irreversible.

4:4 The hydroxide and phosphate (HPO_4^{2-}) ions catalysed hydrolysis of phenyl glycinate.

4:4.1 Aim of studies.

We investigated the basic hydrolysis of phenyl glycinate in the hope of being able to calculate the rate constants for reactions of both the neutral and protonated forms of the ester with hydroxide ion. Such values were previously available only for alkyl esters.

For the phosphate catalysed reactions, we hoped to estimate the maximum rate of catalysis by HPO_4^{2-} on the hydrolysis of phenyl glycinate. This result together with that of the imidazole catalysed hydrolysis of phenyl glycinate (Section 7) could be used in attempting to establish the nature of the catalysis involved for the various catalysts.

4:4.2 Basic hydrolysis

All solutions used in this section were made from freshly boiled, doubly-distilled water, degassed while the solution cools. Appropriate amount of concentrated potassium hydroxide was added to make up to the required pH. The pH of each solution used for kinetic study was recorded before and after the completion of the reaction by Radiometer 28 pH-meter. The results of these runs are shown in Table 4.6.

Table 4.6 The observed rate constants for the basic hydrolysis of phenyl glycinate.

pH	$10^3 k_h$ (s^{-1}) (30^o)
10	.48
10.8	21
11.0	28
11.2	50
11.6	125
12.0	300

The rate of catalysed hydrolysis in water can be expressed by the Equation 4.6, ignoring acid catalysis;

$$k_h[E]_T = k_E^o[E] + k_{EH^+}^o[EH^+] + k'_{OH^-}[E][OH^-] + k''_{OH^-}[EH^+][OH^-] \qquad 4.6$$

At sufficiently high pH (> 10) however, the first two terms, representing hydrolysis by water will be negligible in comparison with OH^- terms. Also, at sufficiently high pH (>10), the concentration of the protonated ester (EH^+) will be so

low (the pK of the ester is 7.15) that the last term of Equation 4.6 will probably be negligible. Therefore $[E]_T$ approximates to [E]. Under these conditions, Equation 4.6 reduces

$$k_h = k'_{OH^-}\,[OH^-] \qquad 4.7$$

to Equation 4.7.

The criterion for the validity of this relationship and the assumptions made above is a linear relationship between k_h and $[OH^-]$. We tested and found such linearity, using values of k_h at various pH > 10 (Table 4.7 and plot, Figure 4.3).

Table 4.7 Data for and result of the plot of k_h vs $[OH^-]$.*

pH	10^3 $[OH^-]$ mol l^{-1}	10^2 k_h s^{-1}	Gradient $l\ mol^{-1}\ s^{-1}$
10.8	0.93	2.1 ± .2	
11.0	1.47	2.8 ± .3	
11.2	2.33	5.0 ± .5	
11.6	5.85	12.5 ± 1.5	
12.0	14.6	30.0 ± 3	21 ± 3

*see *Note* on page 64.

The values of $[OH^-]$ were calculated using pK_w = 13.833 at 30°C [112] based on pH meter readings on reaction solutions. The gradient of the plot k_h vs $[OH^-]$ gives the rate constant k'_{OH^-} for the neutral ester, a value of 21 ± 3 $l\ mol^{-1}\ s^{-1}$ at 30°C.

To determine the rate constant for the protonated ester k''_{OH^-} (Equation 4.6), rate studies are required at a pH at which a higher proportion of the ester is present in the

protonated form , EH^+, so that the last term $k''_{OH^-}[EH^+][OH^-]$ of Equation 4.6 makes a significant contribution to the overall rate. We have used the uncatalysed rate constant values from the pH region of 6.1 - 7.4, in which the bicarbonate ion catalysis was studied (Section 4.3). These values were determined from the intercepts of plots of k_h vs $[C_T]$ by least square treatment (Table 4.8).

Table 4.8 Data for and result of the calculation of k''_{OH^-} .*

pH	$10^4 k_h$ s^{-1}	$10^8[OH^-]$ mol l^{-1}	k''_{OH^-} (30°C) 1 mol^{-1} s^{-1}
7.37	8.54 ± .43	34.4	6100 ± 500
6.91	4.56 ± .23	11.9	5800 ± 450
6.57	2.96 ± .15	5.46	6700 ± 600
6.14	1.49 ± .07	2.03	8000 ± 800

*see Note on page 64.

From Equation 4.6, assuming as a first approximation, that the first two water hydrolysis terms are still negligible, we have;

$$k_h = k'_{OH^-} f_E [OH^-] + k''_{OH^-} f_{EH^+} [OH^-] \qquad 4.8$$

Having established that $k'_{OH^-} = 21 \pm 3$ 1 mol^{-1} s^{-1} (30°C), k''_{OH^-} is the only unknown in this equation. Rearranging Equation 4.8;

$$k''_{OH^-} = (k_h - (k'_{OH^-}) x f_E x [OH^-]) / (f_{EH^+}) x [OH^-] \qquad 4.9$$

where the values of f_E and f_{EH^+} are found in Section 4.3.

The value of k''_{OH^-} is calculated at four different pHs (Table 4.8). Constancy in the calculated values of k''_{OH^-} would provide support for the assumptions made in arriving at Equation 4.9. The values are in fact reasonably constant within experimental error down to pH 6.57. The value at pH 6.14 is significantly higher. This suggests that at pH 6.14, a significant contribution to the overall rate comes from one or the

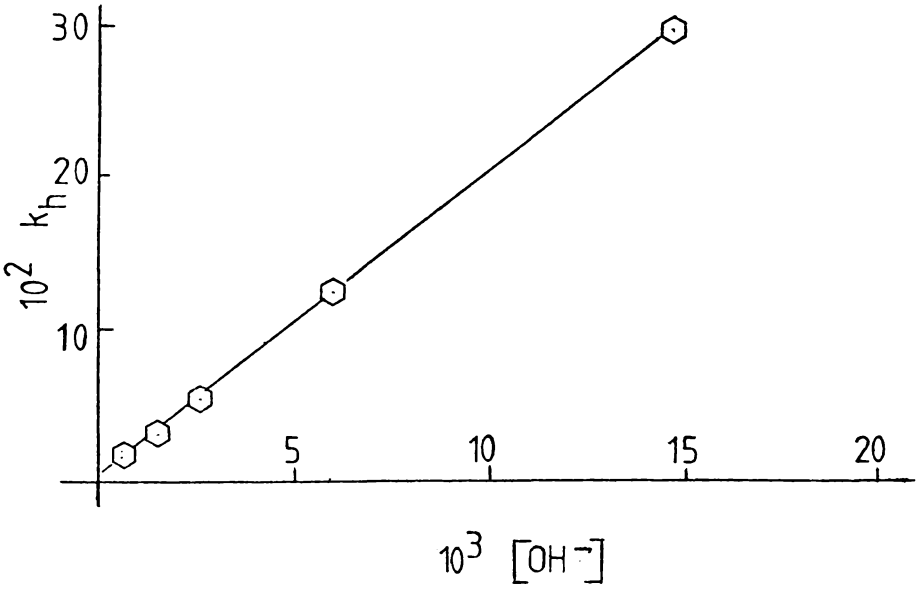


Figure 4.3 The plot of k_h vs $[OH^-]$.

other or both terms that we assumed to be negligible in Equation 4.6. It could be that water hydrolysis becomes significant, or possibly even that some acid catalysis (not covered by Equation 4.6) is setting in. This may also apply for the results of pH 6.57 but to a lesser extent, since k''_{OH^-} is only slightly higher here than at the other two higher pH

values. We have therefore taken the value of k''_{OH^-} at these two pHs as being the most acceptable for the rate constant, i.e., k''_{OH^-} is about 6000 1 mol^{-1} s^{-1} (30°C).

By comparison with the value of k'_{OH^-} (21 1 mol^{-1} s^{-1} at 30°C) for the neutral ester, E, it is apparent that the hydroxide ion hydrolyses the protonated ester about 300 times more rapidly than the neutral ester. This is in line with relative rates for the protonated and neutral forms of alkyl esters of some amino acids previously studied [71], where rate enhancements by factors of 10^2- 10^3 were attributed to the increased electrostatic and inductive effects exhibited by the protonated esters over the neutral counterparts [71].

Note added after oral examination: since results in section 4:4.2 are based on pH measurements of hydrogen ion activity at ionic strengths greater than 0.1 mol l^{-1}, hydroxide ion is properly expressed in units of activity $^{-1}$ rather than mol l^{-1} and k'_{OH^-} and k''_{OH^-} in units of activity $^{-1}s^{-1}$.

4:4.3 Phosphate ion catalysis.

The rate of catalysed hydrolysis in phosphate solutions can be expressed by Equation 4.10, assuming that HPO_4^{2-} is the active species as in the catalysed hydrolysis of 4-nitrophenyl acetate [14, 15]. The concentration $[P_T]$ is the total phosphate concentration ($[H_2PO_4^-]+[HPO_4^{2-}]$).

$$k_c[E]_T[P_T] = (k_1[E] + k'_1[EH^+])x[HPO_4^{2-}] \qquad 4.10$$

Rearranging Equation 4.10, we have;

$$k_c[P_T]/[HPO_4^{2-}] = k_1[E]/[E]_T + k'_1[EH^+]/[E]_T \qquad 4.11$$

since $[P_T]/[HPO_4^{2-}] = 1/f_{HPO_4^{2-}}$,

$[E]/[E]_T = f_E$,

and $[EH^+]/[E]_T = f_{EH^+}$

where f is fraction of the species concerned

Therefore; $k_c/f_{HPO_4}^{2-} = k_1 f_E + k_1' f_{EH^+}$ 4.12

but $f_{EH^+} = 1 - f_E$

and substituting f_{EH^+} into Equation 4.12 we have;

$$k_c/f_{HPO_4}^{2-} = k_1 f_E + k_1'(1 - f_E)$$
$$= (k_1 - k_1')f f_E + k_1' \qquad 4.13$$

A plot of $k_c/f_{HPO_4}^{2-}$ vs f_E would enable the rate constants k_1 and k_1' (for the neutral and protonated ester, respectively) to be calculated. If it were found that a non-linear plot resulted, this would show that Equation 4.10 is not valid and suggest that $H_2PO_4^-$ might be involved in catalysis. However, as seen in the next section a linear plot was obtained.

4:4.3 (a) Results and discussion.

The catalytic rate constant k_c is the gradient of the plot k_h vs $[P_T]$ where k_h is the observed rate constant and $[P_T]$ the total phosphate concentration (Table 4.9). A representative plot of k_h vs $[P_T]$ is shown in Figure 4.4.

Table 4.9 Data for and results of the plots of k_h vs $[P_T]$.

pH	$[P_T]$ mol l^{-1}	10^4 k_h s^{-1}	k_c $l\ mol^{-1}\ s^{-1}$
8.25	.070	5.5	
	.140	6.8	
	.180	8.0	

continued

	.220	8.26	
	.360	11.8	$2.0 \pm .2 \times 10^{-3}$
6.6	.123	3.1	
	.203	4.4	
	.203	4.4	
	.243	4.7	
	.405	6.4	
	.405	6.3	$1.1 \pm .1 \times 10^{-3}$
5.95	.030	.85	
	.270	1.7	
	.340	2	
	.410	2.6	
	.680	3.2	$3.7 \pm .3 \times 10^{-4}$

From Equation 4.13, a plot of $k_c/f_{HPO_4^{2-}}$ vs f_E (Table 4.10 and Figure 4.5) would give an intercept value equal to the rate constant k_1' (for the protonated ester) and a gradient

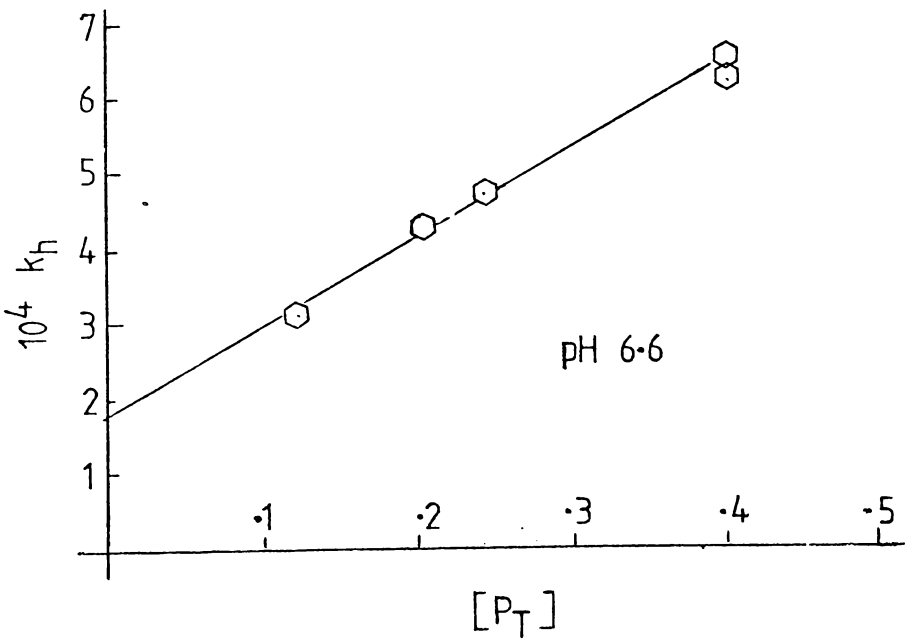


Figure 4.4 The plot of k_h vs $[P_T]$.

value equal to $(k_1 - k_1')$ where k_1 (the rate constant for the neutral ester) can be evaluated.

Table 4.10 Data for the plot of $k_c/f_{HPO_4^{2-}}$ vs f_E.

$pK_a = 7.1$ (phosphate [112])

pH	$f_{HPO_4^{2-}}$	$10^3 k_c$ (1 mol^{-1} s^{-1})	10^3 $k_c/f_{HPO_4^{2-}}$ (1 $mol^{-1}s^{-1}$)	f_E
8.25	.934	2.0 ± .2	2.1 ± .2	.926
6.6	.240	1.1 ± .1	4.6 ± .4	.22
5.95	.066	.37 ± .03	5.6 ± .5	.06

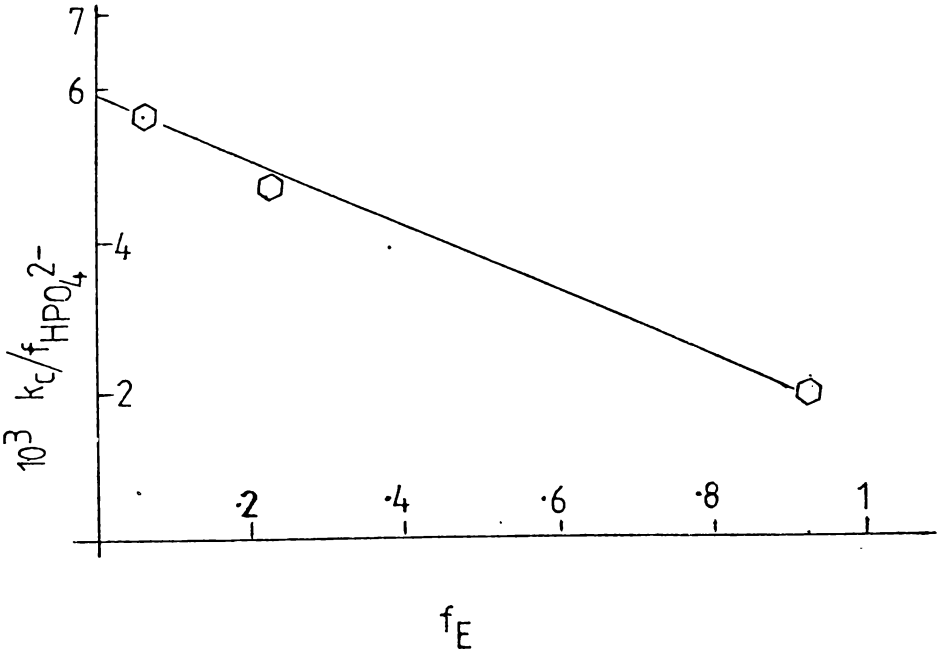


Figure 4.5 The plot of $k_c/f_{HPO_4^{2-}}$ vs f_E.

The values of k_1 and k_1' are 1.6 ± .2 $x10^{-3}$ 1 mol^{-1} s^{-1} and 5.9 ± .5 $x10^{-3}$ 1 mol^{-1} s^{-1}, respectively (Table 4.11).

Table 4.11 Summary of rate constants for the catalysed hydrolysis of phenyl glycinate.

Catalyst	$k_2(E)$ 30°C	$k_2(EH^+)$ 30°C
HCO_3^-	0.87 ± .07	-
*HCO_3^-	0.9 ± .1	-
OH^-	21 ± 3	6000 ± 500
HPO_4^{2-}	1.6 ± .2 x10^{-3}	5.9 ± .5 x10^{-3}
**Imidazole	2.4 ± .3 x10^{-3}	19 ± 3 x10^{-3}

* $k_2(E)$ was estimated from the result of Wieland and Jaenicke [100] at pH 7.5 and 25°C.

** the rate constant for the first order imidazole term only from Section 7 of this thesis.

From Table 4.11, it is quite clear that imidazole and HPO_4^{2-} have similar catalytic reactivity in the hydrolysis of phenyl glycinate as in the corresponding hydrolysis of ethyl dichloroacetate [44] (Table 1.1) which was known to hydrolyse by a general base mechanism.

We think that the phosphate and imidazole catalyse the hydrolysis of phenyl glycinate and 4-methoxyphenyl glycinate by a general base mechanism on the basis of their catalytic reactivities. For imidazole a second order term in catalyst is dominant, however. That probably represents general base catalysis of rate-limiting proton transfer in a nucleophilic catalysis pathway as will be discussed in Chapter 7.

Section 5 The potassium bicarbonate catalysed hydrolysis of 4-nitrophenyl esters of glycine and valine.

5:1 Results and analysis.

5:1.1 Introduction.

The observed rate constants, k_h, were found to be pseudo first order with respect to the ester concentration (Tables 5.1 and 5.2). Plots of k_h against the total concentration of added bicarbonate, $[C_T]$, are linear, showing the reaction to be first order also in one or more carbonate species (CO_2, HCO_3^- and CO_3^{2-}).

The catalytic rate constants, k_c, are calculated from the gradients of plots of k_h vs $[C_T]$ and are shown in the last column of Tables 5.1 and 5.2. Representative plots of k_h vs $[C_T]$ are shown in Figures 5.1 and 5.2.

Table 5.1 The potassium bicarbonate catalysed hydrolysis of 4-nitrophenyl glycinate.

Error in k_h values ± 5%

Temperature 30°C

pH	$10^5[C_T]$ mol l^{-1}	10^3 k_h s^{-1}	k_c l mol^{-1} s^{-1}
8.13	0	7.3, 6.9	
	4.50	7.9	
	9.0	9.1	
	18.0	9.6	

continued

	30.0	9.6	
	45.0	11.2	
	75.0	13.8	8.8 ± .6
7.91	0	5.9, 6.2	
	6.0	6.7	
	12.0	9.1	
	22.5	11.0	
	45.0	14.1	
	90.0	20.2	
	135.0	24.1	
	180.0	32.0	14 ± 1
7.45	0	7.3, 7.5	
	9.0	10.5	
	18.0	13.5	
	30.0	16.5	
	45.0	21.6	
	75.0	31.1	
	105.0	40.3	30 ± 2
6.93	0	7.9, 7.7	
	10.0	12.4	
	20.0	15.9	
	40.0	23.3	
	60.0	32.4	
	70.0	38.8	
	80.0	41.9	
	100.0	54.8	43 ± 3
6.45	0	7.4	
	8.0	9.3	
	10.0	9.9	

continued

15.0	13.1	
20.0	13.8	
30.0	20.2	
60.0	29.9	
80.0	39.8	40 ± 5

Table 5.2 The potassiun bicarbonate catalysed hydrolysis of 4-nitrophenyl valinate.

Error in k_h values ± 5%

Temperature 30°C and 25°C as specified.

Temp. (°C)	pH	$10^5[C_T]$ (mol l^{-1})	$10^3 k_h$ (s^{-1})	k_c ($l\ mol^{-1}\ s^{-1}$)
30	8.13	0	1.75, 1.85	
		8.0	3.0	
		15.0	3.58	
		44.9	5.98	
		74.8	8.20	
		89.8	9.40	
		119.7	11.60	7.6 ± .6
25	8.13	0	1.7	
		26.8	3.5	
		53.6	5.3	
		80.2	7.0	
		107.2	8.8, 8.7	
		134	10.2	6.4 ± .5

continued

30	7.38	0	2.4	
		15.0	7.06	
		29.9	11.3, 11.5	
		67.3	22.3, 23.2	
		89.8	29.0	30 ± 1
25	7.4	0	1.8	
		12.0	7.3	
		26.8	8.3	
		40.2	10.8	
		69.7	17.9	
		75.0	19.4	
		93.8	23.3	22 ± 2
30	6.95	0	1.6	
		14.9	9.2	
		29.9	15.1	
		37.4	16.6	
		53.3	22.4, 22.8	
		59.8	27.4	
		89.9	38.8	44 ± 4
25	6.99	0	1.4	
		6.7	3.4	
		9.4	4.8	
		20.1	8.6	
		40.2	16.4	
		49.6	19.1	
		64.0	24.4	37 ± 3

continued

30	6.51	0	3.5	
		15.0	11.2	
		52.4	25.8	
		52.4	28.1	
		74.8	37.7	
		74.8	39.2	45 ± 5
25	6.54	0	1.3	
		13.4	6.2	
		26.8	11.6	
		40.2	15.0	
		80.0	32.8	
		94.0	35.5	
		138.0	51.8	
		161.0	64.0	37 ± 4

5:1.2 Analysis.

Previous studies, in particular, those of Main and Hay [39] have suggested that carbamates are formed in the bicarbonate catalysed hydrolysis of some amino acid esters. In this study we have found the catalysis by bicarbonate to be consistent with the rate form;

$$\text{Rate} = k_2[E][CO_2] \qquad 5.1$$

for 4-nitrophenyl esters, as previously observed [39], but differing from the rate for phenyl and 4-methoxyphenyl

glycinate (Chapter 4 of this thesis).

Equation 5.1 implies that the reactive species in the catalytic process are CO_2 and the neutral ester, E . The basis for this comes from equating the experimentally established rate equation (Equation 5.2) with Equation 5.1;

$$\text{Rate} = k_c[E]_T[C_T] \qquad 5.2$$

we can show that;

$$k_2 = k_c x[E]_T/[E]x[C_T]/[CO_2]$$
$$= k_c/f_E x f_{CO_2} \qquad 5.3$$

The factors, f_E and f_{CO_2} at a particular pH are the fractions of the total ester present as the neutral ester (E) and carbon dioxide, respectively. The f_E values were calculated from pK (E) 7.1 for the glycine ester and pK (E) 6.98 for the valine ester. (The pK (E) values are in fact self-consistent values over the pH region of kinetic study). The f_{CO_2} values were calculated from pK_a (CO_2) 6.366 [112] at 30°C and a pK_a (CO_2) 6.37 [101] at 25°C.

Our experimental results (Table 5.3) show that the expression, $k_c/f_E x f_{CO_2}$ is invariant over the specified pH range, thus confirming the validity of Equation 5.1 for the catalysed hydrolysis of 4-nitrophenyl glycinate and 4-nitrophenyl valinate in bicarbonate solutions.

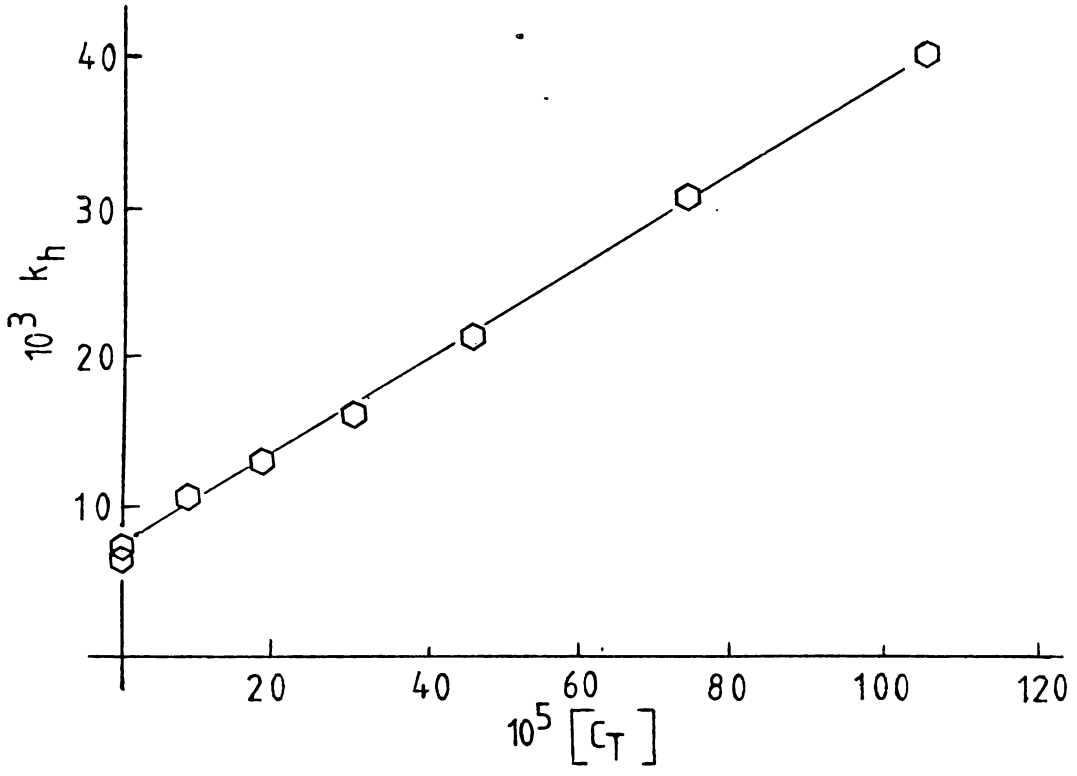


Figure 5.1 The plot of k_h vs [C_T].
4-nitrophenyl glycinate, pH 7.45

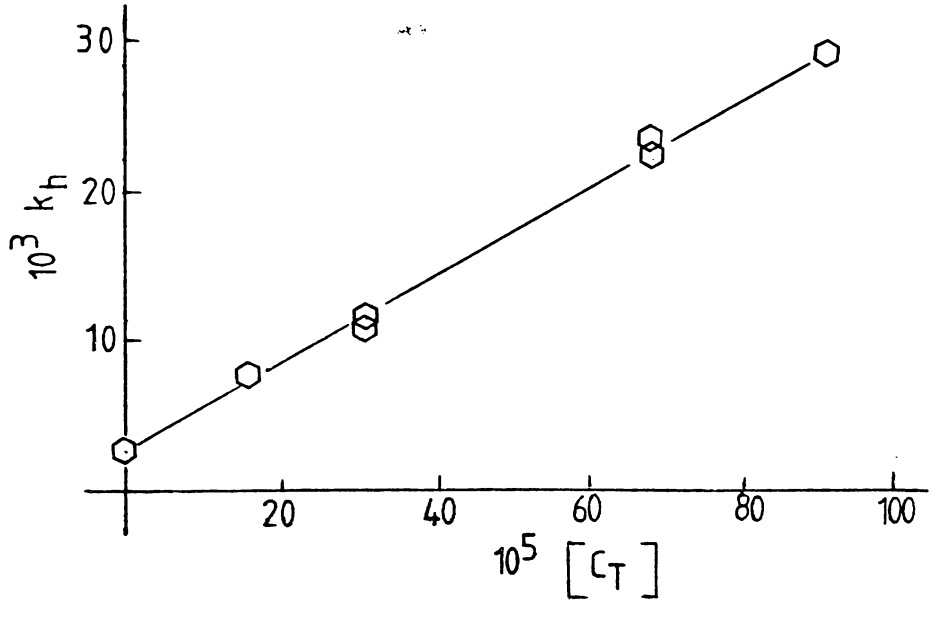


Figure 5.2 The plot of k_h vs [C_T].
4-nitrophenyl valinate, pH 7.38.

Table 5.3 The effect of pH on the potassium bicarbonate catalysed hydrolysis of 4-nitrophenyl esters of glycine and valine.

4-nitrophenyl glycinate (pK (E) 7.1) 30°C

pH	k_c $l\ mol^{-1}\ s^{-1}$	f_E	f_{CO_2}	k_2 $l\ mol^{-1}\ s^{-1}$
8.13	8.8 ± .6	.915	.017	560 ± 40
7.91	14 ± 1	.866	.028	580 ± 40
7.45	30 ± 2	.691	.076	570 ±40
6.93	43 ± 5	.403	.214	500 ± 60
6.45	40 ± 5	.183	.452	480 ± 60

4-nitrophenyl valinate (pK (E) 6.98) 30°C

pH	k_c $l\ mol^{-1}\ s^{-1}$	f_E	f_{CO_2}	k_2 $l\ mol^{-1}\ s^{-1}$
8.13	7.6 ± .6	.934	.017	480 ± 40
7.38	30 ± 1	.725	.085	490 ± 25
6.95	44 ± 4	.483	.207	440 ± 40
6.51	45 ± 5	.253	.418	430 ± 50

continued

4-nitrophenyl valinate 25°C

pH	k_c $l\ mol^{-1}\ s^{-1}$	f_E	f_{CO_2}	k_2 $l\ mol^{-1}\ s^{-1}$
8.13	6.4 ± .5	.934	.017	400 ± 30
7.4	22 ± 2	.715	.085	360 ± 30
6.99	37 ± 3	.483	.193	390 ± 30
6.54	37 ± 4	.253	.403	360 ± 40

Table 5.4 The mean rate constant ($\bar{k}$) and its standard deviation (σ).

Ester	$\bar{k}$ ± σ	
4-nitrophenyl glycinate	530 ± 45	(30°C)
4-nitrophenyl valinate	450 ± 40	(30°C)
4-nitrophenyl valinate	370 ± 25	(25°C)

5:2 Discussion.

The mechanism suggested by Hay and Main [39] to account for CO_2 catalysis is shown in Diagram 5.1. On the basis of relative rates for 4-nitrophenyl esters of different amino acids at one pH, these authors suggested that it is

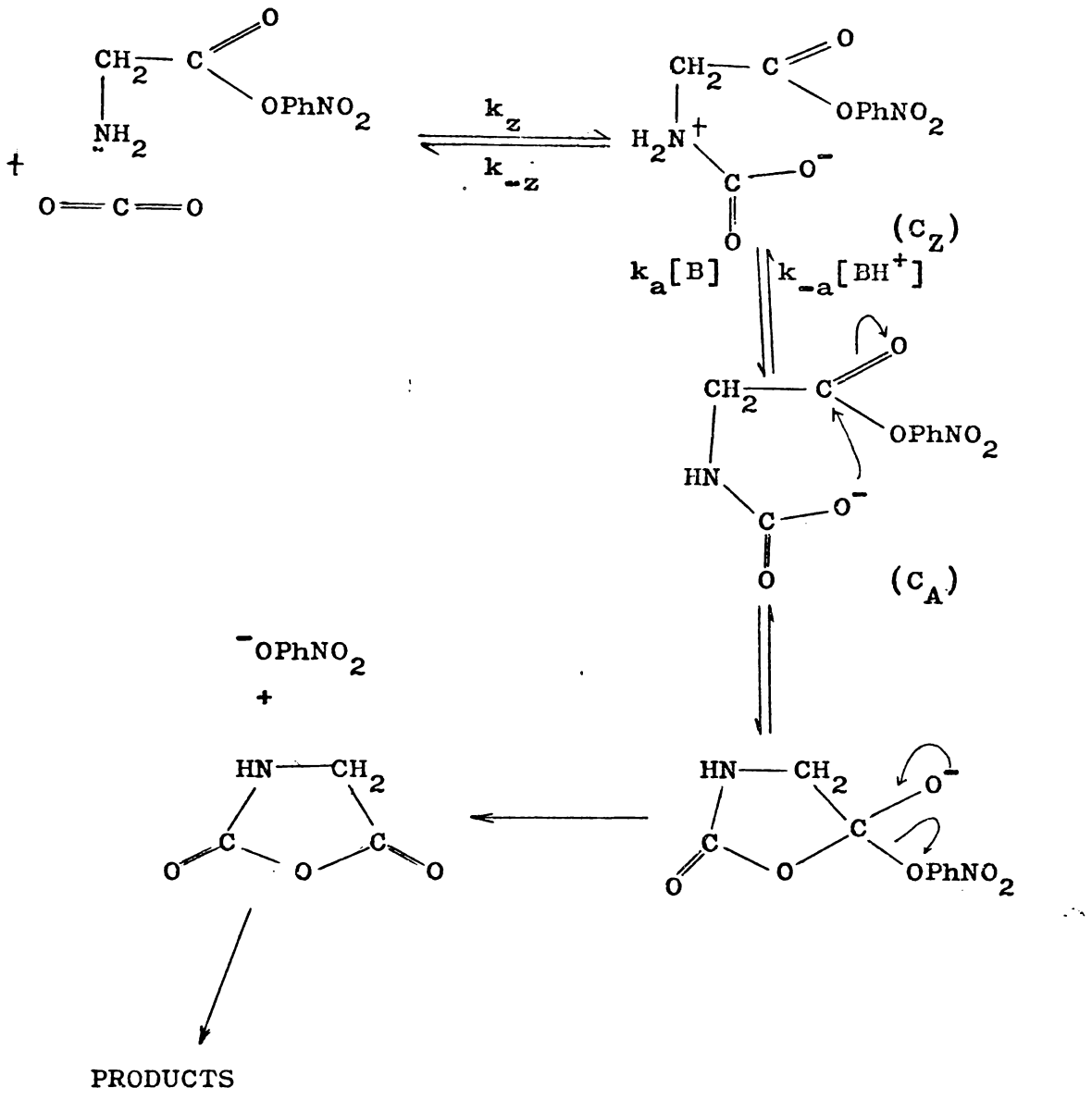


Diagram 5.1 The proposed mechanism for the carbon dioxide catalysed hydrolysis of 4-nitrophenyl esters of some amino acids according to Hay and Main [39].

probably carbamate (C_A) formation rather than decomposition which is rate-determining. However, on the basis of the very small temperature dependence of the experimental rate constant, k_2, for the valine ester (Table 5.4, based on Equation 5.1) we think that there is at least one equilibrium step

before the rate-determining step and that this most probably indicates rate-determining decomposition of a carbamate species.

The basis for this is as follows. If the rate-determining step of a reaction follows one or more equilibria, the overall rate constant is the product of the rate constant for the rate-determining step and the equilibrium constant(s) for the preceding step(s); for instance , if decomposition of C_A in Diagram 5.1 is rate determining, then the overall rate constant k_2 is given by

$$k_2 = kK_AK_Z \qquad 5.4$$

where equilibrium constant subscripts refer to the two equilibria in Diagram 5.1. Unlike a rate constant for a single stage reaction, which as is consistent with transition state theory, will increase sharply with temperature, a composite rate constant like k_2 in Equation 5.4 may increase only little or even decrease with increasing temperature if the increase in the rate constant component (k) is partially or more than compensated by decrease(s) in the associated equilibrium constant(s) (K_A and K_Z in this case).

An example comes from Milstein and Fife's study [119] of the imidazole catalysed hydrolysis of 4-nitrophenyl acetate, a reaction which is accepted as involving pre-equilibrium formation of an acetylimidazolium intermediate [11, 7, 116, 121] which decomposes in a rate-determining step. Here a rate increase of only 50% when the temperature is increased from 20° to 30° was reported.

In the present study a comparable rate increase of 25% between 25°C and 30° is observed for the CO_2-catalysed

hydrolysis of 4-nitrophenyl valinate (Table 5.4). This therefore clearly indicates at least one equilibrium step before the rate-determining step.

We can now discuss the various stages in the scheme (Diagram 5.1) from a rate-determining point of view. If formation of the zwitterion, C_Z, were rate-determining, then $k_2 = k_z$ and k_2 would be expected to show a marked temperature dependence, which is not observed. If the formation of C_A from C_Z is rate-determining, then the requirement for a pre-equilibrium step is met so that this in itself is not inconsistent with the small temperature dependence. However, k_2 is given in this case by

$$\begin{aligned} k_2 &= k_A[B][C_Z] \\ &= k_A[B]K_Z[E][CO_2] \end{aligned} \qquad 5.5$$

where B represents the base responsible for the rate-determining proton transfer, which could easily be water. Since we observed, however, no base catalysis in this reaction, the kinetics being consistent with Equation 5.1 independent of varying concentrations of the buffer base (HPO_4^{2-}) and independent of pH (varying OH^-), we feel that rate-limiting proton transfer plays an unimportant part kinetically in this reaction, even though it is of importance in carbamate formation itself in some cases (Caplow [120]). This leaves the carbamate anion C_A being formed reversibly from reagents, with a subsequent step being the rate-determining one. One possible alternative is that C_Z, in equilibrium with ester and CO_2, cyclizes in a manner analogous to that indicated for C_A, yielding products directly without C_A being formed by proton loss at all. This is probably not likely as C_Z would be expected to have a very short lifetime by analogy with

carbinolamine zwitterion [86, 87] and further the carbamate anion centre of C_Z would be weakly basic and nucleophilic owing to electron withdrawing by the cationic nitrogen. Conjugate acids of the carbamate ions (e.g. C_A) have pK_a's in the region of 6 and for the zwitterion form it would be expected to be several orders of magnitude lower. Such a species would be a very poor nucleophile.

At this point, it is worth diverging to reconsider the kinetic form for the catalysis of hydrolysis of phenyl glycinate in bicarbonate solutions and to make some comments about this in relation to the 4-nitrophenyl ester case. The kinetic form for the phenyl glycinate case is (from Section 4) given by

$$\text{Rate} = k_2[E][HCO_3^-] \qquad 4.3$$

the kinetically equivalent form with the product term $[EH^+][CO_3^{2-}]$ having been excluded. From the equilibria

$$CO_2 + H_2O \overset{K_C}{\rightleftharpoons} H^+ + HCO_3^-$$

and $$H^+ + OH^- \overset{K_W}{\rightleftharpoons} H_2O$$

we have $$CO_2 + OH^- \overset{K_CK_W}{\rightleftharpoons} HCO_3^-$$

and Equation 4.3 could be written in another kinetically equivalent form as follows

$$\text{Rate} = k_2[E]K_CK_W[CO_2][OH^-] \qquad 5.6$$

indicating the possibility of a reaction involving E and CO_2 and OH^- rather than with HCO_3^-.

This kinetic form can be seen to be identical with that based on rate-determining proton transfer from C_Z to give C_A.

(Diagram 5.1 and Equation 5.5) in the special case that the concentration of base, [B], in Equation 5.5 is the concentration of hydroxide [OH^-]. This at first appears to be a sensible and logical explanation for the kinetic form for phenyl glycinate, given the known dependence of rates of carbamate formation on [OH^-] from Caplow's work [120]. However, we are forced to exclude this possibility for the following reasons. Firstly, OH^- catalysis in previously studied cases [120] is only significant at pH 11 upwards so it is hardly likely to be important in the phenyl glycinate case at pH 6 to pH 7.4, where [OH^-] is so low. Secondly, if the hydroxide ion at such low concentrations were able to catalyse the reaction via proton transfer, then the good base HPO_4^{2-}, present as a buffer component in relatively high concentration, should be much more effective, but we have no evidence for any buffer catalysis at all. Nor is there kinetic evidence for the weaker base HCO_3^- taking any base role.

A consideration of the comparative importance of rate-determining proton transfer ($C_Z \longrightarrow C_A$, Diagram 5.1) for phenyl glycinate also clearly eliminates this possibility. The important point here is that if proton transfer to base B were sufficiently slow to be rate-determining for 4-methoxyphenyl glycinate and phenyl glycinate, then it must necessarily be more than sufficiently slow to be rate-determining for 4-nitrophenyl glycinate. The rationale for this is in the 4-nitrophenyl case where the leaving group is very good , decomposition of C_A must be much faster than for the phenyl case where the leaving group is poorer. In other words, if the proton transfer to form C_A is sufficiently slow to be rate-determining when the product C_A is relatively slow to react (phenyl case) it must also be rate-determining when C_A reacts faster (4-nitrophenyl case). However, as is clear from this

and previous studies the kinetic form for the 4-nitrophenyl case is given by Equation 5.1 and a kinetic term analogous to Equation 5.5 incorporating the $[OH^-]$ factor is not observed.

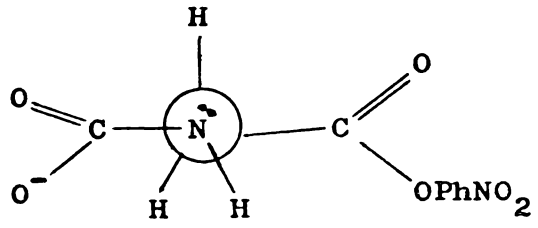
The only possible circumstance under which this conclusion would be invalidated is, if in the case of 4-nitrophenyl ester but not the phenyl ester, the zwitterion C_Z resulted in hydrolysis without C_A being form by proton transfer, a possibility which is discussed above. For the phenyl ester, rate-determining proton transfer from C_Z to OH^- would provide a feasible explanation in principle. However, as discussed above this is not consistent with the lack of catalysis by other bases and the pH is probably much too low for significant concentrations of OH^- to allow such a kinetic term to appear.

In summary, then, we are left with CO_2 catalysis of 4-nitrophenyl esters involving rate-determining breakdown of carbamate, while for catalysis in bicarbonate solutions of phenyl glycinate, HCO_3^- and not CO_2, is the catalytically active species, mechanisms having been discussed in Section 4.

A comparison of the extent of CO_2 catalysis for the glycine and valine esters is now made. The aim of carrying out this comparative study in the first instance was to determine, given the known susceptibility of carbamate formation to steric effects [122], whether the valine ester with the bulky isopropyl α-alkyl substituent might react much slower than the glycine ester, the view being held at that stage that carbamate formation, rather than decomposition, was probably rate-determining. However, the catalytic rate constants were found to be very similar (530 and 450 $1\ mol^{-1}\ s^{-1}$ at 30°C for the glycine and valine ester, respectively) and if cyclization of C_A (Diagram 5.1) is rate-determining, then the similarity of the catalytic rate constants must indicate that any significant differences in the overall equilibrium constants for

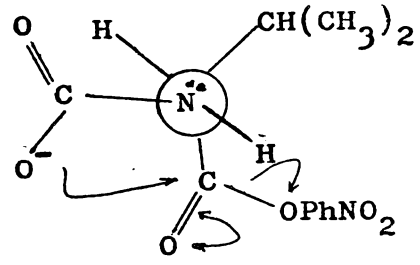
carbamate formation are approximately compensated by differences in the rates of reaction of C_A once formed.

If carbamate formation is sterically hindered by the α-substituent ($-CH(CH_3)_2$)in the valine case, as appears possible from the studies of Frahn and Mills [122], this could very well be compensated by a greater rate of cyclization of C_A in the valine case. The conformational diagrams below (Diagrams 5.2 and 5.3) show that for the glycine case the preferred



(C_A)

Diagram 5.2 The likely conformation for the carbamate of the glycine ester.



(C_A)

Diagram 5.3 One likely conformation for the carbamate of the valine ester.

conformation for C_A would be one in which the groups are relatively far apart for a fast reaction, but for the valine case the conformation in which these groups are relatively

close is relatively much more likely to be a major conformation, allowing for a faster reaction.

Finally, having suggested that CO_2 catalysis of the hydrolysis of 4-nitrophenyl esters involves rate-determining decomposition rather than formation of carbamate, this section concludes with a dampener which takes the form of an estimate of the rate constant for carbamate formation from an amine pK (of conjugate acid) equal to 7.15 (phenyl glycinate). In his report on rates of uncatalysed carbamate formation, Caplow [120] published an approximately linear Brønsted plot of log k vs pK_a for a limited series of amines. The value of k for pK_a equal to 7.15 is approximately 90 l mol^{-1} s^{-1} at 10°C. This is lower but of the same order of magnitude as the experimentally determined catalytic rate constant for CO_2 catalysis of 4-nitrophenyl glycinate at 30°C (k_2 = 530 l mol^{-1} s^{-1}). Even taking the temperature difference into account, carbamate formation could at best not be predicted to be very much if at all faster than the overall rate of CO_2 catalysis of 4-nitrophenyl ester hydrolysis. These data do raise the query whether carbamate formation is really sufficiently rapid to be a true pre-equilibrium.

So whereas this comparison might well have provided clear support for carbamate formation being rapid relative to overall rate, it does not do so and the answer to the question whether carbamate decomposition really is rate-determining is still somewhat uncertain.

5:3 The possibility of an E|cB mechanism of CO_2-catalysis of 4-nitrophenyl ester hydrolysis.

In the previous section we have considered the more likely mechanisms for CO_2 catalysis of ester hydrolysis. We now turn our attention to a mechanism which has not been considered for the role of CO_2 in catalysis.

Evidence for the E|cB mechanism was revealed by Bruice et al for ester hydrolysis [104, 125-127]. The kinetics of their hydrolysis reactions involving α-acyl substituted 2- and 4-nitrophenyl acetates provided evidence for a reactive carbanion species which decomposes to products probably by way of a short-lived ketene intermediate (Diagram 5.4).

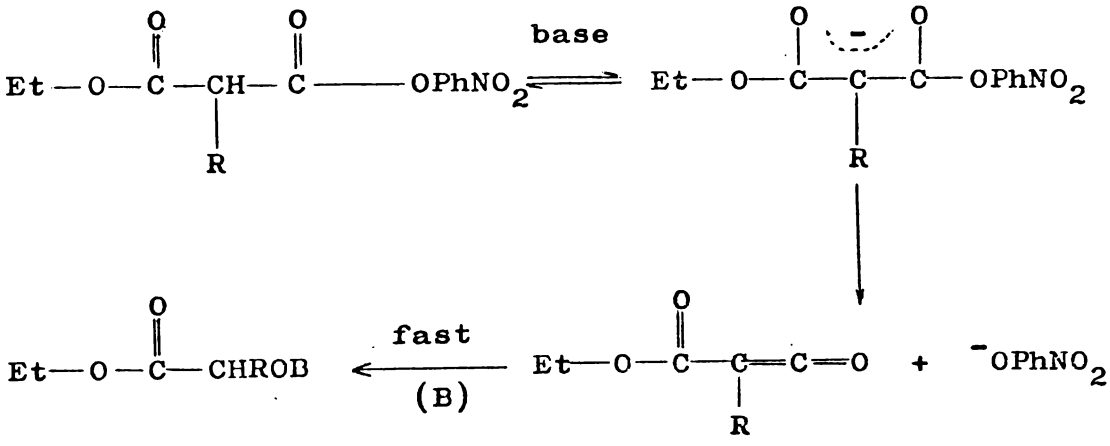


Diagram 5.4 The base catalysed hydrolysis of α-acyl substituted 2- and 4- nitrophenyl acetate (according to Bruice et al [125-127]).

The compounds used by Bruice et al are malonate diesters and the α-proton is very acidic, the carbanion formed being highly

stabilized and thus favouring the E|cB mechanism according to Diagram 5.4.

We have attempted to investigate whether a similar mechanism could in fact be applicable to the CO_2-catalysed hydrolysis of 4-nitrophenyl esters of amino acids(Diagram 5.5).

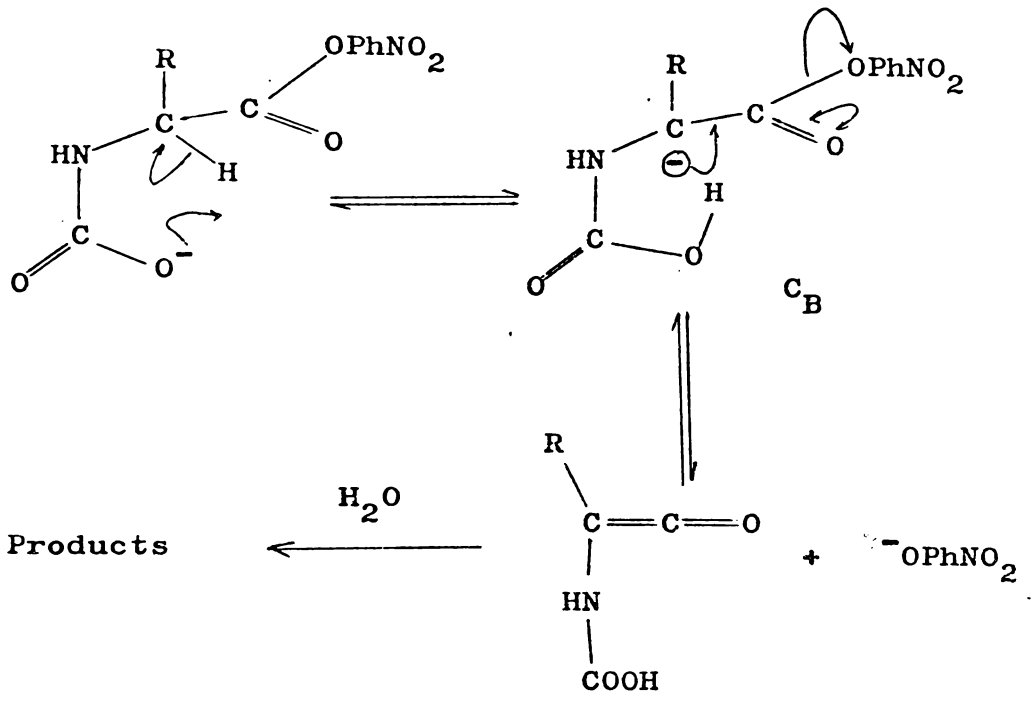


Diagram 5.5 Possible carbanion mechanion for CO_2-catalysed hydrolysis of 4-nitrophenyl amino acid esters.

In favour of this mechanism is the intramolecular nature of the proton abstraction. Against it is the weak basicity of the carbamate ion (pK_a of the carbamate being about 6 [120]) relative to that of the carbanion, C_B, which is certain to be more strongly basic than carbanions involved in malonate ester hydrolyses (Diagram 5.4).

To test the feasibility of this mechanism we wished to determine whether the exchange of an α-proton of an amino acid

ester with solvent, specifically D_2O, is catalysed by CO_2. If this was found to be the case it would support the viability of a species such as C_B as a reaction intermediate in the CO_2-catalysed hydrolysis.

Such a test is not possible for a 4-nitrophenyl ester, which would of course hydrolyse rapidly, so we chose to use an alkyl ester, for which α-proton exchange would be slightly more difficult to achieve. Since we wished to observe the proton exchange by ^{1}H-NMR studies, a system was required in which preferably only one α-proton was present and for which H to D exchange would give clearly detectable spectral changes.

We chose to use L-ethyl phenylalaninate (Diagram 5.6) as the model compound. Ethyl esters do not hydrolyse at significant rates at pH 7.2 (pD 7.6) of the study, a half-life of about 15 hours being estimated [71] at a much higher pH of 9.5. The ^{1}H-NMR spectrum of L-ethyl phenylalaninate is characterized by a clearly distinguishable doublet representing the methylene

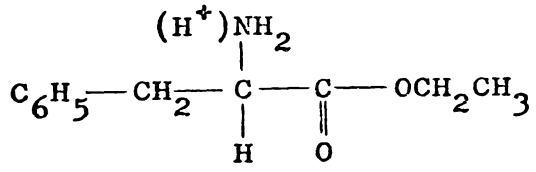


Diagram 5.6 L-ethyl phenylalaninate.

protons of the α-benzyl group. The α-proton signal itself is not separable from those of the other protons, so exchange of this proton for deuterium is not readily distinguishable. However, if the α-proton is exchanged for deuterium its spin-spin splitting of the methylene proton signal to give a doublet would be eliminated and the doublet would become a singlet.

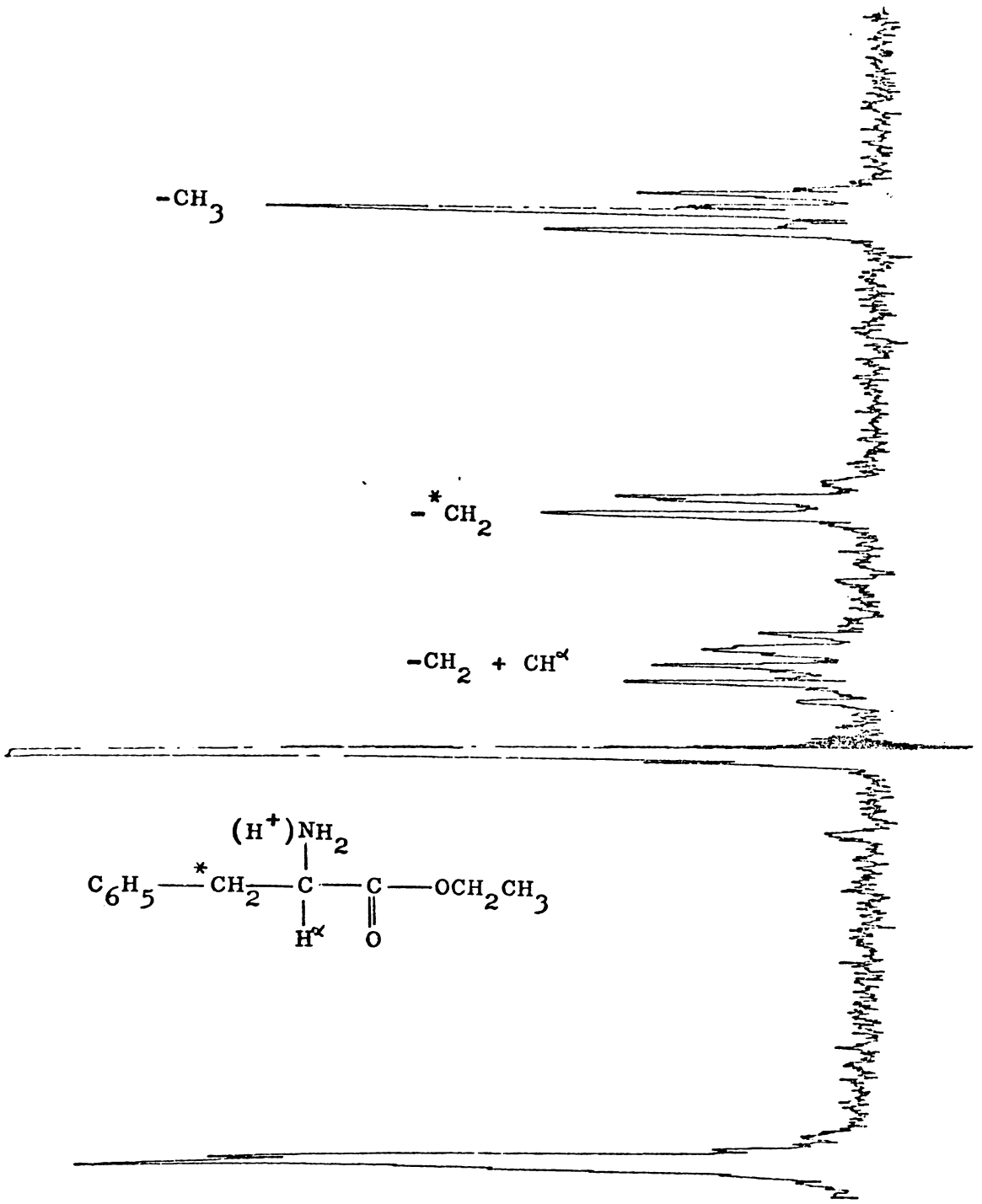
It is possible, therefore, to study the α-proton exchange by following the collapse of the methylene doublet to a singlet.

The experiment was carried out by dissolving in D_2O amounts of dried L-ethyl phenylalaninate hydrochloride and dried potassium carbonate calculated to give pD 7.6 (checked by pH measurements with appropriate correction) and to ensure that the total carbonate (present as CO_2 and HCO_3^-) was at least 200 times more concentrated than for the studies of CO_2-catalysed hydrolysis of 4-nitrophenyl esters. The aim was simply to enhance the chances for CO_2-catalysis of proton exchange so that it could become more readily apparent.

The experiment was carried out at 34°C (probe temperature), ^{1}H-NMR spetra being recorded initially at short and then longer time intervals. Since 4-nitrophenyl esters hydrolyse at pH 7.6 in the presence of CO_2 at concentrations about 200 times lower than the present study with half-lifes of the order of 20 - 40 seconds, we would expect very rapid α-proton exchange and doublet collapse if carbanion formation (Diagram 5.5) is sufficiently rapid to be on the pathway to ester hydrolysis. However, as the following sequence of spectra shows, the doublet is maintained even over the very long time intervals in which the ester is apparently undergoing other reaction(s).

From the sequence of spectra it is evident that the doublet nature of the methylene proton signal is maintained over a long time period, indicating clearly that CO_2 does not catalyse carbanion formation of the type which would be essential for hydrolysis via the E1cB mechanism (Diagram 5.5).

Rigorously, this experiment does not exclude the possibility of a concerted elimination without discrete carbanion formation (Diagram 5.7), but the lack of any measurable α-proton exchange in the present study suggests that this proton is hardly likely to be sufficiently acidic to permit this reaction,

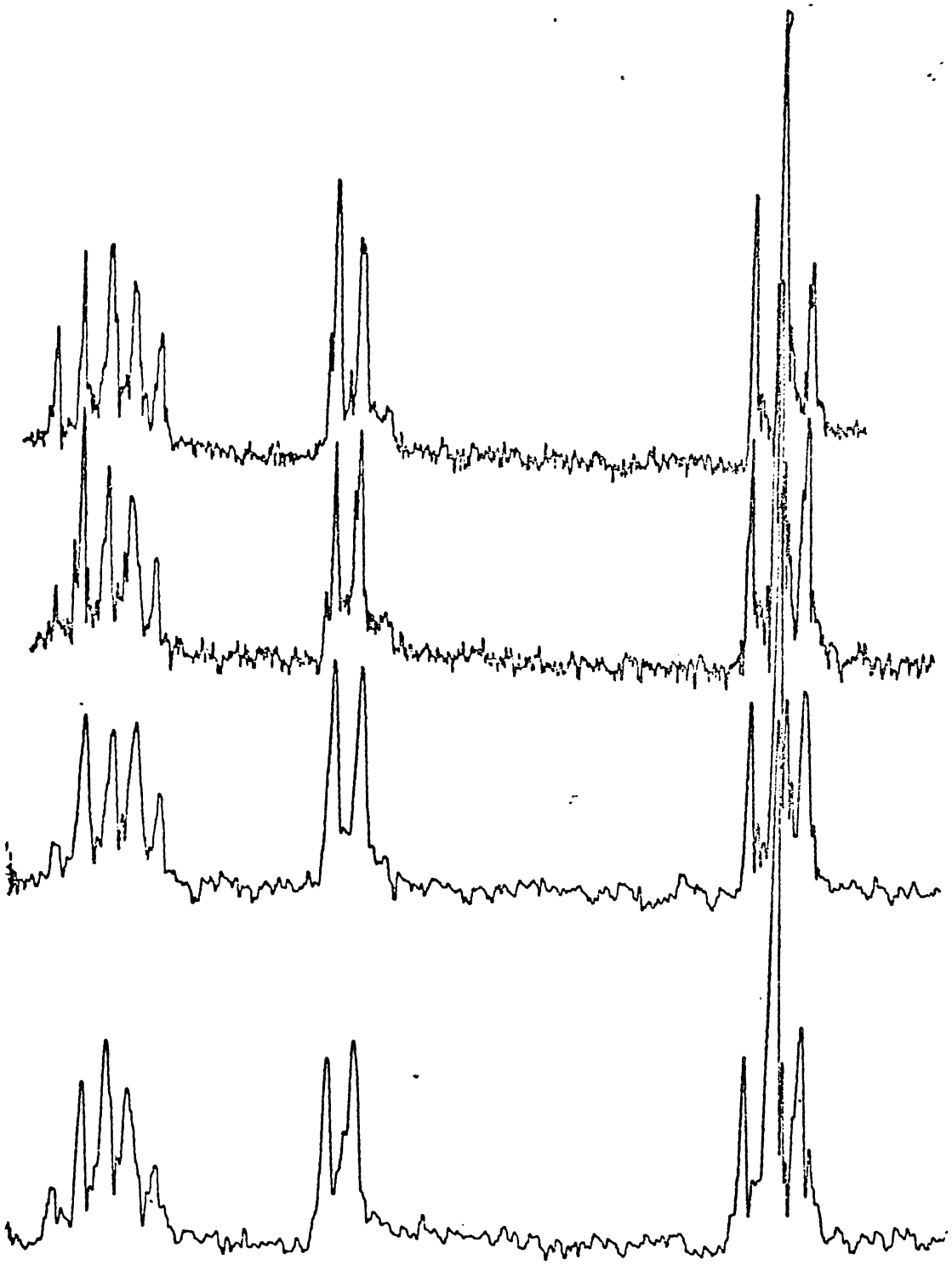


Spectrum I Taken when potassium carbonate is added.

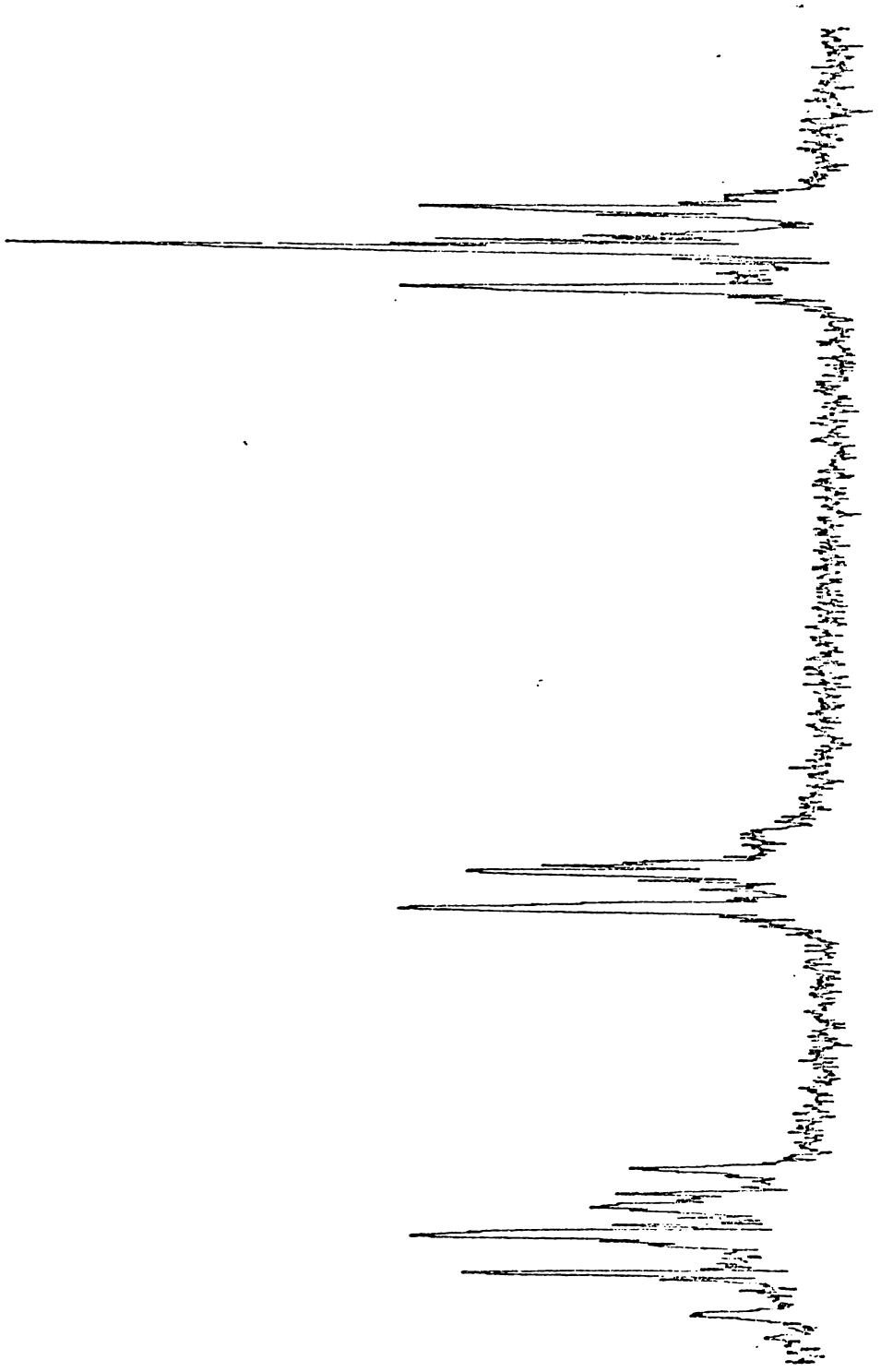
(Zero time)

D_2O = 0.5 ml, pD 7.6,

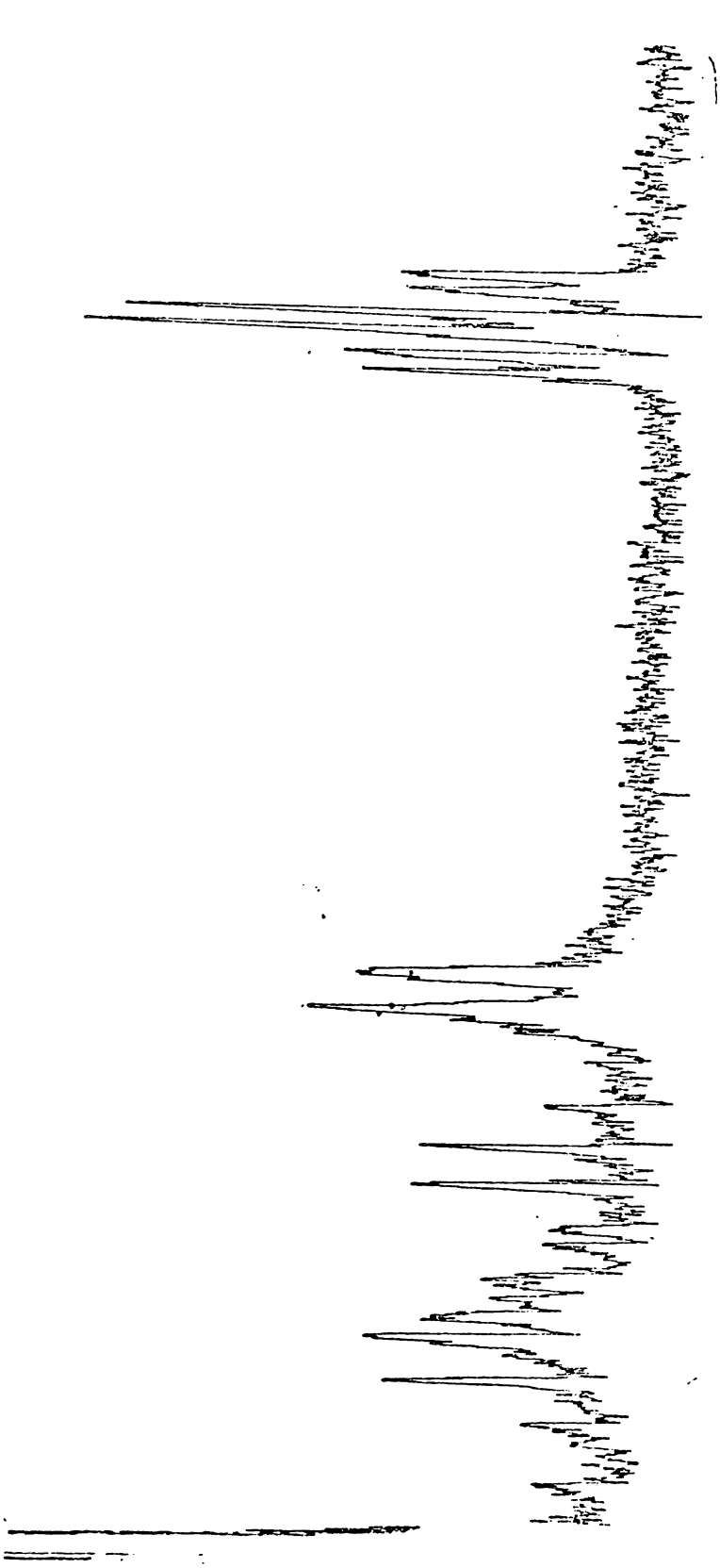
K_2CO_3 = 46 mg, Ester = 14 mg



Spectrum 2 Recorded at hourly intervals(superimposed).
Spectra were unchanged.



Spectrum 3 No apparent change after 24 hours.



Spectrum 4 Taken after one week - appearance of new triplet and quartet (CH_3CH_2OH presumably formed; see text).

even though its coupling to release the 4-nitrophenolate ion, a very good leaving group, would facilitate such a process.

In conclusion, then, we feel it is unlikely that elimination mechanisms offer a feasible alternative to mechanisms suggested in the previous section for CO_2-catalysis of 4-nitrophenyl ester hydrolysis or for that matter HCO_3^- catalysis in the case of phenyl ester hydrolysis.

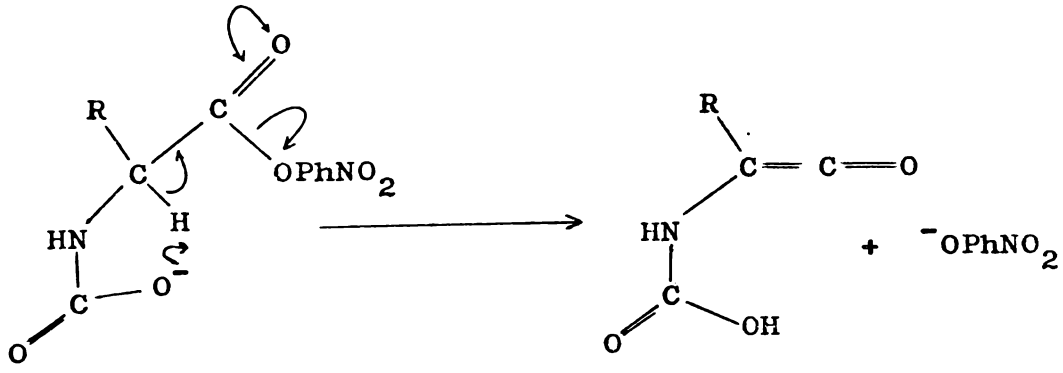


Diagram 5.7 Concerted elimination process of carbamate.

A brief mention of the significance of ^{1}H-NMR spectral changes in the above experiment is worthwhile.

Finally, we have mentioned that the doublet remained unchanged as shown in ^{1}H-NMR spectra 1-3 over a period of one week. There is however an apparent change in Spectrum 4, which shows up new triplet and quartet signals consistent with ethanol resonance, a by-product of the possible mechanism according to Diagram 5.8.

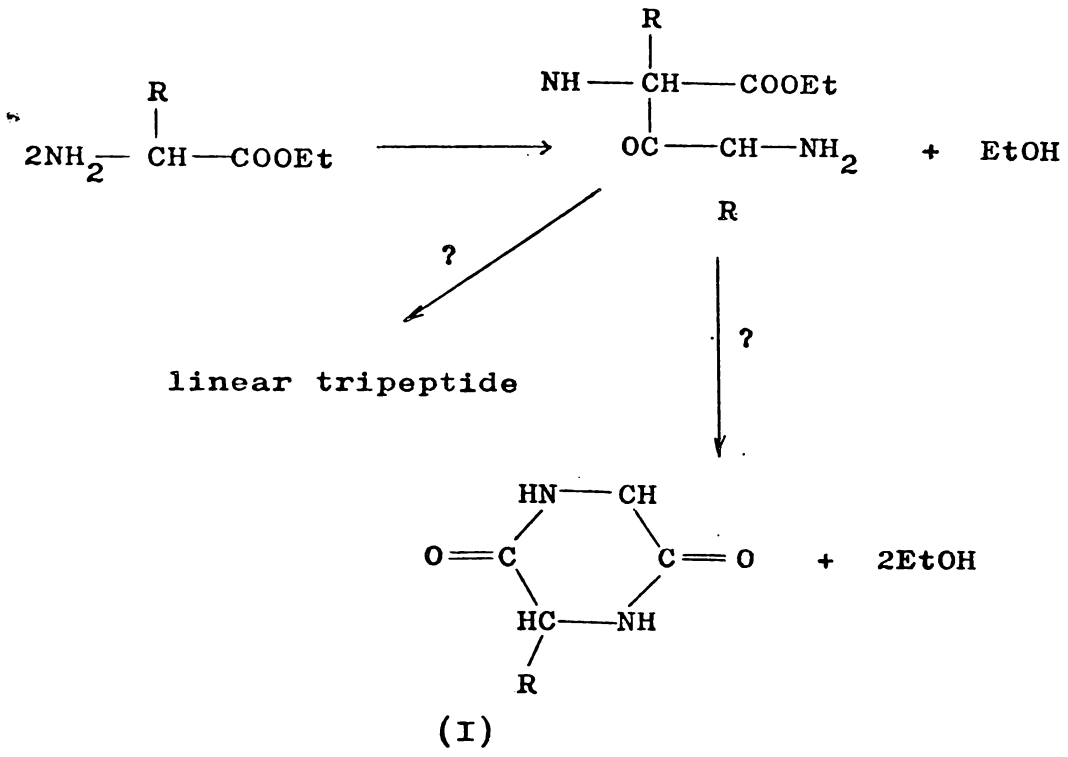


Diagram 5.8 Polymerization reaction to a di-keto compound (I) (or possibly a linear tripeptide).

Section 6 The 4-nitrobenzaldehyde catalysed hydrolysis of 4-nitrophenyl esters of Glycine, Valine, Proline, Phenyl Glycinate and 4-methoxyphenyl Glycinate.

Section 6:1.1 Results

The 4-nitrobenzaldehyde (NB) catalysed hydrolysis of the following amino acid esters ; 4-nitrophenyl esters of glycine, valine, proline, phenyl glycinate and 4-methoxyphenyl glycinate have been investigated. At fixed concentration of catalyst, the hydrolysis was always first order in ester, as shown by the pseudo first order rate constants, k_h, in Tables 6.1 to 6.5.

The plots of k_h vs [NB] at constant pH were linear. That implied a first order dependence on the catalyst, the gradient being defined as the catalytic rate constant, k_c (see Section 4.1). Representative plots of k_h vs [NB] are shown in Figures 6.1 to 6.5.

Table 6.6 shows the results of the pH variation study. The catalytic rate constants, k_c, are listed in the third column with the fraction of the neutral amino acid ester in the second column. The ratio k_c/f_E over the pH range for each of the esters appeared to be constant, (the factor, f_E, is defined for the fraction of the neutral ester). These results are consistent, therefore, with the neutral amino acid ester (E) being the reactive species as can be seen in the following equations:

$$\text{Rate} = k_c[E_T][NB] = k_2[E][NB]$$

therefore,

$$k_2 = k_c[E_T]/[E]$$

but, $[E_T]/[E] = 1/f_E$

therefore, $k_2 = k_c/f_E$

Values of k_2 should be constant with pH if only the neutral ester reacts.

6:1.2 Analysis

It is well established that a carbinolamine intermediate is formed when a carbonyl compound reacts with the aldehyde [67, 78 - 81]. Carbinolamine intermediates were thought to be involved in the aromatic aldehyde catalysed hydrolysis of some amino acid esters [92, 94].

Our results, Table 6.6, show that the kinetic form for hydrolysis catalysed by 4-nitrobenzaldehyde is :

$$d[\text{Product}]/dt = k_2[NB][E] \qquad 6.1$$

where [E] is the concentration of the neutral ester and [NB] the concentration of 4-nitrobenzaldehyde. The protonated form of the amino acid esters was present in varying proportion over the pH range under study but was not involved in the catalysed hydrolysis presumably because it is not able to undergo the carbonyl addition necessary for carbinolamine formation.

The generalized reaction involving carbinolamine (C_x) formation is given by Equation 6.2. Application of the steady

state hypothesis [105] to the intermediate, C_x, gives Equation 6.3

$$NB + E \underset{k''}{\overset{k'}{\rightleftharpoons}} C_x \xrightarrow{k'''} \text{Product} \qquad 6.2$$

$$d[\text{Product}]/dt = k'k'''/(k'' + k''')\,[NB][E] \qquad 6.3$$

Two cases arise from Equation 6.3. In the first instance, where $k'' \ll k'''$ with formation of C_x as rate limiting, Equation 6.3 simplifies to Equation 6.4

$$d[\text{Product}]/dt = d[C_x]/dt = k'[NB][E] \qquad 6.4$$

The rate constant, k_2 defined in Equation 6.1 is then equal to k' in Equation 6.4. In the second instance, where $k'' \gg k'''$, the decomposition of C_x is rate limiting, then,

$$d[\text{Product}]/dt = k'''\,[C_x] \qquad 6.5$$

$$\text{but } [C_x] = k'/k''\,[NB][E]$$

Substituting $[C_x]$ into Equation 6.5, we have,

$$d[\text{Product}]/dt = k'''(k'/k'')[NB][E]$$
$$= k'''K[NB][E] \qquad 6.6$$

where K is defined as the equilibrium constant for the carbinolamine formation. The rate constant, k_2, defined in Equation 6.1 in this case equals $k'''K$ in Equation 6.6.

It is clear therefore that both rate limiting formation and rate limiting decomposition of the carbinolamine, C_x, are consistent with the observed kinetic form but which applies merits consideration.

Table 6.1 The 4-nitrobenzaldehyde (NB) catalysed hydrolysis of 4-nitrophenyl glycinate.

Error in k_h values ± 5%

Temperature 30°C (Ionic strength =.2 mol l^{-1})

pH	10^5 [NB] (mol l^{-1})	10^3 k_h (s^{-1})	k_c (l mol^{-1} s^{-1})
8.13	0	7.3	
	1	10	
	3	19.5	
	3.5	20.5	
	8	40.8	
	12	53.7	430 ± 25
7.91	0	6.54	
	1	10.9	
	3	19.3	
	3.5	21.9	
	6	30.4	
	6.5	33.9	
	10	48.9	
	15	67.5	410 ± 25
7.45	0	8.5	
	1.5	13.1	
	3	18.8	
	3.5	20.0	
	7	30.5	
	8	35.7	
	9	48.0	330 ± 15

continued

7.0	0	7.7	
	1	10.3	
	3	14.6	
	3.5	15.6	
	7	23	
	8	26.6	
	9	27.3	
	12	31.7	220 ± 15
6.52	0	6.1	
	1	8.38	
	4	9.56	
	5	11.9	
	9	15.5	
	10	17.6	
	15	22.2	105 ± 10

Table 6.2 The 4-nitrobenzaldehyde (NB) catalysed hydrolysis of 4-nitrophenyl valinate.

Error in k_h values ± 5%

Temperature 30°C

pH	10^5 [NB] (mol l^{-1})	10^3 k_h (s^{-1})	k_c (l mol^{-1} s^{-1})
8.13	0	1.7	
	3	6.9	
	3	8.15	
	5	11.94	
	5	11.6	
	8	18.6	200 ± 15

continued

7.4	0	2.1	
	3.7	7.12	
	7.0	13.22	
	8.0	14.1	
	10.0	16.6	
	12.0	18.9	150 ± 10
6.99	0	1.5	
	1.0	2.7	
	3.0	4.4	
	6.0	7.02	
	9.0	10.4	
	12.0	13.5	100 ± 10
6.55	0	1.3	
	4	2.95	
	7.9	4.9	
	8.0	5.2	
	10.0	6.14	
	14.5	8.54	
	19.0	11.3	55 ± 5

Table 6.3 The 4-nitrobenzaldehyde (NB) catalysed hydrolysis of 4-nitrophenyl-L-prolinate.

Error in k_h values ± 8%

Temperature 30°C

pH	10^5 [NB] (mol l^{-1})	10^3 k_h (s^{-1})	k_c (l mol^{-1} s^{-1})
8.1	0	51.58 51.13	
	0.5	51.52	
	1.0	58.3	
	1.5	59.4	
	3.0	74.2	
	6.0	99.0	
	7.0	103.5	
	9.0	118.9	780 ± 60
7.38	0	42.4	
	1.0	45.4	
	2.5	47.8	
	3.5	52.7	
	6.5	59.7	
	7.0	61.1	
	7.5	66.6	
	10.0	74.6	290 ± 30
6.96	0	36.1	
	1.5	37.8	
	1.9	39.4	
	2.4	40	
	5.0	43.9	
	7.5	46.9	
	9.0	47.8	
	10.0	49.5	

continued

	13.0	53.3	
	15.0	56.3	
	17.0	58.7	
	20.0	62.7	130 ± 12
6.56	0	25.6, 25.9	
	0.5	27.1	
	1.0	27.6	
	2.0	28.8	
	2.5	28.3	
	3.5	28.8	
	4.0	29.5	
	8.5	32, 30.6	
	10.0	32.4, 31	
	13.0	34.3	50 ± 5

Table 6.4 The 4-nitrobenzaldehyde (NB) catalysed hydrolysis of phenyl glycinate.

Error in k_h values ± 5%

Temperature 30°C

pH	10^5 [NB] (mol l^{-1})	10^4 k_h (s^{-1})	k_c ($l\ mol^{-1}\ s^{-1}$)
7.43	0	4.3	
	1.0	4.5	
	3.0	5	
	5.0	5.6	
	8.0	6.3	
	10.0	7.1	2.6 ± 0.15

continued

6.91	0	2.9	
	1.0	3.04	
	2.0	3.13	
	4.0	3.4	
	5.0	3.5	
	8.0	3.93	
	10.0	4.13	1.3 ± 0.06
6.42	0	2.3	
	0.5	2.4	
	1.0	2.47	
	3.0	2.5	
	4.0	2.56	
	5.95	2.7	0.55 ± 0.05

Table 6.5 The 4-nitrobenzaldehyde (NB) catalysed hydrolysis of 4-methoxyphenyl glycinate.

Error in k_h values ± 5%

Temperature 30°C

pH	10^5[NB] (mol l^{-1})	10^4 k_h (s^{-1})	k_c (l mol^{-1} s^{-1})
7.8	0	3	
	3.0	5.3	
	5.0	6.9	
	9.0	10.1	
	10.0	10.6	
	13.0	13.2	
	15.0	15.1	0.70± 0.04

continued

7.3	0	3.5	
	5.0	4.9	
	12.0	7.8	
	15.0	9.4	
	18.0	10.2	
	22.0	11.6	0.44 ± .03
6.6	0	2.8, 3.0	
	5.0	3.52	
	10.0	4.09	
	14.0	4.6	
	18.0	5.2	0.14 ± .03

Table 6.6 The effect of pH on the 4-nitrobenzaldehyde catalysed hydrolysis of Phenyl Glycinate, 4-methoxyphenyl Glycinate, and 4-nitrophenyl esters of Glycine, Valine and Proline.

Phenyl Glycinate pK(ester) = 7.15 Temp. = 30°C

pH	f_E	k_c ($l\ mol^{-1}\ s^{-1}$)	$k_2 = k_c/f_E$ ($l\ mol^{-1}\ s^{-1}$)
7.43	.656	2.6 ± .15	3.9 ± .2
6.91	.365	1.3 ± .06	3.6 ± .2
6.41	.154	0.55 ± .05	3.6 ± .3

4-Nitrophenyl Glycinate pK(ester) = 7.1 Temp. = 30°C

pH	f_E	k_c ($l\ mol^{-1}\ s^{-1}$)	$k_2 = k_c/f_E$ ($l\ mol^{-1}\ s^{-1}$)
8.13	.914	430 ± 30	470 ± 30
7.91	.866	410 ± 30	470 ± 30
7.45	.691	330 ± 15	480 ± 20
7.0	.443	220 ± 15	500 ± 35
6.52	.208	105 ± 10	500 ± 50

4-Methoxyphenyl Glycinate pK(ester) = 7.4 Temp. = 30°C

7.8	.715	0.7 ± .04	.97 ± .06
7.3	.443	0.44 ± .03	.99 ± .07
6.6	.137	0.14 ± .03	1.0 ± .2

4-Nitrophenyl Valinate pK(ester) = 6.98 Temp. = 30°C

8.13	.934	200 ± 15	215 ± 15
7.34	.696	150 ± 10	215 ± 15
6.99	.506	100 ± 10	200 ± 20
6.55	.271	55 ± 5	200 ± 20

4-Nitrophenyl Valinate pK(ester) = 6.98 Temp.= 25°C

8.1	.929	180 ± 10	190 ± 10
7.36	.706	140 ± 10	195 ± 10
6.95	.483	90 ± 5	190 ± 10
6.51	.253	45 ± 5	180 ± 15

4-Nitrophenyl Prolinate pK(ester) = 8.03 Temp. = 30°C

8.1	.5402	780 ± 60	1450 ± 100
7.38	.183	290 ± 30	1500 ± 150
6.96	.0784	130 ± 15	1650 ± 200
6.56	.033	50 ± 5	1500 ± 150

4-Nitrophenyl Prolinate pK(ester) = 8.03 Temp. = 25°C

8.1	.5402	870 ± 40	1600 ± 90
7.38	.183	290 ± 20	1600 ± 120
6.96	.0784	125 ± 10	1600 ± 120
6.56	.033	50 ± 5	1600 ± 160

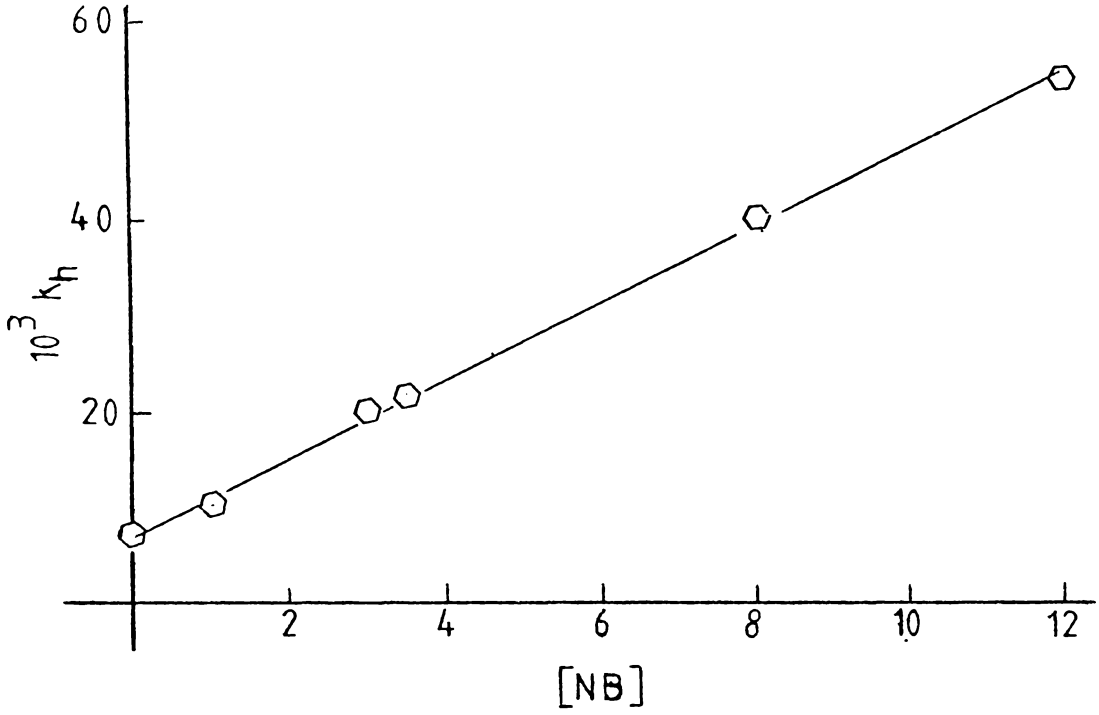


Figure 6.1 The plot of k_h vs [NB] for the catalysed hydrolysis of 4-nitrophenyl glycinate (pH = 8.13)

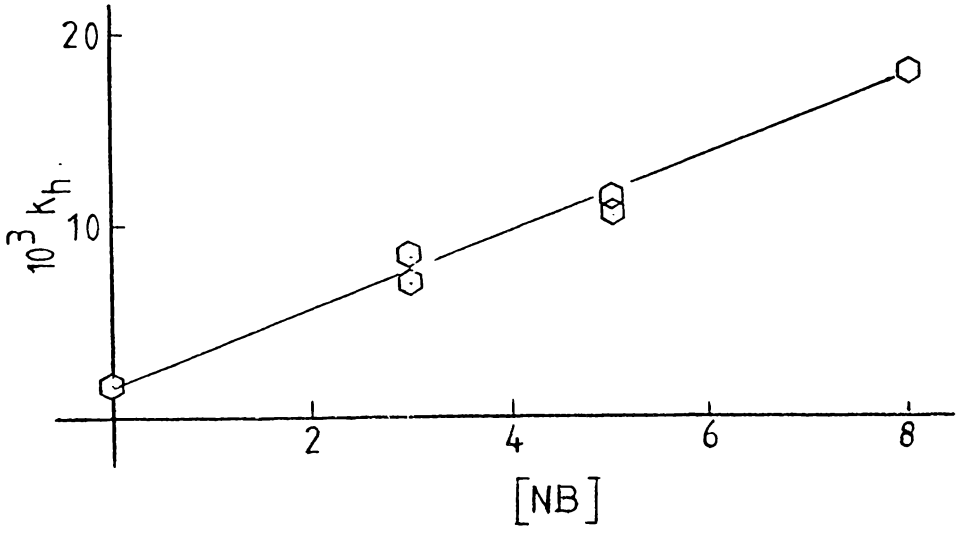


Figure 6.2 The plot of k_h vs [NB] for the catalysed hydrolysis of 4-nitrophenyl valinate (pH = 8.13)

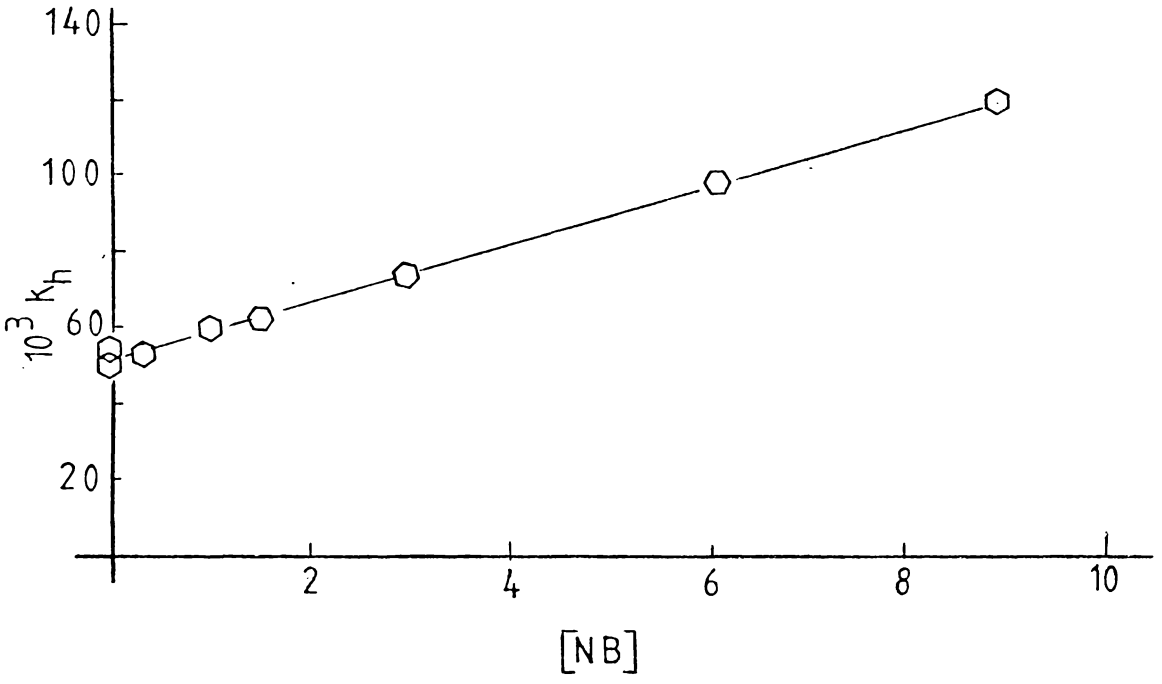


Figure 6.3 The plot of k_h vs [NB] for the catalysed hydrolysis of 4-nitrophenyl prolinate (pH = 8.1)

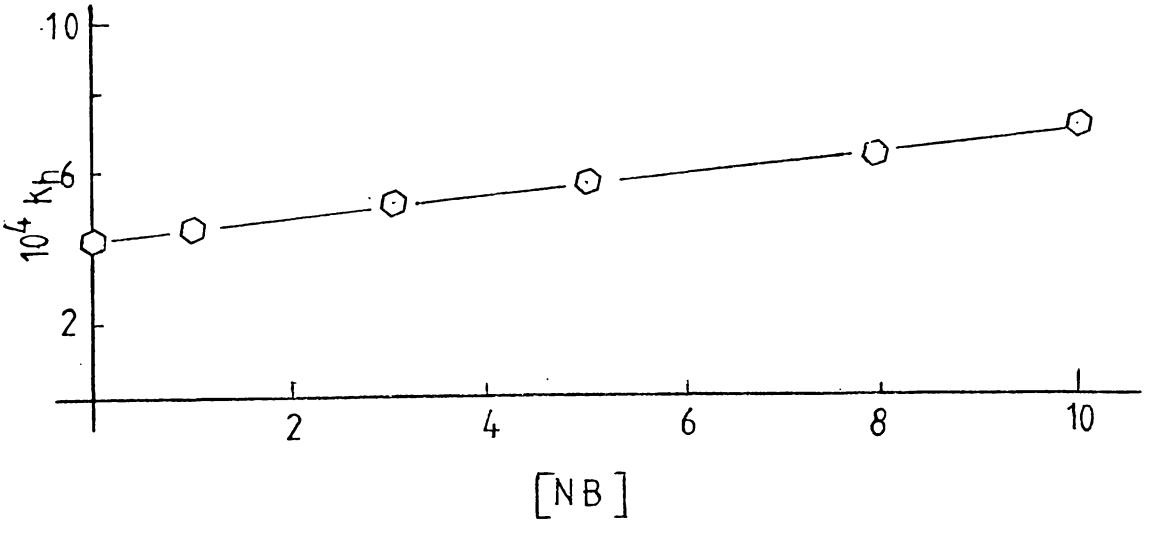


Figure 6.4 The plot of k_h vs [NB] for the catalysed hydrolysis of phenyl glycinate (pH = 7.33).

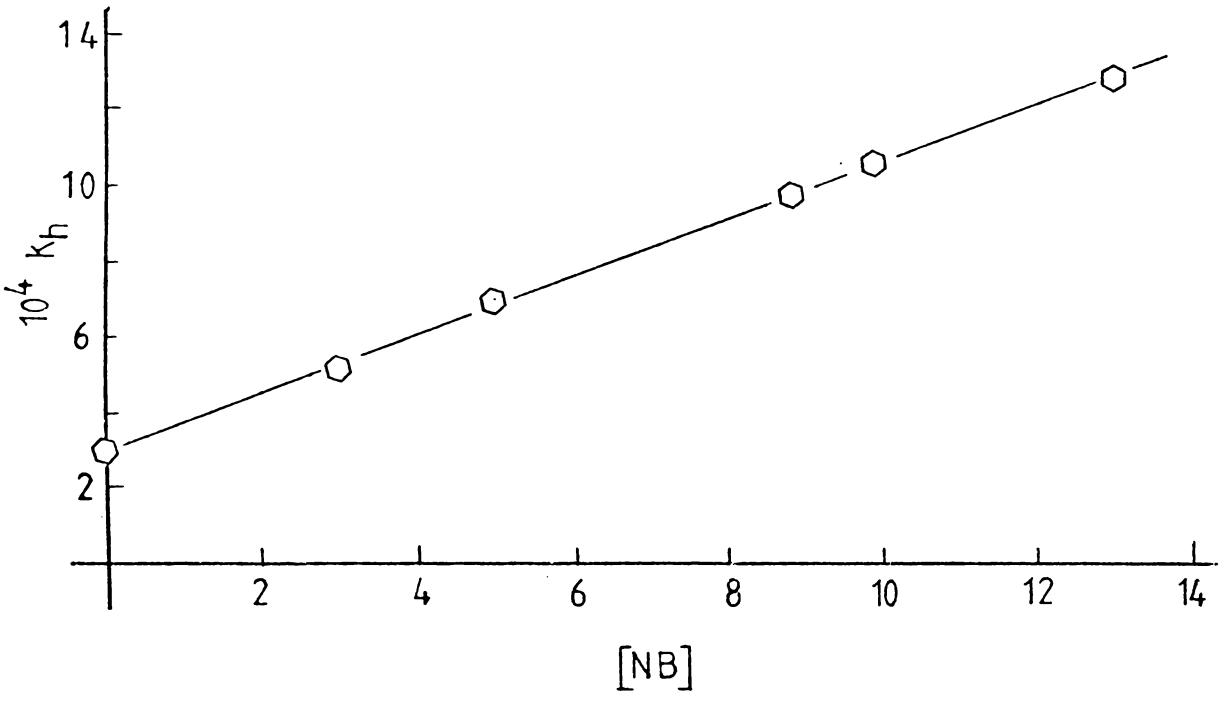


Figure 6.5 The plot of k_h vs [NB] for the catalysed hydrolysis of 4-methoxyphenyl glycinate (pH = 7.66).

Various intermediates that were likely to be involved in the aldehyde catalysed hydrolysis of some amino acid esters have been dealt with in depth in earlier studies [92, 94]. Diagram 6.1 shows the two likely paths where the intermediates, a cyclized form in one case and an imine in the other are formed and each pathway is described in turn. Path I shows the carbinolamine anion, C^-, in equilibrium with the tetrahedral addition intermediate, T_A. The partitioning of T_A favours the forward direction to form the cyclized intermediate on the basis of leaving group ability, where in this case phenolate ions ($4\text{-}NO_2C_6H_4O^-$ or $C_6H_5O^-$) would be better leaving groups than the carbinolamine anion (C^-). The cyclized intermediate would be expected to hydrolyse rapidly to amino acids, regenerating the catalyst 4-nitrobenzaldehyde. This path will now be referred to as the Cyclization Pathway. Path II involves the neutral carbinolamine, C. Here the carbinolamine forms imine, T_B, by the expulsion of OH^-, the latter being accepted by the ester carbonyl group in the intramolecular fashion indicated. Once OH^- has been transfered, the reaction becomes essentially irreversible, the partitioning of T_B favouring 4-nitrophenolate ion rather than OH^- loss.

6:1.3 Results and discussion

The results (Table 6.6) show that k_2 (Equation 6.1) has the mean value of 485, 3.8, 0.9 ($1\ mol^{-1}\ s^{-1}$) for 4-nitrophenyl glycinate, phenyl glycinate and 4-methoxyphenyl glycinate, respectively. When these values are compared with the second order rate constants for hydrolysis by OH^- of the corresponding substituted phenyl acetates (k_2 = 34,000 $1\ mol^{-1}$

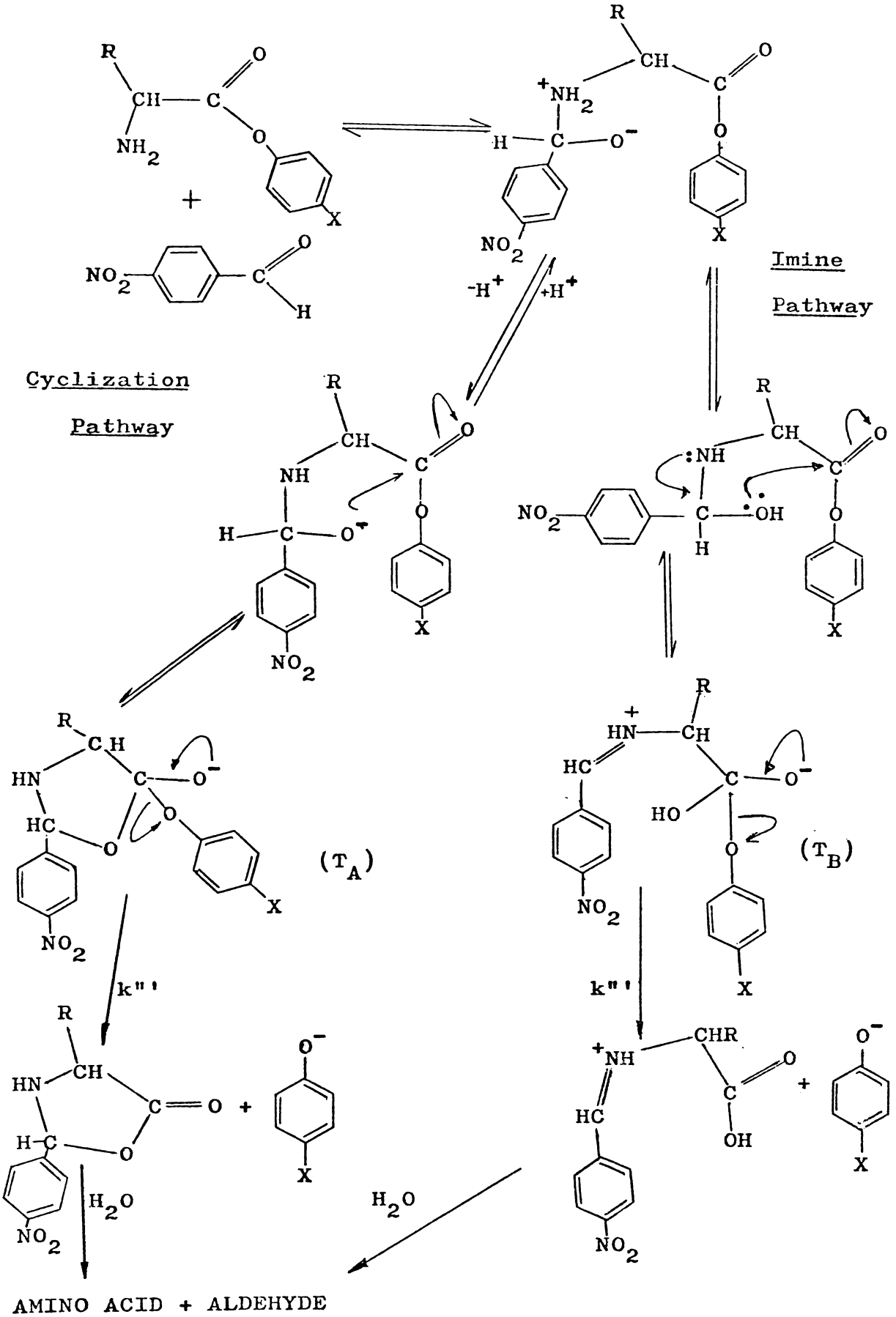


Diagram 6.1 Possible mechanistic paths for Aromatic Aldehyde catalysed hydrolysis of Amino Acid Esters.

s^{-1}, 4600 l mol^{-1} s^{-1} and 3800 l mol^{-1} s^{-1}), it is clear that 4-nitrobenzaldehyde catalysed hydrolysis is more sensitive to changes in substituents in the leaving group than OH^- catalysed reactions. This is clear from Figure 6.6, where the logs of the rate constants of the two processes have been plotted against each other, Table 6.7

Table 6.7 Data for the plot of log $k_2(NB)$ vs log $k_2(OH^-)$

Ester	$k_2(NB)$ (l mol^{-1} s^{-1})	log $k_2(NB)$	$k_2(OH^-)$ (l mol^{-1} s^{-1})	log $k_2(OH^-)$
Phenyl Acetates				
4-NO_2	-	-	34,000	4.53
H	-	-	4560	3.65
4-OCH_3	-	-	3780	3.58
Phenyl Glycinates				
4-NO_2	485	2.69	-	-
H	3.8	0.58	-	-
4-OCH_3	0.99	0.00	-	-

This high sensitivity to the nature of the leaving group is hardly to be expected if the formation of carbinolamine (by attack of the amino group of the ester on the catalyst) is rate determining. Indeed, the rates of formation of carbinolamine would be expected to be of the same order of magnitude for all three esters since their pK_b values are similar and if anything, the slight electron withdrawing effect of the distant nitro group of 4-nitrophenyl

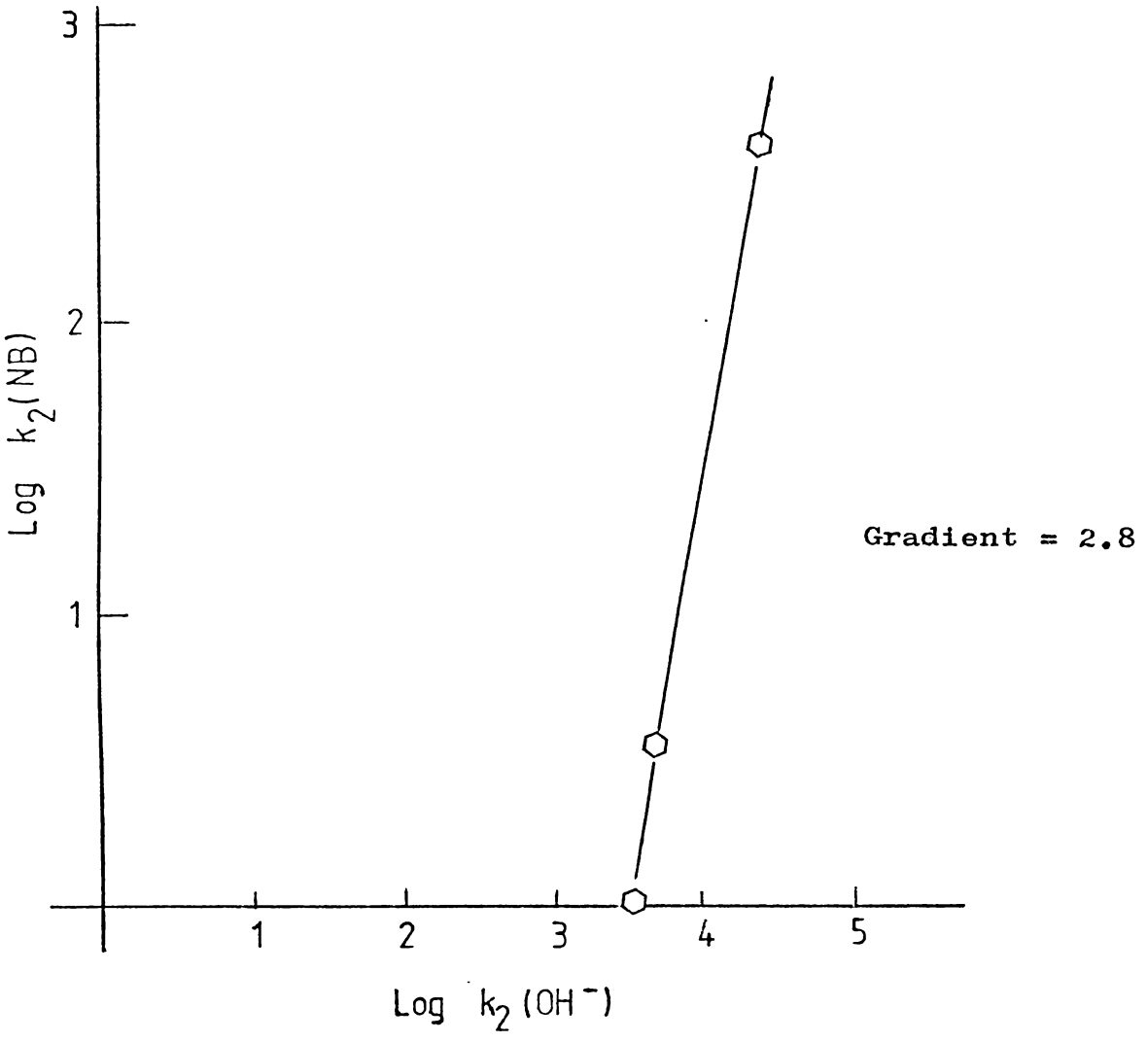


Figure 6.6 The plot of log k_2(NB) vs log k_2(OH^-).

substituted amino acid esters would be expected to hinder rather than facilitate carbinolamine formation.

In terms of rate limiting reaction of either of the carbinolamine intermediates, $C^-(T_A)$ or $C(T_B)$, the high sensitivity to leaving group is anticipated by comparison with the sensitivity in the case of the OH^- attack at the corresponding ester carbonyl function in the hydrolysis of phenyl acetates.

The results clearly support, then, rate limiting decomposition rather than formation of the carbinolamine. It is worth

noting that if decomposition is rate determining, the rate constant, k_2, from Equation 6.6 is given by k"'K. So values of k_2 depend on both the rate constant for decomposition (k"') and the equilibrium constant (K) for the formation of C_x. As noted above, the values of K would not be expected to vary markedly for the various esters and the major effect on rate differences must result from differences in k"'. Nevertheless, it should be realized that the linearity of the log k plot (Figure 6.6) might slightly improve if k"' were available in place of the composite k"'K values. In fact K for 4-nitrophenyl glycinate would be expected to be somewhat lower than the values for phenyl glycinate and 4-methoxyphenyl glycinate. Taking this into account, a plot of log k"', were the k"' values available, would probably give a better fit to a straight line.

6:1.3(a) Relative rates for 4-nitrobenzaldehyde catalysis of the hydrolysis of 4-nitrophenyl esters of glycine, valine and proline.

Catalytic rate constants, k_2, of about 480, 200 and 1600 $1\ mol^{-1}\ s^{-1}$ (30°C), for the esters of glycine, valine and proline, respectively, can be seen in Table 6.6. To rationalized the relative rates, it is necessary to recognize that if the decomposition of the carbinolamine is rate determining, these values represent k"'K (Equation 6.6) and the effect of the structure of the amino acid ester on rate must

be considered in light of both k"' and K.

(i) K: No literature values are available for the equilibrium constants for carbinolamine formation from amino acid esters. Values are available for the corresponding acids, but then only sporadically [88 - 90], (Table 6.8).

Table 6.8 Equilibrium constants for the reactions of some amino acids and aldehydes.

Amino acid	pK_a	Aldehyde	K ($l\ mol^{-1}$)
L - leucine		Formaldehyde	20
L - Alanine	9.86	"	21
DL- Valine	9.78	"	13
DL- Proline	10.68	"	76
L - Alanine		Pyridine-4-carboxaldehyde	2.1
Glycine		"	6.9

From those values listed above, one can estimate the very rough approximation;

$$5\ K(\text{4-nitrophenyl valinate}) = K\ (\text{4-nitrophenyl glycinate}) = K(\text{4-nitrophenyl prolinate}) \qquad 6.7$$

It must be noted that the sequence above is based on values for two different aldehydes, which are furthermore both quite

different from the catalyst in the present study.

It is even more tenuous, then, to extend this approximation to the amino acid esters, the latter, of course, not existing in zwitterionic forms. Nevertheless, the relative rates of catalysed hydrolysis of the ester will be briefly discussed taking the view that K for the valine ester may be significantly smaller than for the other two esters to see if a sensible interpretation results.

(ii) $k"'$: Experimentally, $k"'$ for the valine ester is about half that of the glycine ester. In terms of relative contributions of $k"'$ and K, this could be interpreted as follows. If K for the valine ester is about a fifth that of the glycine ester, then, $k"'$ must be about two and a half times larger for the valine ester than for the glycine ester. Is there any rationale for this ? The rate constant, $k"'$, represents the rate of reaction of either of the two carbinolamines (C and C^-) to release the 4-nitrophenolate ion, Diagram 6.1.

We commence the discussion of $k"'$ for the carbinolamines derived from the glycine and valine esters (primary amine function) and leave consideration of the proline case (secondary amine function) till later. According to the two mechanisms already suggested (Section 6:1.2) what is required for carbinolamine decomposition to products is reaction of the carbinolamine oxygen with the ester carbonyl group, either the oxygen of the neutral carbinolamine (C) in the so called imine pathway (Diagram 6.2) or the anionic oxygen of the anionic carbinolamine (C^-) in the cyclization pathway (Diagram 6.3).

We are concerned then with electronic and steric effects of

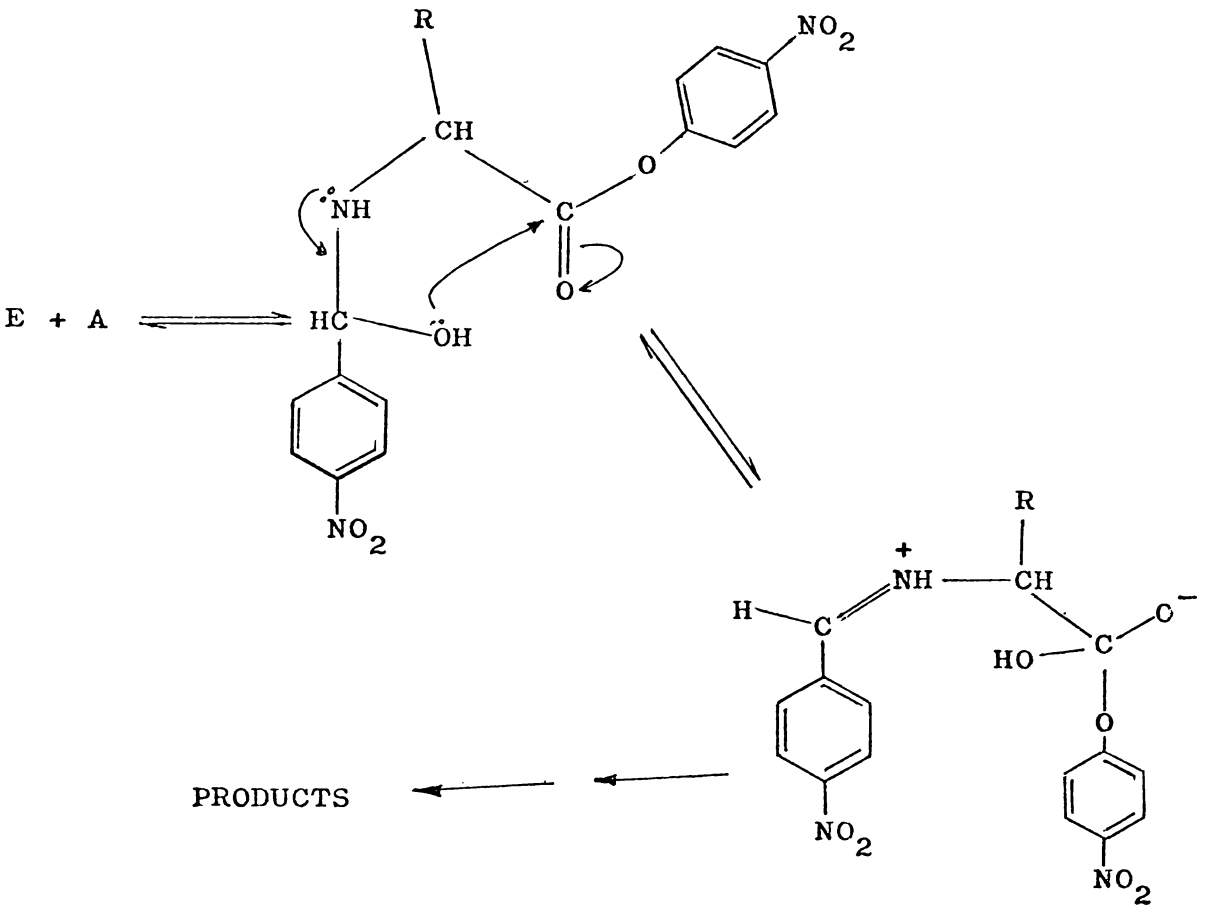


Diagram 6.2 The decomposition of the neutral carbinolamine C via the Imine Pathway.

the group R (R = H for the glycinate case, R = $CH(CH_3)_2$ for the valinate case) which might promote the reactions outlined in Diagram 6.2 and 6.3.

Consider the inductive effect of the alkyl group in the valine case. It is clear that although the +I effect will increase the nucleophilic power of the oxygen function (and also stabilize the resulting cationic imine centre in the imine pathway case), the effect will also reduce the electrophilicity of the ester carbonyl. It is impossible, then, to

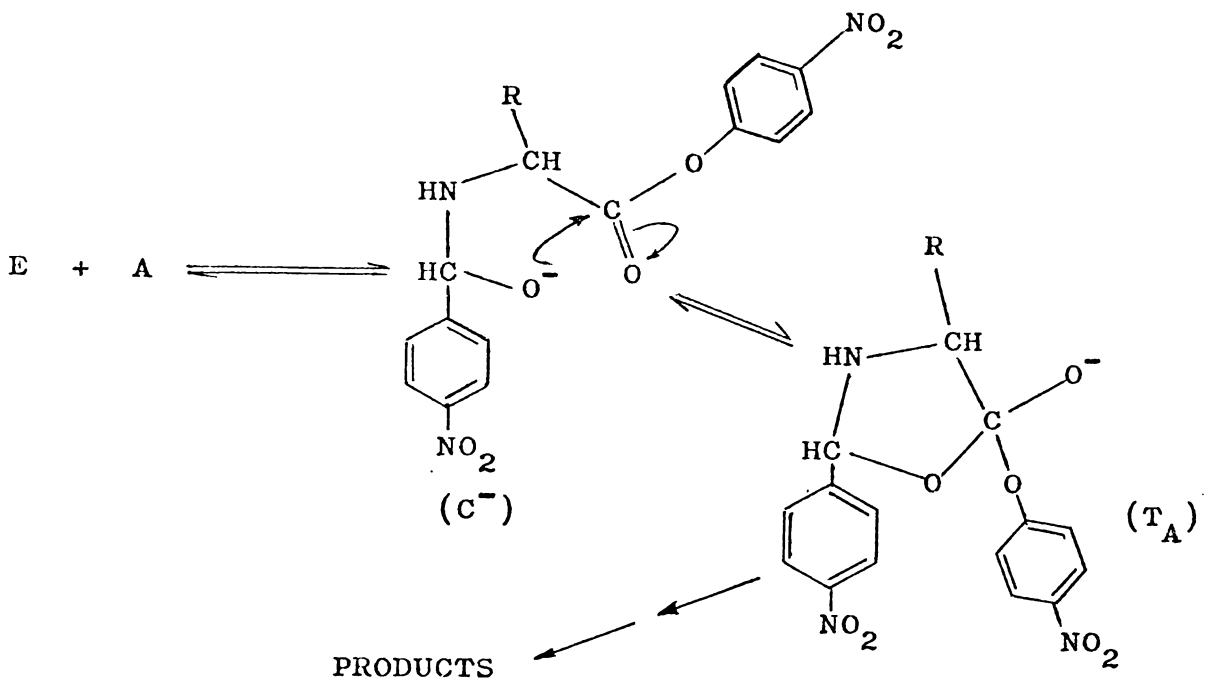


Diagram 6.3 The decomposition of the anionic carbinolamine C^- via the Cyclization Pathway.

assess the nett effect of the alkyl substituent on the value of k"' and impossible to predict whether the inductive effect will increase or decrease k"' for the valine case as compared with the glycine case. The second factor to consider, the steric effect, gives a more clearcut interpretation. As suggested by Main [92], the most favoured conformation about the N—C^{α} bond of the carbinolamine from the glycine ester would be one in which, as a result of steric repulsion, the bulky groups are at a dihedral angle of near 180° (Diagram 6.4) a conformation in which the very groups which are required to react are geometrically inaccessible to each other. For the valine ester, on the other hand, a conformation of the type shown in Diagram 6.5, becomes relatively much more stable and it is from such a conformation that ester hydrolysis can ensue.

It is reasonable to expect then that the value of k"' for the valine case might be significantly larger than that for the

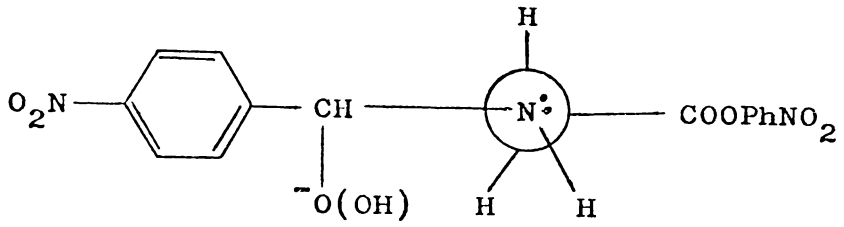


Diagram 6.4 The likely conformation for the carbinolamine of the glycine ester.

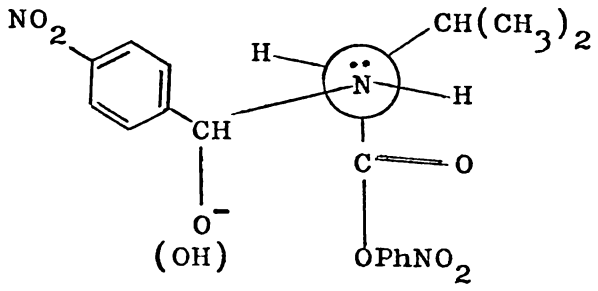


Diagram 6.5 One likely conformation for the carbinolamine of the valine ester.

glycine case simply on the basis of conformation population considerations. Since the overall rate constant (k"'K) for the valine ester is about half that of the glycine ester (Table 6.6) and assuming that K for the valine ester is about one fifth that of the glycine case , it can be deduced that k"' is two and a half times greater for the valine carbinolamine species than for the glycine one, a result consistent with the deduction above based on the conformation populations. This analysis then is self consistent, but it is, as mentioned earlier rather precariously based on crude estimations of K

values and it is limited, as discussed above, by a lack of knowledge of the nett result of differences in inductive effects.

Coming now to a comparison of the overall rate constants for the glycine and proline esters, we find that the proline ester is about four times more reactive than the glycine ester (Table 6.6). If the values of the equilibrium constant K are taken to be about the same (as discussed earlier) then the value of k"', the rate constant for decomposition of the carbinolamine is about four times greater for the proline ester than for the glycine ester. The proline carbinolamine species responsible for ester hydrolysis (C or C^-) is of course subject to cis - trans isomerism, associated with inversion at nitrogen (Diagram 6.6) and it is only from the cis isomer that ester hydrolysis could proceed. The cis isomer is expected to be less stable than the trans form. In spite of this unfavourable isomerism aspect, we estimate k"' to be larger than for the glycine case. It should be noted that once the cis isomer is attained the reacting groups (Diagran 6.6) are fairly rigidly held, as a result of the proline ring system, in such proximity as to allow rapid reaction. Presumably, the high reactivity of the cis isomer is sufficient, in spite of its small percentage, to ensure a nett rate of decomposition for the proline carbinolamine greater than that for the glycine case. Inversion at N is known to be a rapid reaction so this could have no bearing on the overall reaction rate.

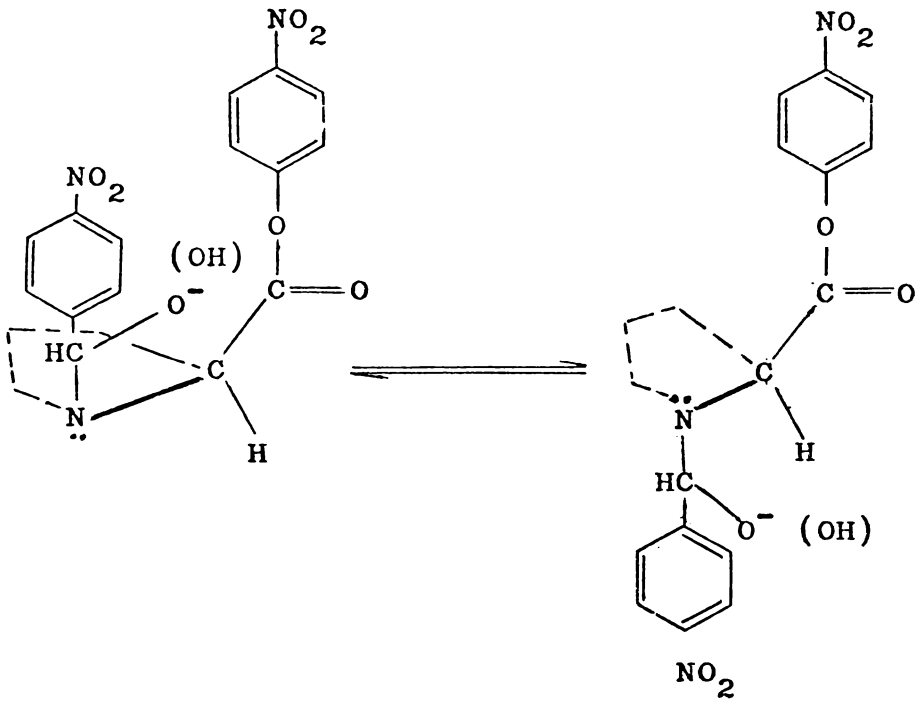


Diagram 6.6 The cis - trans isomerism of the carbinolamine of 4-nitrophenyl prolinate.

6:2 Temperature variation study

The temperature variation study on the catalysed hydrolysis of 4-nitrophenyl valinate is interesting as well as providing evidence for a two or multi steps process in which the overall rate constant, k_2, is of the form k"'K, as shown in Equation 6.6. From Table 6.6, we have k_2 at 30°C= 200 ± 18 1 mol^{-1} s^{-1} and at 25°C = 190 ± 15 1 mol^{-1} s^{-1}. These two results show clearly how little temperature variation affects the overall rate constant. This would be unusual for a single step reaction but not necessarily so for a two-step or multi-step reaction where a pre-equilibrium exists.

It will be consistent with our results if the composite rate constant, k"'K, is such that the increase in k"' with temperature is being compensated by a decrease in the equilibrium constant, K. It is well known that equilibrium constants can decrease with increasing temperature (Johnson [2]). In the case of carbinolamine formation between formaldehyde and glycine (Ionic strenght= 1 mol l^{-1}), a value of K = 6.9 1 mol^{-1} at 25° was reported by Sander and Jencks [90] and a value of K = 3.8 1 mol^{-1} at 30° was reported by French and Bruice [89] a drop of nearly half for a 5°C rise in temperature. We believe that a similar decrease could easily apply for the equilibrium constant, K, for the carbinolamine formation between the valine ester and 4-nitrobenzaldehyde.

In fact, this is the only logical interpretation that can be placed on the observation that the overall rate constant k_2 increases only slightly with temperature. We suggest that the expected increase in k"' with temperature is nearly compensated by a decrease in the equilibrium constant K. This supports the concept that the decomposition of the carbinolamine species rather than its formation, is the rate determining step.

Section 7 The Imidazole Catalysed Hydrolysis of Phenyl Glycinate.

7:1 Introduction.

The imidazole catalysed hydrolysis of 4-nitrophenyl acetate had been extensively studied(for reviews, see section 1:1 and 1:2). There is one brief report of a similar study for amino acid esters in which the suggestion is made that imidazole could catalyse the hydrolysis of 4-nitrophenyl glycinate via an N-acylimidazole intermediate [39]. Kinetic studies showed a first order dependence on imidazole in that study.

7:1.1 Results

Our results show that a plot of k_h vs $[Im]_n$ is non-linear(Table 7.1 and Figure 7.1) where k_h is the observed rate constant and $[Im]_n$ the neutral imidazole species, respectively. However, the plot of $k_h/[Im]_n$ vs $[Im]_n$ gave a linear relationship(Figure 7.2), implying a second order dependence on the imidazole but small non-zero intercepts also suggested some contributions by a first order term in imidazole in the overall kinetic form. The analysis on which these inferences are based is in the following section.

7:1.2 Analysis

The kinetic results indicated that the neutral imidazole molecule is the catalytic species, as observed by Jencks and Gilchrist [111] and Bruice and Benkovic[11] for the catalysed hydrolysis of 4-nitrophenyl acetate. The experimental rate

Table 7.1 Data and results of the plots $k_h/[Im]_n$ vs $[Im]_n$.

Temperature 30°C

Error for k_h ± 5% (Ionic strength = 1 mol l^{-1})

Error for $k_h/[Im]_n$ ± 7%

pH	$[Im]_{TOTAL}$ (mol l^{-1})	$[Im]_n$ (mol l^{-1})	10^3 k_h (s^{-1})	10^3 $k_h/[Im]_n$ (1 mol^{-1} s^{-1})
8.0	0.4	0.345	32	92.7
	0.4	0.345	31.4	91.0
	0.2	0.173	8.3	47.9
	0.2	0.173	8.0	46.2
	0.15	0.129	4.7	36.4
	0.05	0.043	0.8	18.3
		Intercept	4 ± 1	1 mol^{-1} s^{-1}
		Gradient	0.25 ± .02	l^2 mol^{-2} s^{-1}
7.65	0.48	0.354	44.8	130
	0.45	0.332	39.8	120
	0.35	0.258	25.6	99.3
	0.35	0.258	24.8	96.1
	0.3	0.221	18.1	82.0
	0.22	0.162	10.0	61.7
	0.048	0.0354	0.64	18.5
		Intercept	6 ± 1.2	1 mol^{-1} s^{-1}
		Gradient	0.35 ± .02	l^2 mol^{-2} s^{-1}
7.0	0.6	0.232	29.2	126
	0.45	0.174	18.6	107
	0.4	0.155	14.2	91.8
	0.35	0.135	10.3	76.3
	0.3	0.116	7.8	67.6
	0.2	0.077	3.98	51.5
		Intercept	12 ± 2.5	1 mol^{-1} s^{-1}
		Gradient	0.51 ± .03	l^2 mol^{-2} s^{-1}

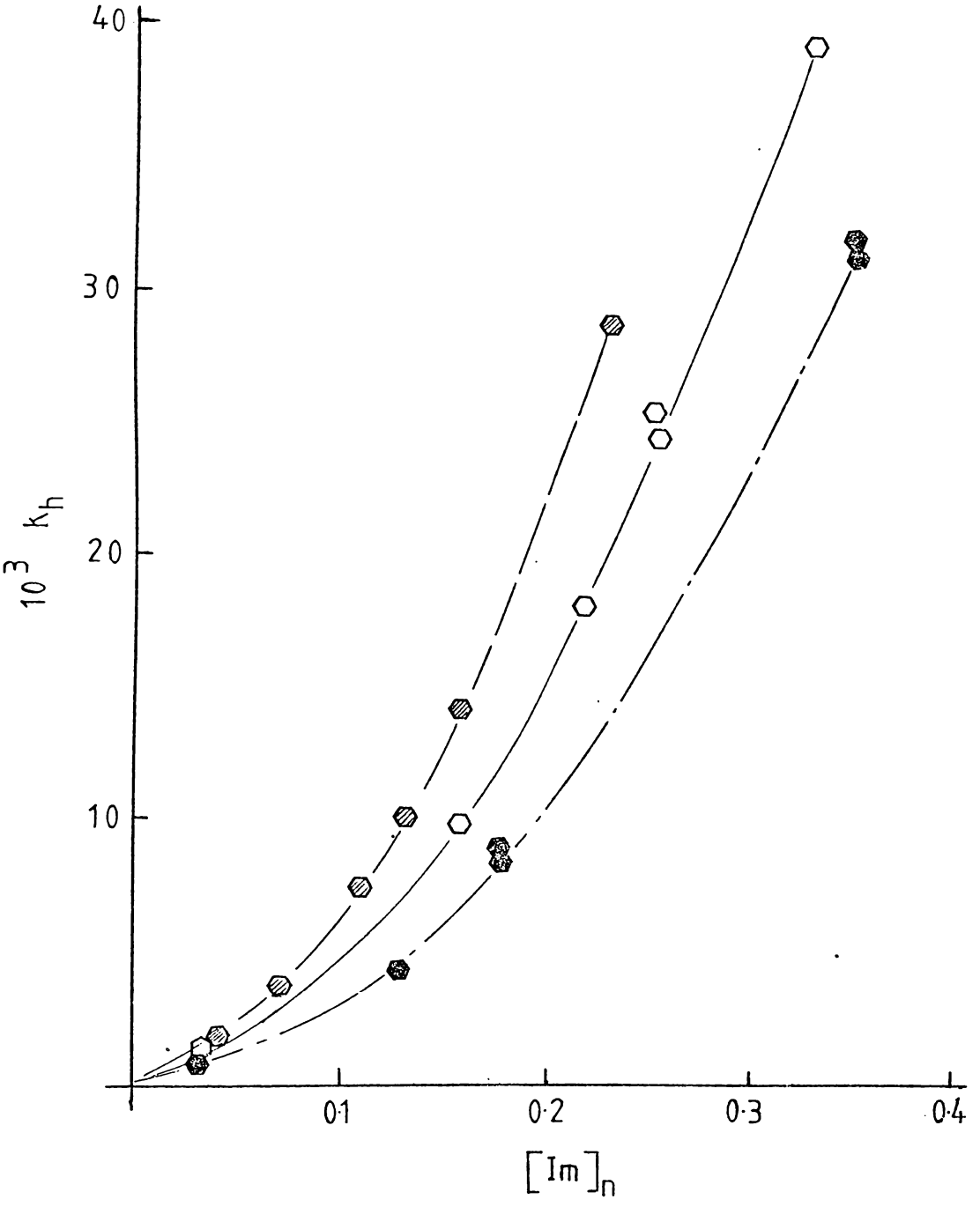


Figure 7.1 The plots of k_h vs $[Im]_n$

(a) pH = 7.0

(b) pH = 7.65

(c) pH = 8.0

equation which incorporates both first and second imidazole terms is expressed by Equation 7.1.

$$k_h [E_T] = k_1[E][Im]_n + k_1'[EH^+][Im]_n + k_2[E][Im]_n^2 + k_2'[EH^+][Im]_n^2 \qquad 7.1$$

where; k_1 and k_2 are rate constants for first and second order terms in imidazole, respectively, with the neutral phenyl glycinate (E).

k_1' and k_2' are rate constants for first and second order terms in imidazole, respectively, with the protonated phenyl glycinate (EH^+).

[E], $[EH^+]$ and $[E_T]$ are the concentrations of neutral, protonated and total phenyl glycinate in the solution.

Rearranging Equation 7.1,

$$k_h = (k_1[E]/[E_T] + k_1'[EH^+]/[E_T])[Im]_n + (k_2[E]/[E_T] + k_2'[EH^+]/[E_T])[Im]_n^2$$

i.e.,

$$k_h/[Im]_n = k_1 f_E + k_1' f_{EH^+} + (k_2 f_E + k_2' f_{EH^+})[Im]_n \qquad 7.2$$

where f_E and f_{EH^+} are fractions of neutral and protonated phenyl glycinate, respectively, calculated at each pH with the pK(ester) value of 7.15 ± .05(from Section 4 of this thesis). The concentration of neutral imidazole is calculated with the pK_a value of 7.14[112]

From Equation 7.2, a plot of $k_h/[Im]_n$ vs $[Im]_n$ gives the intercept value equal to the expression $k_1 f_E + k_1' f_{EH^+}$.

$$\text{Let } \sigma = k_1 f_E + k_1' f_{EH^+} \qquad 7.3$$

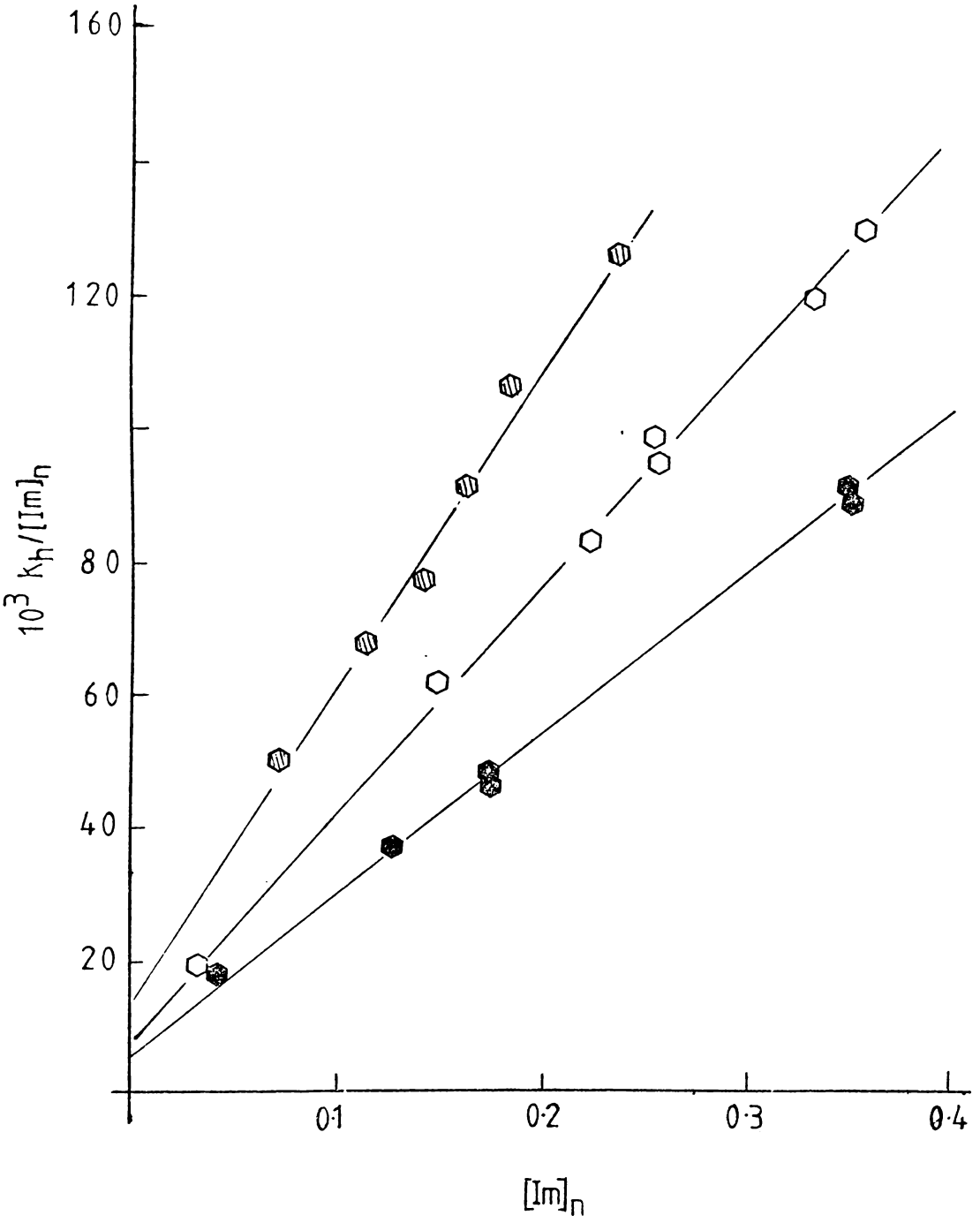


Figure 7.2 The plots of $k_h/[Im]_n$ vs $[Im]_n$

(a) pH = 7.0

(b) pH = 7.65

(c) pH = 8.0

Also, $f_E + f_{EH^+} = 1$ 7.4

From Equations 7.3 and 7.4, we can show that,

$$\sigma = k_1(1-f_{EH^+}) + k_1' f_{EH^+} \quad 7.5$$

Rearranging;

$$\sigma = k_1 + (k_1'-k_1) f_{EH^+} \quad 7.6$$

Therefore a plot of σ vs f_{EH^+} gives the intercept k_1 and a gradient of $(k_1'-k_1)$ (Table 7.2 and Figure 7.3).

Table 7.2 Data for and results from the plot of σ vs f_{EH^+} to evaluate the rate constants k_1 and k_1'.

pH	$10^3\ \sigma$ ($1\ mol^{-1}s^{-1}$)	f_{EH^+}	$10^3\ k_1$ ($1\ mol^{-1}s^{-1}$)	$10^3\ k_1'$ ($1\ mol^{-1}s^{-1}$)
8.0	4 ± 1	0.112		
7.65	6 ± 1.2	0.22		
7.0	12 ± 2.5	0.557	2.4 ± 0.5	19 ± 3

Similarly, from Equation 7.2, the plot of $k_h/[Im]_n$ vs $[Im]_n$ gives a gradient equal to the expression

$$k_2 f_E + k_2' f_{EH^+}$$

$$\text{Let } \delta = k_2 f_E + k_2' f_{EH^+} \quad 7.7$$

Using Equations 7.4 and 7.7, we can show that

$$\delta = k_2 + (k_2'-k_2) f_{EH^+} \quad 7.8$$

A plot of δ vs f_{EH^+} (Figure 7.4) from the values found in Table 7.3 gives the intercept k_2 and gradient $(k_2'-k_2)$.

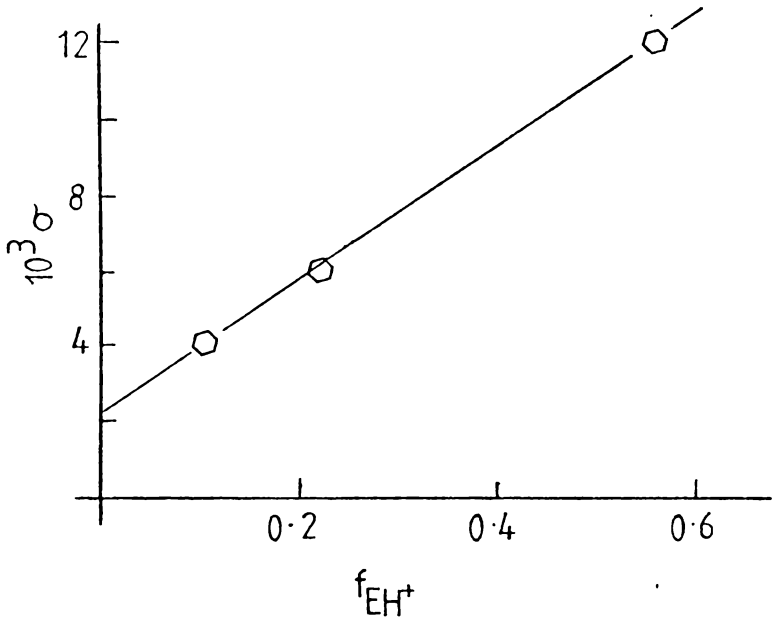


Figure 7.3 The plot of σ vs f_{EH^+}

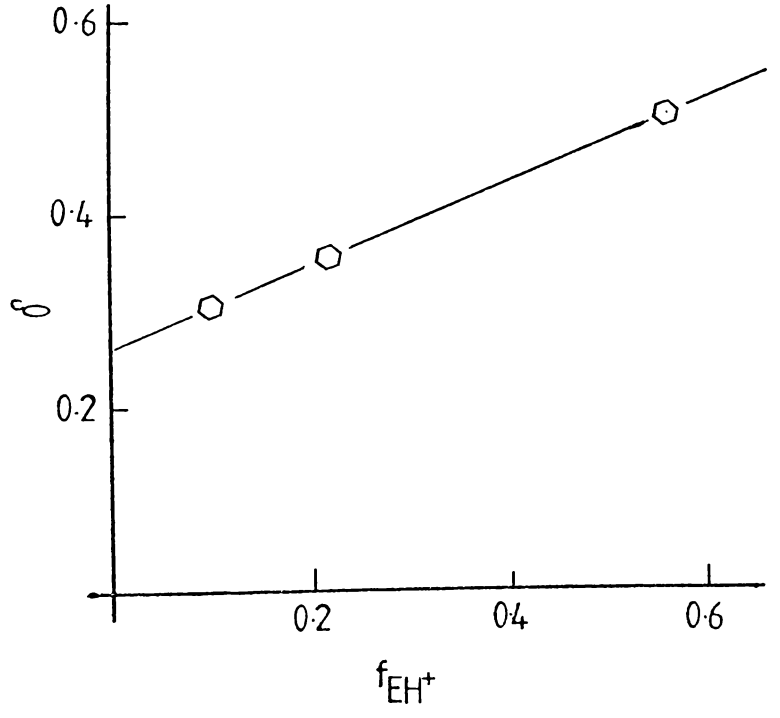


Figure 7.4 The plot of δ vs f_{EH^+}

Since k_2 is known, therefore k_2' can be evaluated (Table 7.3).

Table 7.3 Data for and results from the plot of δ vs f_{EH^+} to evaluate the rate constants k_2 and k_2'.

pH	δ (l^2 $mol^{-2}s^{-1}$)	f_{EH^+}	k_2 (l^2 mol^{-2} s^{-1})	k_2' (l^2 $mol^{-2}s^{-1}$)
8.0	0.25 ± .02	0.112		
7.65	0.35 ± .0"	0.22		
7.0	0.51 ± .03	0.557	0.2 ± .015	0.75 ± .05

7.2 Discussion

As discussed in their papers, Bruice and Benkovic[11] suggested the general equation for imidazole catalysed hydrolysis of 4-nitrophenyl acetate and phenyl acetate shown in Equation 7.9.

$$\text{Rate} = k_n[E][Im]_n + k_{gb}[E][Im]_n^2 \qquad 7.9$$

where; k_n is the nucleophilic rate constant representing nucleophilic catalysis by imidazole.

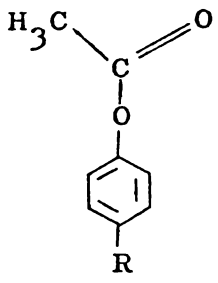
k_{gb} corresponds to the term second order in imidazole representing additional base catalysis by a second imidazole molecule.

It was found that in the catalysed hydrolysis of phenyl and 4-chlorophenyl acetate[11], the second kinetic term(k_{gb}) in Equation 7.9 was not detectable and the rate equation reduces to Equation 7.10.

$$\text{Rate} = k_n[E][Im]_n \qquad 7.10$$

Both terms in Equation 7.9, however, did apply for esters with poorer leaving groups such as 4-methoxyphenyl and 4-methylphenyl acetates (Table 7.4).

Table 7.4 Rate constants for the imidazole catalysed hydrolysis of some Phenyl Acetates [11].

Ester	R	$10^3 k_n$ ($l\ mol^{-1}s^{-1}$)	$10\ k_{gb}$ ($l^2 mol^{-2}s^{-1}$)
	H	16	-
	CH_3	5.5	1.8
	OCH_3	5.5	1.6
	Cl	26.7	-

In our analysis (Section 7:1.2), we found that the imidazole catalysed hydrolysis of phenyl glycinate (unlike phenyl acetate[11]), involved a term second order in imidazole and indeed this far outweighed the minor contribution by a term first order in imidazole (Table 7.2 and 7.3).

The aim of this discussion is to see how the term second order in imidazole arises and to explain why it is important

for phenyl glycinate(and not so for 4-nitrophenyl glycinate, an ester with a better leaving group[39]) when it is not so for phenyl acetate.

First we have to resolve the reason for the existence of the term second order in imidazole. This cannot be associated with the involvement of the second imidazole in decomposition of an N-acyl intermediate. Consider the reaction scheme, Diagram 7.1, discussed by Jencks and Satterthwait[110] to account for tertiary amine catalysed hydrolysis of phenyl acetates but here applied to the imidazole catalysed hydrolysis of phenyl glycinate.

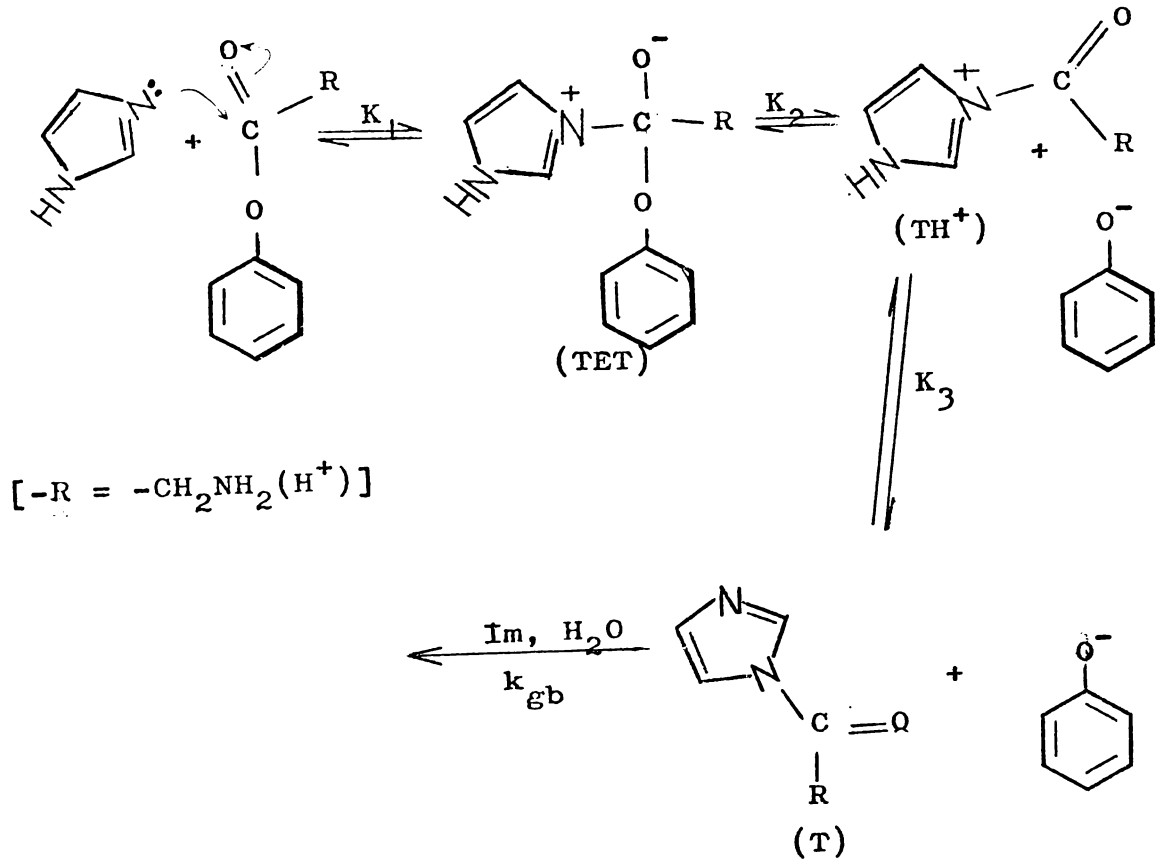


Diagram 7.1 Proposed mechanism to show how the second imidazole molecule might be involved in the rate determining decomposition of an N-acyl intermediate.

Assuming that the term second order in imidazole is associated with the catalysis of the rate determining hydrolysis of the N-acyl intermediate, we expect that the overall rate would be depressed because of the increasing concentration of the phenolate ion(PhO^-) before the second imidazole can participate in the hydrolysis.

Mathematically, we can show that imidazole is not involved in the catalysis of the rate determining hydrolysis of N-acyl intermediate(T in Diagram 7.1).

From Diagram 7.1, we have the following relationships:

$$[TET] = K_1[E][Im]_n \qquad 7.11$$

$$[TH^+] = K_2[TET]/[PhO^-] \qquad 7.12$$

Substituting 7.11 into 7.12

$$[TH^+] = K_2K_1[E][Im]_n/[PhO^-] \qquad 7.13$$

but $$[T] = K_3[TH^+]/[H^+] \qquad 7.14$$

Substituting 7.13 into 7.14, we have,

$$[T] = K_3K_2K_1[E][Im]_n/[H^+][PhO^-] \qquad 7.15$$

If the second imidazole molecule is associated with the rate determining decomposition of the N-acyl intermediate (T), the overall rate is given by Equation 7.16.

$$\text{Rate} = k\,[T][Im]_n \qquad 7.16$$

Substituting 7.15 into 7.16 we have,

$$\text{Rate} = kK_3K_2K_1[E][Im]_n^2/[H^+][PhO^-] \qquad 7.17$$

Now, Equation 7.17 shows that the overall rate would be depressed by the increasing concentration of the phenolate ion. This was not observed in our experiments. Therefore, we believed that the term second order in imidazole cannot be associated with the rate determining breakdown of T in

Diagram 7.1. The second imidazole molecule must be involved in the formation not in the decomposition of the N-acyl intermediate and we can now consider as alternative general basic function of imidazole in the mechanism given in Diagram 7.2

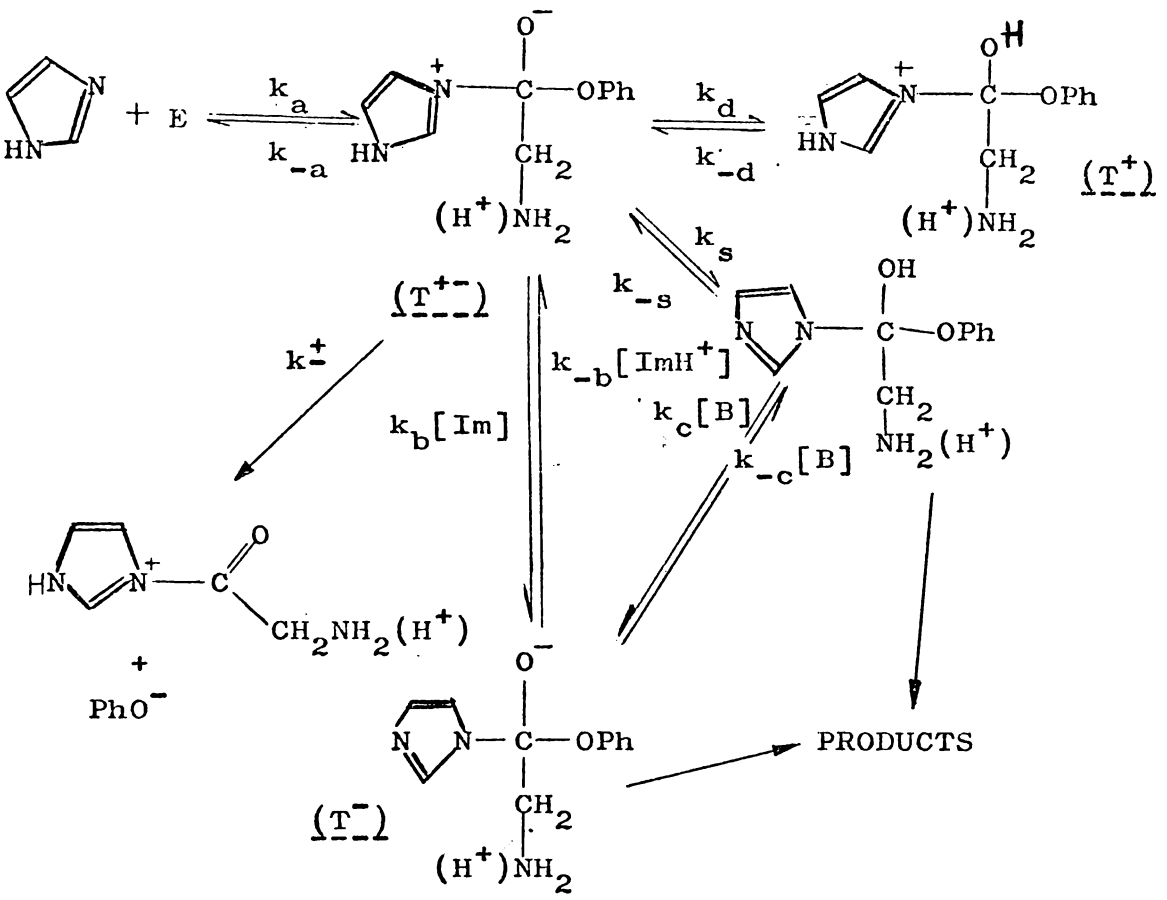


Diagram 7.2 General scheme for catalysis by imidazole of the hydrolysis of phenyl glycinate. (Based on the scheme given by Jencks and Satterthwait to account for tertiary amine catalysed hydrolysis of phenyl acetates[110]).

This mechanism requires that the formation of the zwitterionic intermediate T^{+-} is rapid and reversible. T^{+-} can either

break down directly in a rate determining step(rate constant $k^{\pm}$) to the N-acyl intermediate giving an overall process first order only in imidazole or it can react in an alternative rate determining step with another imidazole molecule ($k_b[B]$) to form another intermediate T^-(by proton removal from T^{+-}) preventing T^{+-} from rapidly reverting to starting materials. This essentially traps the zwitterion T^{+-}.

In fact, T^{+-} could in principle also be trapped by protonation by general acids to T^+(rate constant k_d) but there is no evidence for such involvement by protonated imidazole in our experiments.

Hence, where the term first order in imidazole is involved, the reaction can be accounted for by direct release of PhO^- from the intermediate T^{+-} and the rate can therefore be expressed for the neutral ester as,

$$\text{Rate} = k^{\pm}[T^{+-}] = k^{\pm}k_a/k_{-a}[E][Im]_n \qquad 7.18$$

For the protonated phenyl glycinate, the rate is correspondingly expressed as,

$$\text{Rate} = k^{\pm}k_a'/k_{-a}'[EH^+][Im]_n \qquad 7.19$$

For the term second order in imidazole, for which we think the process involves rate determining proton abstraction from T^{+-} by an imidazole molecule, the rate is given by,

$$\begin{aligned}\text{Rate} &= k_b[Im]_n[T^{+-}] \\ &= k_b k_a/k_{-a}[E][Im]_n^2 \qquad 7.20\end{aligned}$$

For the protonated phenyl glycinate, the rate is likewise expressed as,

$$\text{Rate} = k_b' k_a'/k_{-a}'[EH^+][Im]_n^2 \qquad 7.21$$

We are now in the position to ask why the hydrolysis of phenyl glycinate (both protonated and neutral forms) is dominated by kinetic terms second order in imidazole (Equations 7.1,7.20 and 7.21, Tables 7.2 and 7.3), whereas only a term first order in imidazole applies for phenyl acetate(Equation 7.10 and Table 7.4).

To interpret this difference, we need to know and consider the effect of electron withdrawing acyl substituents in the ester species on the relative contributions of the two competing pathways for the reactions of T^{+-}(Diagram 7.3).

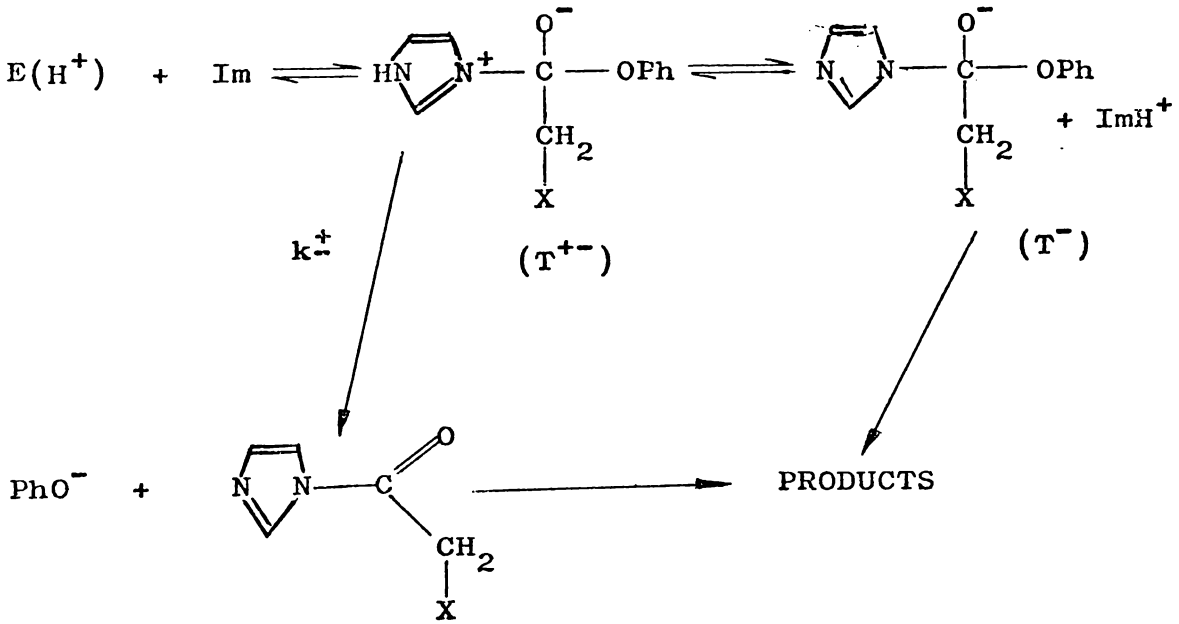


Diagram 7.3 Direct break-down of $T^{+-}(k_{\pm}^{\pm})$ vs rate determining proton abstraction from T^{+-} by imidazole($k_b[Im]$) for phenyl glycinate($X = NH_2$) and its protonated form ($X = {}^{+}NH_3$).

The first pathway ($k^{\pm}$) corresponds to first order imidazole involvement and the second pathway ($k_b[Im]$) corresponds to a general base catalysed process with a term second order in imidazole. From Tables 7.2 and 7.3, the results show that $k_b[Im] > k^{\pm}$ for the catalysed hydrolysis of phenyl glycinate. It is necessary to ask:

(a) whether k_b should be larger for X = NH_2 or NH_3^+ than for X = H

(b) whether $k^{\pm}$ should be smaller for X= NH_2 or NH_3^+ than for X = H

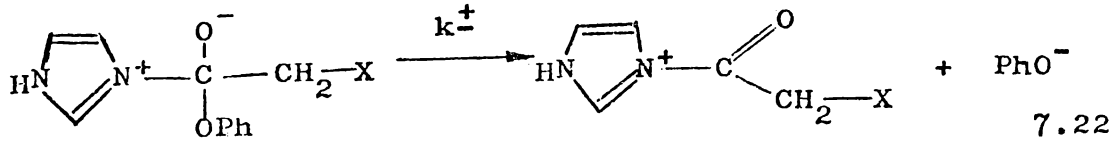
Either of the two effects (a and or b) would serve to promote the term second order in imidazole in the phenyl glycinate kinetic form. We consider the points in turn.

(a) Effect of substituents X on the magnitude of k_b.

It is likely that the rate constant, k_b, would be increased by inductive electron withdrawing by X (= NH_2 and NH_3^+ , in the case of phenyl glycinate species), as it would make the N-H proton abstracted from imidazole moiety of T^{+-} more acidic and thus more readily removed than the case of phenyl acetate[11]. The proton abstraction process, in the case of phenyl acetate, does not effectively compete with the direct break-down of T^{+-} to products ($k^{\pm}$ term). The effect of acyl substituents of phenyl glycinates, then, is to promote the second order imidazole term at the expense of the first order term.

(b) Effect of substituents on the magnitude of k^{+-}.

It is likely that the rate constant $k^{\pm}$ would be smaller for X = NH_2 or NH_3^+ than for X = H (Equation 7.22),



7.22

because electron withdrawal will make the loss of the phenolate ion more difficult. The $\rangle C-OPh$ bond is harder to break to release PhO^- if there is an electron withdrawing group, X, adjacent to it, as in $X-CH_2-C-OPh$. The value of $k_{\pm}^{+}$ could be expected to be significantly higher for phenyl acetate than for phenyl glycinate. These interpretations then are consistent with the first order imidazole term being more important for phenyl acetate and the second order term more important for phenyl glycinate.

We can now compare the results in Tables 7.2 and 7.3. It is to be expected that $k_1' > k_1$ because the carbonyl centre of protonated phenyl glycinate is more reactive to the attack of the nucleophile (imidazole) than the carbonyl centre of the neutral phenyl glycinate, as in the trend, $k(EH^+) > k(E)$, for the alkaline hydrolysis of ethyl glycinate [71]. However, the uncertainty in k_1 and k_1' values make interpretation and comparison quite difficult. We can, however, compare the magnitudes of k_2 and k_2' (Equation 7.1 and Table 7.3) in terms of electron withdrawal effects of $X = NH_2$ and $X = NH_3^+$ and other possible factors (such as steric congestion) that may be associated with the rate-determining general base catalysed process of proton abstraction from T^{+-} (rate constant $k_b[Im]_n$).

From Equations 7.1, 7.20 and 7.21 for the neutral phenyl glycinate,

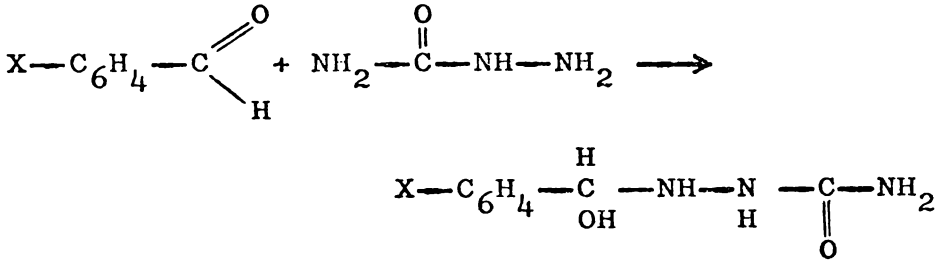
$$k_2 = k_b\, k_a/k_{-a} \qquad 7.23$$

and the protonated phenyl glycinate,

$$k_2' = k_b'\, k_a'/k_{-a}' \qquad 7.24$$

In terms of the electron withdrawing effect, the order of equilibrium constants, $k'_a/k'_{-a} > k_a/k_{-a}$, is to be expected because of a larger -I effect shown by $X = NH_3^+$ over $X = NH_2$. Electron withdrawing acyl substituents are known to facilitate nucleophilic addition at the carbonyl groups (Table 7.5 [113]).

Table 7.5 Equilibrium constants for Semicarbazone formation for a series of substituted benzaldehyde (25°C).



X	Compound	Equilibrium Constants (1 mol^{-1})
$4\text{-}OCH_3$	4-methoxybenzaldehyde	0.34
$4\text{-}CH_3$	4-methylbenzaldehyde	0.62
H	benzaldehyde	1.32
4-Cl	4-chlorobenzaldehyde	4.14
$4\text{-}NO_2$	4-nitrobenzaldehyde	40.1

The order of the rate constants $k'_b > k_b$ is also to be expected because of the larger -I effect operating here which indirectly increases the proton acidity, more effectively for the more strongly electron withdrawing group NH_3^+ than for NH_2.

For the overall rate constants, k_2 and k_2' (Equation 7.20 and 7.21), we would therefore expect the order $k_2' \gg k_2$. Our results show that k_2' is larger than k_2 by a factor of about four which seems a rather small enhancement in terms of electron withdrawing effects. However, if the intermediate for the protonated phenyl glycinate, T^{+-+}(Diagram 7.4), instead of transfering the imidazole proton to a second imidazole molecule, rapidly undergoes intramolecular proton transfer from the protonated amine to the oxyanion to give T^+ before the second imidazole can participate in the rate

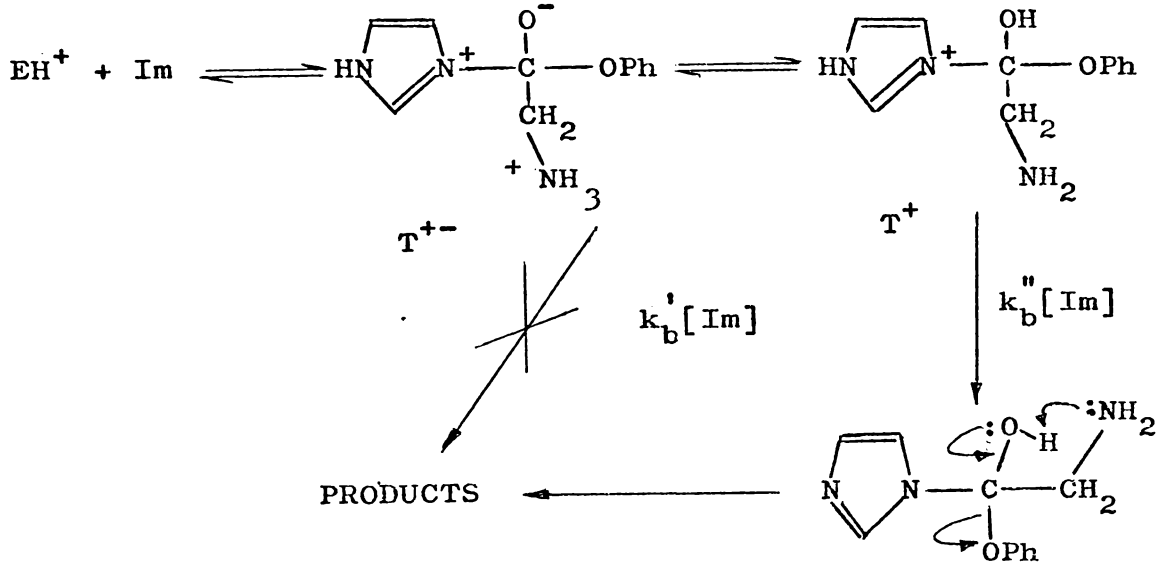


Diagram 7.4 Possible mechanism for imidazole catalysed hydrolysis of protonated phenyl glycinate.

determining proton transfer step(k_b''[Im] term in Diagram 7.4), then the experimentally determined k_2' value is going to reflect k_b'' (instead of k_b') for the phenyl glycinate cation ($X = NH_3^+$) whereas for the neutral phenyl glycinate the

normal $k_b[Im]$ term will apply.

What the relative values of k_b' and k_b'' might be is difficult to assess. Although k_b'' is certainly favoured as it involves conversion of a cationic to neutral species, there will also be significant differences in solvation and other effects for T^{+-} and T^{+}.

In conclusion, there is no simple explanation for the small value of the ratio k_2'/k_2. Clearly, it does suggest less effective catalysis by the second molecule of imidazole for the protonated phenyl glycinate but why this is so is less clear.

The final point to consider is why the term second order in imidazole, though important for catalysis of phenyl glycinate hydrolysis, is entirely absent from the kinetic form for 4-nitrophenyl glycinate [39]. According to Diagram 7.5, the zwitterionic tetrahedral intermediate formed(T^{+-}) can either decompose directly to N-acylimidazole releasing the

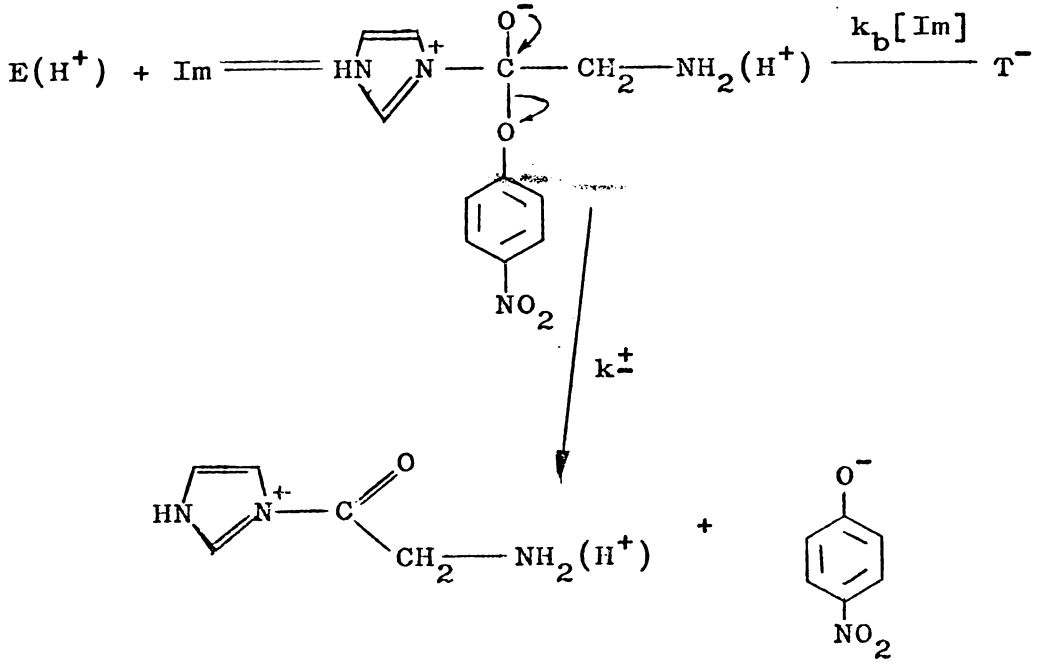


Diagram 7.5 Decomposition of T^{+-} for the catalysed hydrolysis of 4-nitrophenyl glycinate.

4-nitrophenolate ion ($k_{\pm}^{\pm}$ rate constant) or it can as in the phenyl glycinate case, require another imidazole to abstract a proton to form an anionic tetrahedral intermediate, T^{-}. It is clear that the latter process (second order term in imidazole) must not be significant for the 4-nitrophenyl glycinate. There are two possible explanations to account for this result. Either (a) formation of T^{+-} is now rate determining or (b) $k_{\pm}^{\pm}$ is so large relative to k_b (from $k_b[Im]$ pathway) that T^{+-} decomposes releasing 4-nitrophenolate ion without further base catalysis.

We consider these aspects in turn.

(a) Kirsch and Jencks [10] suggested that for imidazole catalysed hydrolysis of phenyl acetates, the attack of the nucleophile (imidazole) is rate determining if the leaving group is good. If the trend carries over to phenyl glycinates, it is feasible that the formation of T^{+-} is rate determining for 4-nitrophenyl glycinate but not for phenyl glycinate which has a poorer leaving group.

(b) Direct decomposition of T^{+-} ($k_{\pm}^{\pm}$ term) will obviously be faster for esters with better leaving groups. Therefore $k_{\pm}^{\pm}$ will clearly be much larger for the hydrolysis of 4-nitrophenyl glycinate than for phenyl glycinate. In the case of the latter, $k_{\pm}^{\pm}$ may be so small that proton abstraction occurs before the release of phenolate ion is possible, thus allowing the appearance of the kinetic term second order in imidazole.

Overall, then, our result are consistent with the view that imidazole is acting both as nucleophile (first stage) and as a general base (second stage) in the hydrolysis of phenyl glycinate for which a term second order in imidazole predominates. For 4-nitrophenyl glycinate, imidazole still acts as a nucleophile in the first step to form a tetrahedral

intermediate. Either the formation or direct decomposition of this intermediate may be rate determining depending on which is the slower step, but further base catalysis by imidazole is unnessary where the good 4-nitrophenolate leaving group is involved.

Section 8 The N-ethylmorpholine catalysed hydrolysis of Phenyl Glycinate.

8.1 Introduction

Complexation reactions between substrates (ester) and ligands (catalyst) are common in the reactions of imidazole and tertiary amines with carbonyl compounds [40, 107, 114].

Electrostatic interaction and hydrophobic forces have been implicated [40, 107, 114]. The former effect describes a molecule with a charge binding to another molecule in aqueous solution. The latter effect also described in terms of "hydrophobic bonds" [40], is defined as non-electrostatic in nature. The driving force for the formation of the hydrophobic bond relates to the difficulty of dissolving a relatively non-polar solute in water. It is not surprising that the properties of the hydrophobic bonds follow from the properties of the solutes in water (for further reading, Jencks [40] pgs 393-436).

Non-linear dependence of the observed first order rate constants on the concentration of the catalyst is common in the reactions of imidazole (positive deviation) and tertiary amines (negative deviation) with carbonyl compounds. Non-linear dependence of the rate constants on the concentration of the catalyst is thought to be caused by complex formation (not necessarily a carbonyl addition compound) between the catalyst (imidazole and morpholine, etc.) and the esters. There are two types of non-linear dependence, namely positive and negative deviations. The latter describes a situation where the calculated second order rate constants decrease with the increase in concentration of the catalyst.

8:2 Analysis and Results

8:2.1 Analysis

For a two-step reaction of the type (Equation 8.1) in which complexation occurs with the reactive catalyst, C_r, it can be shown that the rate at any concentration of C_r is given by Equation 8.2 [106].

$$E(\text{ester}) + C_r \overset{K}{\rightleftharpoons} \text{Complex X} \xrightarrow{k_2} \text{Products} \qquad 8.1$$

$$\text{Rate} = k_2[C_r]K_E/(\ 1 + K[C_r]\ ) \qquad 8.2$$

At sufficiently high catalyst concentration, a further increase in the concentration of C_r does not increase the rate because the observed rate constant, k_h, is simply the rate constant k_2 of the breakdown of the complex.

In this study, reactions involve both the neutral (E) and protonated (EH^+) forms of phenyl glycinate. Extending to this situation, the complete rate equation (Equation 8.3) can be derived from the reaction scheme in Equation 8.4.

$$k_h[E + EH^+] = k_2[C_r]K_E[E]/\ (1 + K_E[C_r]) + k_2'[C_r]K_{EH^+}[EH^+]/\ (1 + K_{EH^+}[C_r]) \qquad 8.3$$

$$E + EH^+ + C_r \overset{K_E\ K_{EH^+}}{\rightleftharpoons} X + XH^+ \xrightarrow{k_2\ k_2'} \text{Products} \qquad 8.4$$

where K_E and K_{EH^+} are equilibrium constants for the formation of the neutral complex (X) and the protonated complex (XH^+), respectively. The rate constants, k_2 and k_2', are decomposition rate constants for X and XH^+, respectively.

Rearranging Equation 8.3, we have;

$$k_h = [C_r]\sigma \qquad 8.5$$

where $\sigma = f_E K_E k_2/(1 + K_E[C_r])$

$+ f_{EH^+} K_{EH^+} k_2'/(1 + K_{EH^+}[C_r])$

A test for complex formation as an explanation for observed negative deviations of the type decribed above is similar to that of the well-known Lineweaver- Burk plot for enzymatic reactions [106]. The test requires that the inverse plot $1/k_h$ vs $1/[C_r]$ be linear. The equilibrium constant, K, for complex formation can be obtained by extrapolation to the intercept of the negative abscissa. The gradient of this plot is given by the value $1/\sigma$ and is of no particular interest in this section.

The equilibrium constant, K_{EXP}, defined for this study is the experimentally determined equilibrium constant with contributions from both neutral and protonated phenyl glycinate and we want to derive a relationship between K_{EXP}, K_E and K_{EH^+}.

By definition,

$$K_E = [X]/[E]x[N\text{-}Etm]_f \quad , \quad K_{EH^+} = [XH^+]/[EH^+]x[N\text{-}Etm]_f \qquad 8.6$$

where $[N\text{-}Etm]_f$ is the concentration of free or reactive N-ethylmorpholine ($[C_r]$ in Equation 8.3).

but;

$$[\text{Total Complex}] = [X] + [XH^+] \qquad 8.7$$

Substituting 8.6 into 8.7

$$[\text{Total Complex}] = K_E[E][N\text{-}Etm]_f + K_{EH^+}[EH^+][N\text{-}Etm]_f$$

$$= (K_E[E] + K_{EH^+}[EH^+)[N\text{-}Etm]_f \qquad 8.8$$

Experimentally; $[\text{Total Complex}] = K_{EXP}[E_T][N\text{-}Etm]_f \qquad 8.9$

where E_T is the total ester species.

Equating 8.8 and 8.9, we have;

$$K_{EXP} = K_E[E]/[E_T] + K_{EH^+}[EH^+]/[E_T]$$
$$= K_E f_E + K_{EH^+} f_{EH^+} \qquad 8.10$$

where f_E and f_{EH^+} are fractions of neutral and protonated phenyl glycinate.

8:2.2 Results

Figures 8.1 and 8.2 show that the plots k_h vs $[N\text{-Etm}]_f$ are not linear, where k_h is the observed rate constant and $[N\text{-Etm}]_f$ is the concentration of free (or reactive) N-ethylmorpholine. However, the inverse plots, $1/k_h$ vs $1/[N\text{-Etm}]_f$ are linear (Figures 8.3 - 8.5), supporting the

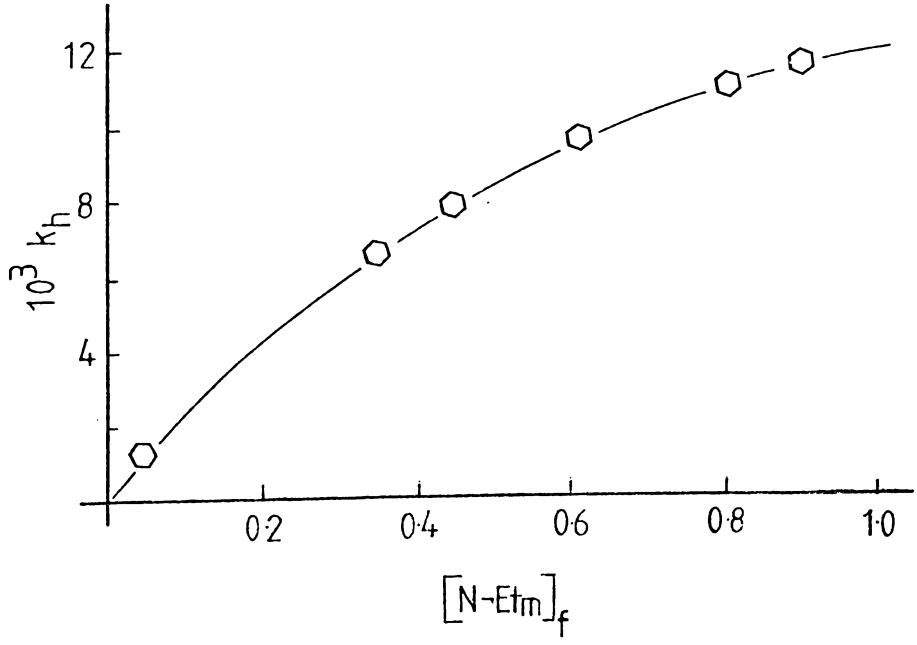


Figure 8.1 The plot of k_h vs $[N\text{-Etm}]_f$ (pH = 8.22)

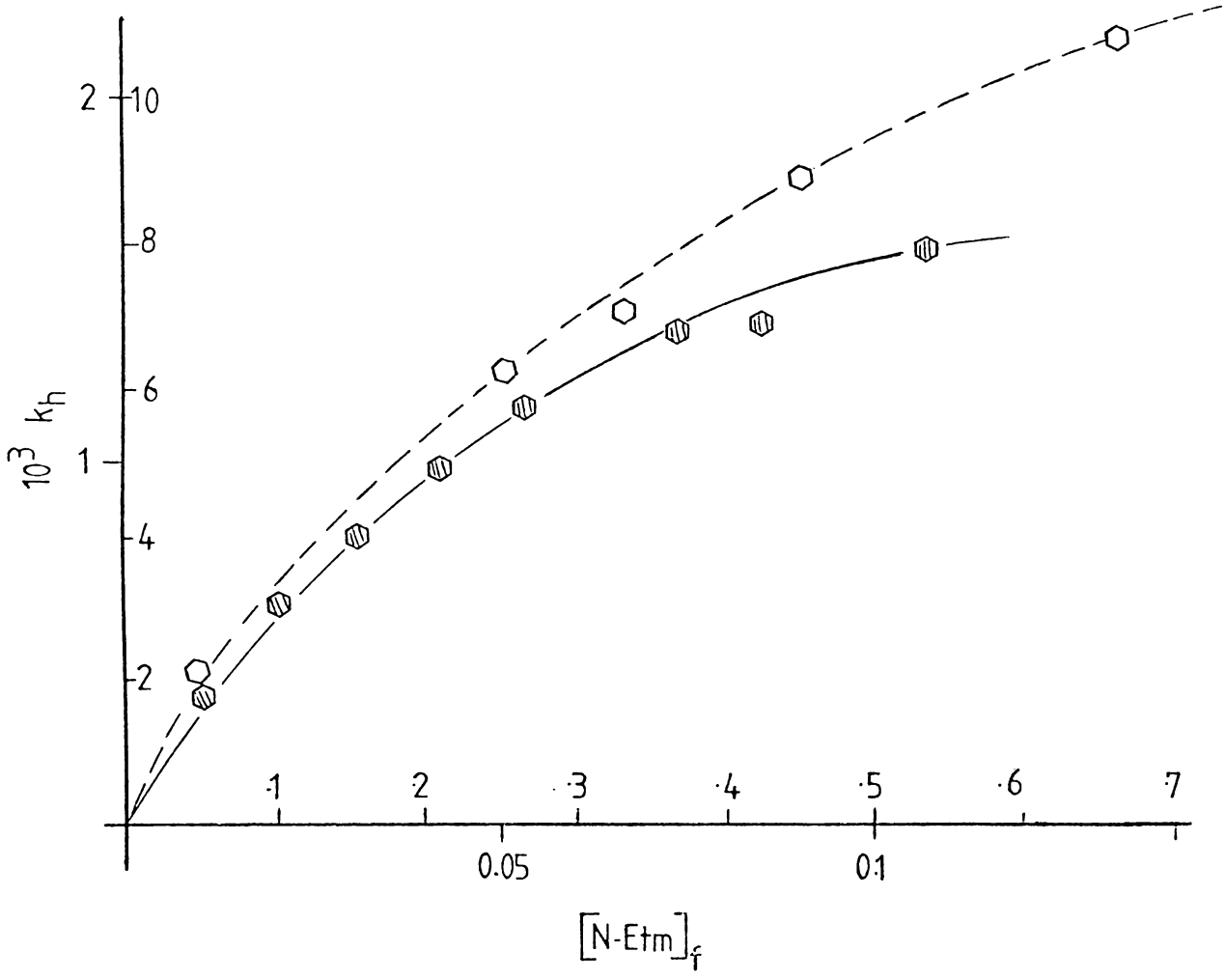


Figure 8.2 The plots of k_h vs $[N\text{-Etm}]_f$

(a) pH = 6.51 ⬡

(b) pH = 7.4 ◍

concept of complexing between the esters (E and EH^+) and N-ethylmorpholine.

The equilibrium constant, K_{EXP}, for each pH is obtained from the appropriate inverse plot (Figures 8.3 - 8.5). Table 8.1 shows the results of such plots.

To obtain an equation to determine the equilibrium constants K_E and K_{EH^+}, we note that;

Table 8.1 Determination of the equilibrium constant K_{EXP} from the inverse plot.

pK_a N-ethylmorpholine = 7.35[112].

pH	$[N\text{-Etm}]_f$ (mol l^{-1})	$1/[N\text{-Etm}]_f$ ($mol^{-1}l$)	$10^3 k_h$ (s^{-1})	$1/k_h$ (s)	K_{EXP} ($l\ mol^{-1}$)
8.22	.881	1.13	11.6	86	
	.793	1.26	10.9	92	
	.617	1.62	9.6	104	
	.441	2.27	7.9	127	
	.352	2.84	6.7	149	
	.044	22.6	1.32	760	1.5 ± .4
7.4	.529	1.89	8	125	
	.423	2.36	7.1	140	
	.37	2.7	6.7	150	
	.264	3.8	5.8	170	
	.212	4.7	5.0	200	
	.159	6.3	4.0	250	
	.106	9.4	3.0	330	
	.053	18.9	1.71	590	3.0 ± .5
6.51	.126	7.94	2.2	450	
	.088	11.36	1.8	560	
	.063	15.87	1.35	740	
	.051	19.8	1.25	800	
	.013	79.3	0.38	2650	6.2 ± 1.2

Error in k_h ± 5%

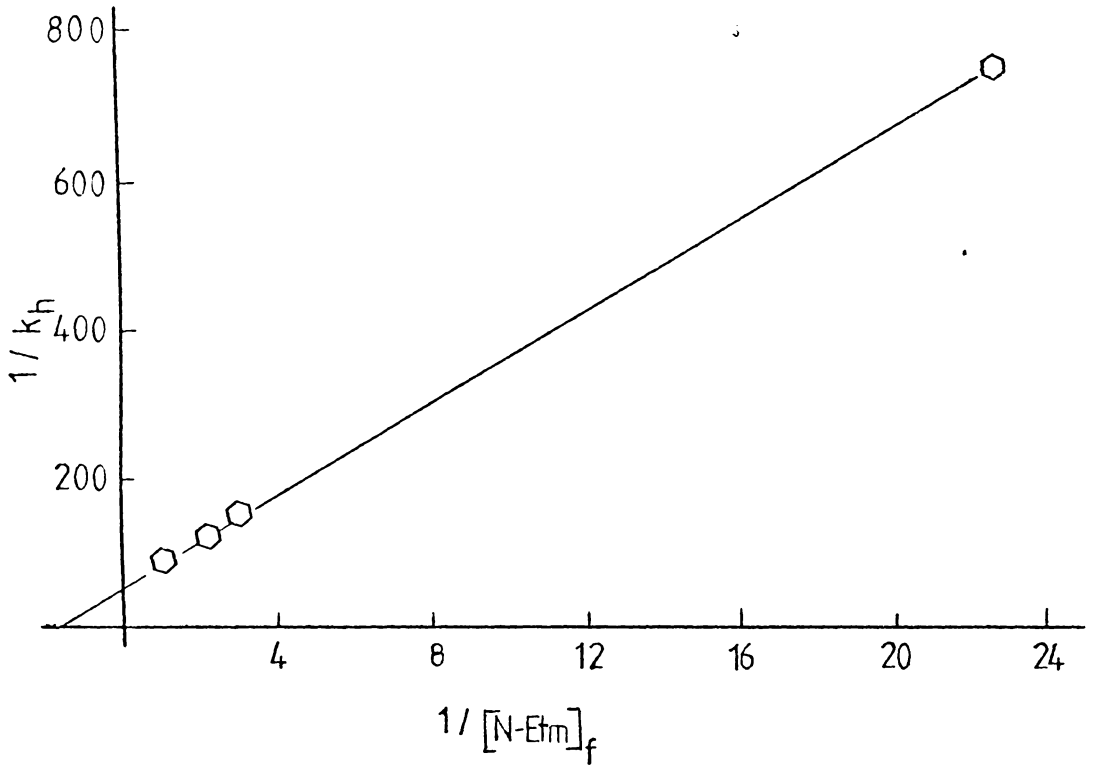


Figure 8.3 The plot of $1/k_h$ vs $1/[N\text{-}Etm]_f$ (pH = 8.22)

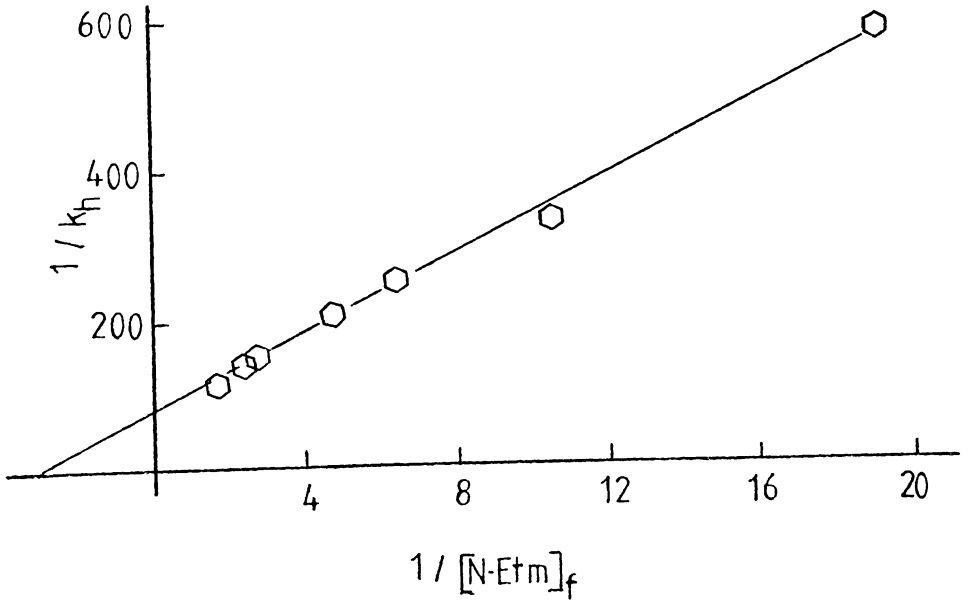


Figure 8.4 The plot of $1/k_h$ vs $1/[N\text{-}Etm]_f$ (pH = 7.4)

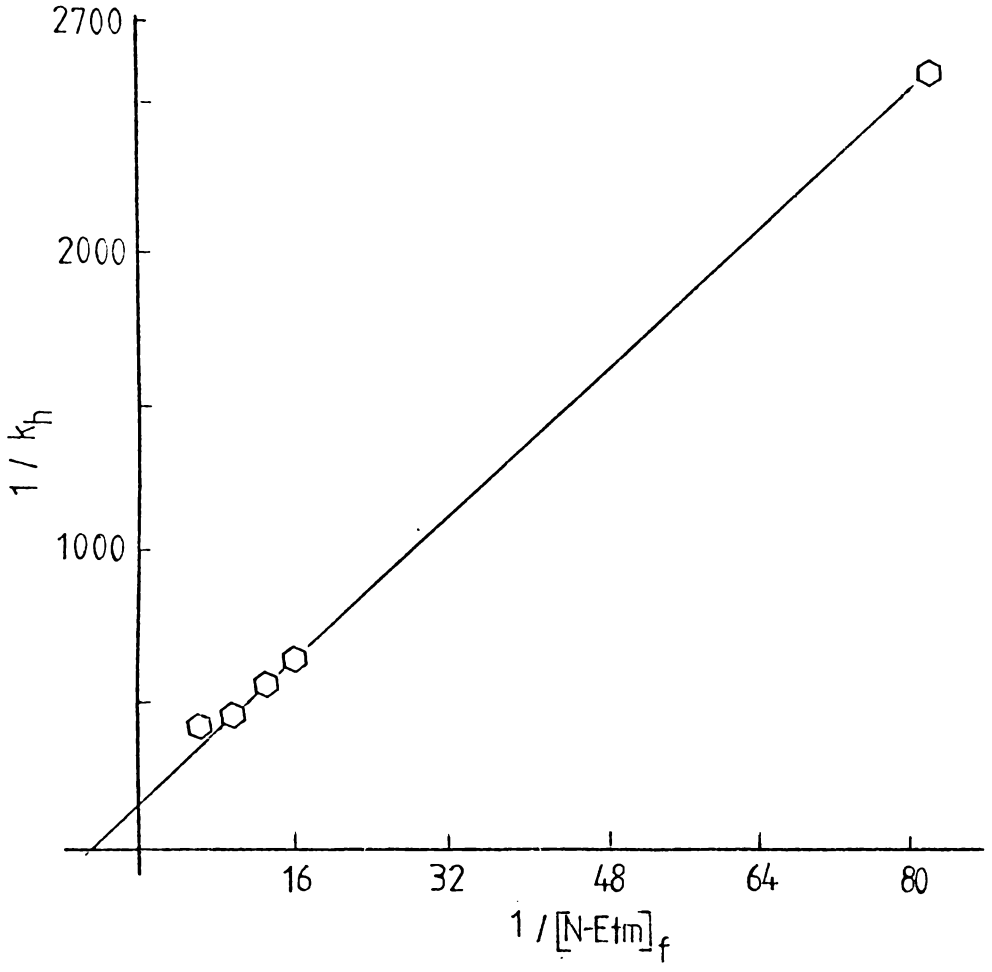


Figure 8.5 The plot of $1/k_h$ vs $1/[N\text{-}Etm]_f$ (pH = 6.51)

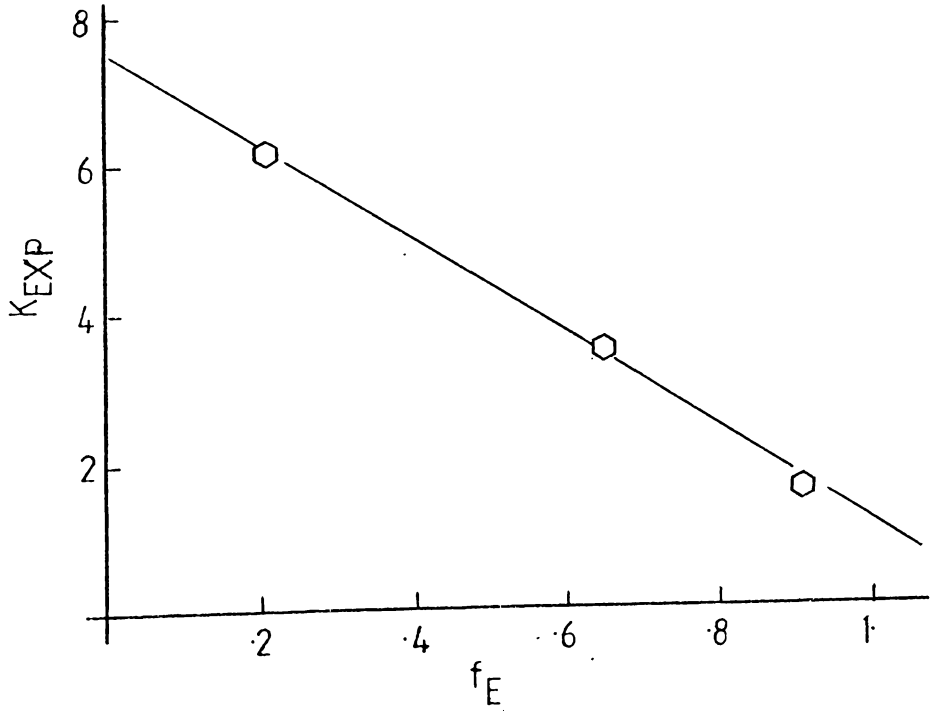


Figure 8.6 The plot of K_{EXP} vs f_E. (To evaluate the equilibrium constants, K_E and K_{EH^+}).

Table 8.2 The data for and result of the plot K_{EXP} vs f_E to evaluate the equilibrium constants K_E and K_{EH^+}.

pH	f_E	K_{EXP} ($1\ mol^{-1}$)	K_E ($1\ mol^{-1}$)	K_{EH^+} ($1\ mol^{-1}$)
8.22	.92	$1.5 \pm .4$		
7.4	.64	$3.0 \pm .5$		
6.51	.19	6.2 ± 1.2	$1.1 \pm .2$	$7.5 \pm .5$

$$f_{EH^+} = 1 - f_E \qquad 8.11$$

Substituting 8.11 into 8,10, we have;

$$K_{EXP} = K_E f_E + (1 - f_E)K_{EH^+} \qquad 8.12$$

Rearranging;

$$K_{EXP} = (K_E - K_{EH^+})f_E + K_{EH^+} \qquad 8.13$$

Therefore a plot of K_{EXP} vs f_E (Figure 8.6) will give the intercept value K_{EH^+} and the gradient, $K_E - K_{EH^+}$, so we can calculate K_E(Table 8.2 for results).

As stated earlier, Equation 8.5 gives the rate constant for the whole process from the reaction scheme(Equation8.4). Substituting $[C_r] = [N\text{-}Etm]_f$ into Equation 8.5, we have,

$$k_h = [N\text{-}Etm]_f(f_E k_2 K_E/(1 + K_E[N\text{-}Etm]_f) + f_{EH^+}k_2' K_{EH^+}/(1 + K_{EH^+}[N\text{-}Etm]_f)) \qquad 8.14$$

Values of k_2 and k_2', the decomposition rate constants for the neutral and protonated complexes used are listed in Table

8.4. They were obtained by trial and error and are found to be self-consistent over the pH range under study. These values together with K_E and K_{EH^+} are used to calculate the value for k_h(calculated), Table 8.3(third column). The observed (experimental) rate constants, k_h(first column) and k_h (calculated) are in good agreement which supports the mechanism involving rate-limiting decomposition of the complexes as given in Equation 8.4.

The summary of equilibrium constants and the decomposition rate constants is shown in Table 8.4

Table 8.3 Comparison between observed rate constant k_h and the calculated rate constant k_h (calculated).

pH	10^3 k_h (s^{-1})	$[N\text{-}Etm]_f$ (mol l^{-1})	10^3 k_h(calculated) (s^{-1})
8.22	11.6	.881	11.7
	10.9	.793	11.1
	9.6	.617	9.77
	7.9	.441	8.02
	6.7	.352	6.9
	1.32	.044	1.23
7.4	8	.53	7.9
	7.1	.433	7.1
	6.7	.37	6.5
	5.8	.263	5.2
	5.0	.212	4.5

	4.0	.159	3.7
	3.0	.106	2.7
	1.71	.053	1.5
6.51	2.2	.126	2.4
	1.8	.088	1.9
	1.35	.063	1.5
	1.25	.051	1.27
	.38	.013	0.39

8.3 Medium Effect.

In the previous section, rate data have been analysed and shown to support a mechanism involving an intermediate complex, as it is evident from the self-consistency of data over a pH range. However, as this study has involved the use of catalyst at moderately high concentrations we have been concerned to know whether such concentrations might affect reaction rates simply by way of medium effects.

An idea of the effects of changes in the nature of the medium caused by reactants and buffer may sometimes be gained by examining the effect of an appropriate model compound. For example, dioxane was used as a model to evaluate the effect of hydrocarbon ether ring of morpholine in the studies of the morpholine catalysed hydrolysis of 4-nitrophenyl phosphate [112]. The result showed that the overall rate constant increases with increasing amount of dioxane. As dioxane is

not a base, the small rate increase was attributed to a solvent effect by dioxane. A similar small medium effect by the catalyst morpholine itself is also possible.

Inhibition by complexation between imidazole with methyl cinnamate[95] in the alkaline hydrolysis of methyl cinnamate was suggested, when the overall rate was decreased after the addition of small amounts of imidazole. The authors[95] suggested that the complexation as well as general solvent effect could have contributed to the decrease in the rate of the alkaline hydrolysis.

To determine whether N-ethylmorpholine or its cation might be having effects which could change reaction rates in our system, we have studied the effects of dioxane and of N,N-diethylmorpholinium iodide on the rate of reaction in the presence of a fixed concentration of the catalyst N-ethylmorpholine and at constant pH.

Table 8.4 Summary of equilibrium constants and decomposition rate constants for N-ethylmorpholine catalysed hydrolysis of phenyl glycinate.

pH	K_E (1 mol^{-1})	K_{EH^+} (1 mol^{-1})	$10^2 k_2$ (s^{-1})	$10^3 k_2'$ (s^{-1})
8.22			2.3	4.3
7.4			2.5	4.3
6.51	1.1 ± 0.2	7.5 ± 0.5	2.5	4.3

Temperature 30°C

Ionic strength = 1 mol l^{-1}

8:3.1 Results and Discussion

In Table 8.5, the effect of dioxane on the observed rate constant is seen to be very dramatic whereas that of N,N-diethylmorpholinium cation is relatively small. Dioxane at concentration of only 0.5 mol l^{-1} in solution depresses the original observed rate constant of 9.8×10^{-3} s^{-1} to 9.3×10^{-4} s^{-1}, a drop to less than one tenth.

Table 8.5 Effect of dioxane on the observed rate constants

[N-Etm] = 1 mol l^{-1} pH = 7.83

Ionic Strength = 1 mol l^{-1} Temperature = 30°C

Dioxane added (mol l^{-1})	10^3 k_h (s^{-1})
0	9.8 ± 0.4
0.12	4.5 ± 0.1
0.25	2.7 ± 0.05
0.5	0.93 ± 0.03

The effect of N,N-diethylmorpholinium cation on the N-ethylmorpholine catalysed hydrolysis of phenyl glycinate is relatively quite small. The decrease in the observed constants could be related to a solvent effect of N,N-diethyl morpholinium cation, through small changes in the observed rate constants when a relatively large cation is added are difficult to interpret in terms of solvent effect(Jencks[112]) because there are other factors that could also affect the change in the observed rate constant.

Table 8.6 Effect of N,N-diethylmorpholinium cation on the observed rate constants

[N-Etm] = 1 mol l^{-1} pH = 6.95

Ionic Strength = 1 mol l^{-1} Temperature = 30°C

[N,N-diethylmorpholinium iodide] added (mol l^{-1})	10^3 k_h (s^{-1})
0.0	6.2 ±.3
0.1	5.6 ±.3
0.2	5.0 ±.2
0.3	4.8 ±.2
0.5	4.6 ±.2

The effect of dioxane on N-ethylmorpholine catalysed hydrolysis of phenyl glycinate is conversely very large. There is no system for direct comparison of this effect reported in the literature but a general solvent effect of rather large sensitivity was reported for the hydrolysis of acetylsalicylic acid anhydride in the presence of dioxane (1 mol l^{-1}). The observed rate constant was reduced by half after 1 mol l^{-1} of dioxane was added.

Our result in Table 8.5 shows a much greater sensitivity to dioxane than in the example quoted. It is probable that we have more than just a general solvent effect. Possibly dioxane competes with N-ethylmorpholine for complexing with phenyl glycinate and competes so effectively that only a little free phenyl glycinate is left at a dioxane concentration of 0.5 mol l^{-1} to react with N-ethylmorpholine. If such

is the case, however, the equilibrium constants for complexing with the phenyl glycinate species must, unexpectedly, be very high.

An alternative is complexing between dioxane and N-ethylmorpholine to prevent the latter from reacting with the phenyl glycinate species. This seems unlikely, however, since the dioxane has a large effect when its molarity is very less than that of N-ethylmorpholine, so a 1 : 1 complex is definitely excluded.

The effect of dioxane is so large that it is unlikely to represent a general medium effect. This counteracts the aim of studying its effect in the first place as a model for the medium effect of N-ethylmorpholine for which we have consequently no guide. The large specific effect of dioxane certainly merits further detailed study.

The effect of the N,N-diethylmorpholinium cation demands careful consideration. The extent of reduction in the observed rate constant value (Table 8.6) with increasing concentration of cation, is similar to the extent of reduction in calculated values of $k_h/[N\text{-}Etm]_f$ with increasing concentration of N-ethylmorpholinium cation at the pH value of 6.51, suggesting that possibly, the change in $k_h/[N\text{-}Etm]_f$ at this pH is a function of concentration of N-ethylmorpholinium cation, via some type of medium effect. However, the similarity of this effect does not carry over to higher pH values. This is clear in Figure 8.7, where the values of the apparent second order rate constants, $k_h/[N\text{-}Etm]_f$, are plotted against $[N\text{-}EtmH^+]$ (curves 1, 2, and 3) and where values of $k_h/[N\text{-}Etm]_f$ are plotted against [N,N-diethylmorpholinium cation] (curve 4, this is superimposed on Figure 8.7 for comparison only). The marked variation between the curves which represent apparent effect of cation shows clearly the cation concentration

Table 8.6 Data for the plots of $k_h/[N\text{-}Etm]_f$ vs $[N\text{-}EtmH^+]$

pH	$10^2\ k_h/[N\text{-}Etm]_f$ ($1\ mol^{-1}\ s^{-1}$)	$[N\text{-}EtmH^+]$ ($mol\ 1^{-1}$)
8.22	1.31	.077
	1.37	.069
	1.56	.054
	1.79	.038
	1.9	.031
	3.0	.004
7.4	1.51	.3
	1.64	.243
	1.81	.208
	2.2	.148
	2.36	.119
	2.52	.089
	2.83	.06
	3.22	.03
6.51	1.74	.537
	2.04	.375
	2.14	.268
	2.47	.215
	2.99	.054

can not be responsible for the variation in $k_h/[N\text{-}Etm]_f$ at all pH values. This does not mean that the cation does not have some effect nor that this effect does not vary from pH to pH. It does mean that whatever is responsible for decreasing values of $k_h/[N\text{-}Etm]_f$ at higher catalyst concentrations, this is not solely a medium effect of N-ethylmorpholinium

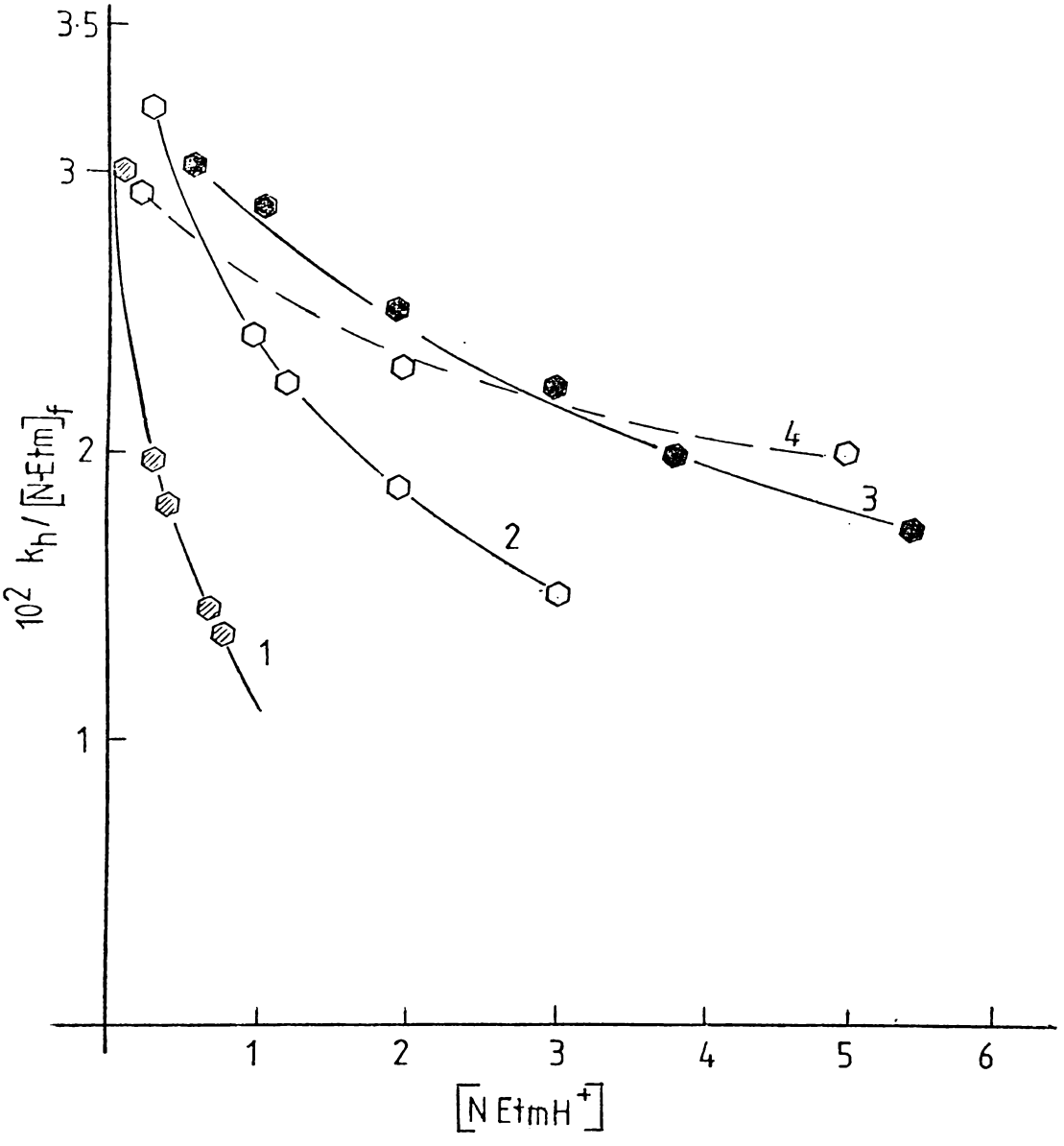


Figure 8.7 Plots of $k_h/[N\text{-}Etm]_f$ vs $[N\text{-}EtmH^+]$.

cation and certainly this can not be a major effect at pH 8.22 for instance, the curve here being very different from that at lower pH values.

Our attempts to determine the medium effects of N-ethylmorpholine and its cations by the use of model compounds has therefore not really provided us with conclusive results.

Dioxane as a model for N-ethylmorpholine has an apparent very specific effect of its own and we are left none the wiser as to what general medium effect it might have which N-ethylmorpholine might have too. The N,N-diethylmorpholinium cation does appear to have an effect which could be a general medium effect. Such an effect operating in similar fashion for the N-ethylmorpholinium cation is, however, insufficient to account for experimentally observed variations in apparent second order rate constant $k_h/[N\text{-}Etm]_f$ for the N-ethylmorpholine catalysed hydrolysis of phenyl glycinate, as is particularly clear at high pH. There may be some general solvent effect of the N-ethylmorpholinium cation, but if so there must be some other major contributing effect also.

This brings us back to the analysis in Section 8.2 where entirely self-consistent results were obtained based on the general scheme involving intermediate complexes in the reaction and excluding consideration of medium effects. This suggested that medium effects were unimportant, but the later studies now reported suggest this need not necessarily be so.

This leaves us with the choice of assuming that medium effects of N-ethylmorpholine and its cation are not important even though, in the case of the cation especially, experiments with model compounds suggest this is unlikely; or we could assume that medium effects possibly compensated in some way by other effects are built in to the values of equilibrium and rate constants calculated (Section 8.2). To make either assumption is not entirely satisfactory but we feel that the latter assumption is more likely correct. This means that the absolute values of calculated equilibrium and rate constants are subject to some uncertainty, but not necessarily that the trends in the values would in the absence of medium effects not be preserved. In the next section, equilibrium and rate

constants are tentatively compared to see if their values are meaningful in terms of the scheme involving complex formation, medium effects, which are unknown, being ignored. The validity of the discussion which follows, then, must be tempered with the view that other factors are being neglected.

8.4 Further discussion

We shall attempt to discuss the results of Table 8.4 in turn with respect to the equilibrium constants (K_E and K_{EH^+}) and the decomposition rate constants (k_2 and k_2').

8:4.1 The equilibrium constants K_E and K_{EH^+}

In Table 8.4, we have $K_{EH^+} > K_E$ by a factor of about 7, which suggests that the charged species (EH^+) complexed more readily than the uncharged species (E) with N-ethylmorpholine. It is not altogether unexpected for N-ethylmorpholine to be a dipolar molecule. We, therefore, suspect that electrostatic interactions assisted in complex formation between EH^+ and N-ethylmorpholine but to a lesser extent between E and N-ethylmorpholine.

The highly soluble EH^+ cation would not be expected to be the type that would complex with N-ethylmorpholine as a result of hydrophobic bonding (refer Section 8.1). Consequently, it is likely that electrostatic interactions between the protonated phenyl glycinate and the tertiary amine center of the N-ethylmorpholine are responsible for complexing. Strong electrostatic interactions are not possible for the

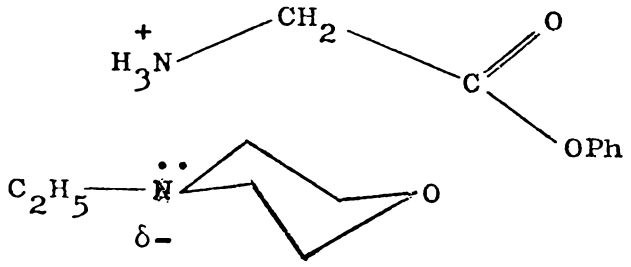
neutral phenyl glycinate with N- ethylmorpholine, for which weak electrostatic interactions and /or hydrophobic interactions could be responsible for complexing.

8:4.2 The decomposition rate constants k_2 and k_2'

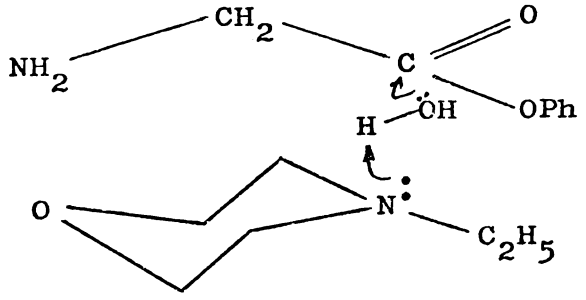
The rate constants k_2 and k_2' are decomposition rate constants for the complexes with N-ethylmorpholine of neutral and protonated phenyl glycinate, respectively.

Table 8.4 shows that $k_2 > k_2'$ by a factor of almost 5 . This is unusual in view of the fact that k_2' describes the decomposition of the protonated ester complex. In terms of electrostatic and/or inductive effects of the protonated amino group over the neutral amino group one would expect that $k_2 \ll k_2'$. For example in Section 1.4, where in the alkaline hydrolysis of ethyl glycinate(Hay et al [71]), the result shows that the protonated ethyl glycinate hydrolysed about 150 times faster than the neutral counterpart.

However, as the protonated ester complex decomposes much slower than the neutral ester complex, this suggests that either the activating effect of the NH_3^+ group, whether it be electrostatic or inductive electron withdrawing in nature, is nullified by complexing with N-ethylmorpholine or that in the protonated ester complex the tertiary amine centre of the N-ethylmorpholine is so orientated as to be only weakly effective as a base in assisting water attack at the ester carbonyl centre. Both of the above effects might apply if the complex were assumed to have something of the geometry indicated in the diagram below.



For the neutral ester, E, one could envisage a different type of complex in which the tertiary amine centre, not being attracted to the now neutral amino group of the ester, is better positioned to act as a base to catalyse water attack at the ester carbonyl as shown in the diagram below or to act as a nucleophilic catalyst.



To some extent, then, the larger equilibrium constant for complexing by the protonated ester, implying as it does electrostatic interactions, is consistent with a slower rate of ester hydrolysis from within the complex if the essential base centre is tied up in electrostatic bonding at some distance from ester function.

In conclusion, there is some rationale for the values of equilibrium and rate constants for the two ester species. However, as mentioned earlier, this analysis ignores possible medium effect contributions to values of equilibrium and rate constants and the value of interpretation is limited by these uncertainties.

Section 9 Summary.

This section summarizes the results and conclusions from the earlier chapters in more detail than the size restriction on the abstract at the start of the thesis allows. It produces nothing new, its aim being simply to summarize the main findings of the thesis in a form useful and readily intelligible particularly to future workers in the field.

9:1 (a) Phenyl and 4-methoxyphenyl glycinate.

We found that it was bicarbonate ion that catalysed the hydrolysis of phenyl and 4-methoxyphenyl glycinate, according to the equation;

$$\text{Rate} = k_2[E][HCO_3^-] \qquad 9.1$$

We consider it unlikely for HCO_3^- to catalyse the hydrolysis of phenyl glycinate by a general base mechanism because HCO_3^- is about 550 times a better catalyst than HPO_4^{2-} although the latter is a stronger base than the former. This is contrary to expectation for general base catalysis.

Nucleophilic catalysis via a direct nucleophilic addition to the ester carbonyl is also considered as an unlikely process for the following reasons;

(i) although a nucleophilic addition by HCO_3^- and HPO_4^{2-} to the ester carbonyl is possible, our results show that HPO_4^{2-} has no similar high catalytic activity as HCO_3^- in spite of the expectation that HPO_4^{2-}, as a stronger base, would be a better nucleophile.

(ii) no catalysis of hydrolysis of the protonated ester was observed, whereas it would be expected to be more susceptible to nucleophilic attack than the neutral ester.

We are left to consider assisted nucleophilic catalysis involving the free amino group of the ester because the protonated ester (EH^+) is unreactive. There are two possible mechanisms involving reaction of the amino group: the general acid assisted addition mechanism (Diagram 4.5) and the nucleophilic, proton-transfer mechanism (Diagram 4.6). We prefer the former mechanism because the latter has the unfavourable requirement of a correct geometry (7-membered ring system) before hydrolysis can take place.

We have also considered as alternatives to the HCO_3^- catalysis implied by Equation 9.1 the kinetically equivalent forms (Equations 9.2 and 9.3) and have excluded these possibilities

$$\text{Rate} = k_2'[EH^+][CO_3^{2-}] \qquad 9.2$$

$$\text{Rate} = k_2''[E][CO_2][OH^-] \qquad 9.3$$

for the following reasons.

(i) We have calculated the overall rate constant (k_2'), based on Equation 9.2, as about 1200 ± 100 $1\ mol^{-1}\ s^{-1}$ ($30^\circ C$). The corresponding value from an analogous study, the carbonate catalysed hydrolysis of a cationic 4-nitrophenyl ester, reported in the literature is $160\ 1\ mol^{-1}\ s^{-1}$ ($25^\circ C$). It is not conceivable for the rate of hydrolysis of a phenyl ester to be 8 times faster than that of a 4-nitrophenyl ester on the basis of leaving group ability, so Equation 9.2 is excluded.

(ii) Equation 9.3 is a special form of the rate equation derived in Chapter 5 of this thesis, i.e.,

$$\text{Rate} = k_2[E]K_cK_w[CO_2][B]$$

where B represents any base, which in the case of Equation 9.3 is the hydroxide ion, so that $k_2'' = k_2K_cK_w$ in this case.

Firstly, we think that OH^- catalysis of carbamate formation is unlikely to be important at neutral pH, Caplow [120] having shown that OH^- catalysis becomes effective at pH > 11. Secondly, if OH^- at such a low concentration (neutral pH) can in fact catalyse the hydrolysis, the relatively high concentration of HPO_4^{2-} (buffer species) should lead to much more effective catalysis by this base but we found no evidence of buffer catalysis at all.

9:1 (b) 4-nitrophenyl esters of glycine and valine.

Our results support the conclusion of Hay and Main[39] that it is CO_2 and not HCO_3^- that catalyses the hydrolysis of the 4-nitrophenyl esters according to the rate equation;

$$\text{Rate} = k_2[E][CO_2] \qquad 9.4$$

A temperature variation study on the valine ester suggests a composite overall rate constant, because the rate constant increase with increasing temperature was unexpectedly low, too low for a single step reaction (such as rate-determining formation of the zwitterionic carbamate, C_Z). The overall rate constant must therefore include one or more equilibrium constants representing equilibria prior to the rate-determining step.

If the formation of the carbamate anion C_A from the zwitterion C_Z is rate-determining, then the requirement for a pre-equilibrium step is satisfied, but the overall rate equation is as expressed below. We did not detect any base (B) catalysis. However, our analysis showed that the kinetics according to Equation 9.4 are independent of varying concentrations of buf-

fer base HPO_4^{2-}, of HCO_3^- and of OH^- (pH variation). On that

$$\text{Rate} = k_A[B]K_Z[E][CO_2] \qquad 9.5$$

basis, rate-determining proton transfer from C_Z to C_A (Equation 9.5) does not apply in the reaction.

We are now left with two alternatives, i.e. the rate-determining cyclization of either the zwitterion C_Z or the anion C_A. Against cyclization of C_Z is the probability that C_Z is very short-lived (by analogy with a carbinolamine zwitterion with half-life of 10^7 s^{-1} [86, 87]). Secondly and more importantly, the electron withdrawing power of the cationic nitrogen would cause strong polarization of electrons away from the carbamate oxygen anion therefore making it less nucleophilic which would not favour cyclization. This leaves us with the more likely mechanism for CO_2-catalysed hydrolysis of 4-nitrophenyl esters, a rate determining cyclization of C_A in which there is much reduced inductive electron withdrawal from the carbamate anion, so that it should be reasonably nucleophilic.

9:2 4-nitrobenzaldehyde catalysed hydrolysis of amino acid esters.

Unlike the case for bicarbonate catalysed hydrolysis, 4-nitrobenzaldehyde (NB) catalysed hydrolyses of phenyl and 4-methoxyphenyl glycinate shows the same kinetic form as for previously studied 4-nitrophenyl esters. The rate equation is given by,

$$\text{Rate} = k_2[E][NB] \qquad 9.6$$

which is consistent with carbinolamine formation.

The formation of the carbinolamine is suggested not to be

rate-determining for the following reasons:

(i) the overall rate constant for 4-nitrophenyl glycinate is about 250 times greater than that for phenyl glycinate (and 500 times greater than that of 4-methoxyphenyl glycinate). This implies rate-determining attack on the ester carbonyl group. It is not consistent with rate-determining formation of carbinolamine because the slightly more basic phenyl ester should, if anything, add to the aldehyde carbonyl to form carbinolamine faster than the 4-nitrophenyl ester.

(ii) a temperature variation study on the catalysed hydrolysis of 4-nitrophenyl valinate suggests a two-step or multi-step reaction. The rate constant must incorporate an equilibrium constant factor because the overall increase with temperature is very small.

On the basis of a rate-determining decomposition of the carbinolamine intermediate, we have considered the two possible pathways previously suggested, i.e. the cyclization and imine pathways, but the present results still do not allow any distinction to be made between them.

9:3 The imidazole catalysed hydrolysis of phenyl glycinate.

Imidazole catalysed the hydrolysis of both neutral and protonated phenyl glycinate. Results show that the term second order in imidazole strongly predominates over the term first order in imidazole.

The first order rate constants (k_1 and k_1' for the neutral and protonated esters, respectively) are quite comparable with the corresponding HPO_4^{2-} catalysed rate constants (Table 4.11). On the basis of the similar catalytic reactivities of HPO_4^{2-} and imidazole, we suggest that the terms first order in

imidazole represent catalysis by a general base mechanism rather than a nucleophilic mechanism.

The term second order in imidazole on analysis indicates a rate-determining proton abstraction process by a second imidazole molecule, from the zwitterionic intermediate T^{+-}, which is first formed from a nucleophilic attack by the first imidazole on the ester carbonyl. The rate equation is given below.

$$\text{Rate} = k_b[\text{Im}]_n[T^{+-}] \qquad 9.7$$

9:4 The N-ethylmorpholine catalysed hydrolysis of phenyl glycinate.

Non-linear dependence of the observed first order rate constants on the concentration of the catalyst was evident in the N-ethylmorpholine catalysed hydrolysis of phenyl glycinate.

The effect was shown to be consistent with complexation between the ester and the free amine. Linearity of the inverse plot $1/k_h$ vs $1/[\text{N-Etm}]_f$ provides a means of obtaining the equilibrium constant for complexation. We have calculated, assuming that there are no medium effects, the equilibrium constants, K_E and K_{EH^+}, for complexing of the neutral and protonated esters, respectively, and the decomposition rate constants k_2 (for E) and k_2' (for EH^+). Such values were found to be consistent over the range pH 6.5 to pH 8.2 with the overall experimentally determined constants (Equation 9.8).

$$k_h = f_E k_2 K_E[\text{N-Etm}]_f/(1 + K_E[\text{N-Etm}]_f) + f_{EH^+}k_2' K_{EH^+}[\text{N-Etm}]_f/(1 + K_{EH^+}[\text{N-Etm}]_f) \qquad 9.8$$

However, consideration of whether it was realistic to neglect possible medium effects by N-ethylmorpholine and its cation led to some tentative experiments which produced interesting results.

We used dioxane as a model for N-ethylmorpholine in an attempt to determine the likely magnitude of general medium effects of such heterocyclic compounds on the catalysed hydrolysis and were surprised to find a dramatic rate-depressing effect by dioxane which presumably reflects some specific effect of this species. N,N-diethylmorpholinium cation as a model for N-ethylmorpholium cation had an effect on hydrolysis rate but this was mild as more consistent with a general medium effect. Nevertheless, this is not negligible from the point of view of the effect of N-ethylmorpholinium cation on the N-ethylmorpholine catalysed hydrolysis of phenyl glycinate.

However,in spite of anticipated medium effects by N-ethylmorpholine and its cation on the basis of the effects of the model compounds dioxane and N,N-diethylmorpholinium cation, the analysis carried out assuming the unusual kinetic effects observed were due solely to complexing, as shown above, was self-consistent. It must be asked whether this was fortuitous and the answer must await the results from further studies.

List of references

The following system has been used to denote volume, page number and year of publication. The author or authors names are given first. The volume number is given after the abbreviation of the title of the periodical. This is followed by the appropriate page and finally the year of publication.

1 Bender, M.L. Chem. Review 53, 1960

2 Johnson, S.L. Adv. Phys.Org. Chem. 5, 237, 1967

3 Patai, S. "The Chemistry of Carboxylic Acids and Esters" Interscience. John Wiley and Sons Ltd. 1969

4 Bruice, T.C. and S.J. Benkovic "Bioorganic Mechanisms" W.A. Benjamin, Inc. (NY) 1966

5 Fife, T.H. Adv. Phys. Org. Chem. 11, 1, 1975

6 Lazduski, M. Progr. Bioorg. Chem. 3, 1, 1974

7 Coleman, J.E. Progr. Bioorg. Chem. 1, 159, 1971

8 Bender, M.L. and B.W. Turnquest J. Am. Chem. Soc. 79, 1656, 1957

9 Bender, M.L. J. Am. Chem. Soc. 73, 1626, 1951

10 Kirsch, J.F. and W.P. Jencks J. Am. Chem. Soc. 86, 837, 1964

11 Bruice, T.C. and S.J. Benkovic J. Am. Chem. Soc. 86, 418, 1964

12 Caplow, M. and W.P. Jencks Biochem. 1, 773, 1964

13 Perrin, D.D. Aust. J. Chem. 16, 572, 1963

14 Bruice, T.C. and R. Lapinsky J. Am. Chem. Soc. 80, 2265, 1958

15 Koshland,D.E. (Jr) J. Am. Chem. Soc. 74,2282,1952

16 Stadtman, E.R. "In the Mechanism of Enzyme Action" W.D. M^{c}Elroy and B. Glass (Eds) John Hopkins Press (Balt.) 1954

17 Stadtman, E. R. and E.H. White J. Am. Chem. Soc. 75, 2022,1953

18 Bafna, S.L. and V. Gold J. Chem. Soc. 1406, 1953

19 Gold V. and E.G. Jefferson J. Chem. Soc. 1409, 1953

20 Bender, M.L. and B.W. Turnquest J. Am. Chem. Soc. 79, 1652,1957

21 Bruice, T.C. and G.L. Schmir J. Am. Chem. Soc. 80,2265,1958

22 Bruice, T.C. and S.J. Benkovic J. Am. Chem. Soc. 85, 1, 1963

23 Bender, M.L. "Proceedings of the International Congress of Catalysis 1956" Advances in Catalysis Vol IX Academic Press (NY) 1957

24 Hartley, B.S. and V. Massey "Ann. Rept. on Progr. Chem." Chem. Soc. London. 51, 311, 1954

25 Menger, F.M. and J.H. Smith J. Am. Chem. Soc. 94,3824, 1972

26 Menger, F.M. and A.C. Vitale J. Am. Chem. Soc. 95,4931,1973

27 Doherty, D.G. and F. Vaslow J. Am. Chem. Soc. 74, 931, 1952

28 Wilson, I.B. and J. Bergman J. Biol.Chem. 186, 683, 1950

29 Gutfreund, H. Trans. Far. Soc. 51, 441, 1955

30 Gutfreund, H. and B.R. Hammond J. Biol. Chem. 61,187, 1955

31 Weil, L., S. James and A.R. Buchert
Arch. Biochem. Biophys. 266, 1953

32 Wagner-Jauregg, T. and B.E. Hackey
J. Am. Chem. Soc. 75, 2125, 1953

33 Brecher, A.S. and A.K. Bolls
J. Biol. Chem. 227, 845, 1957

34 Bergman, M. and L. Zervas
Z. Physil. Chem. 175, 145, 1928

35 Langerbeck, W. and R. Mahrwald Chem. Ber. 90, 2423,1957

36 Waltenburg, G., J. Boger and P. Haake
J. Am. Chem. Soc. 93, 4938, 1971

37 Rivetti, F. and U. Tonelatto
J. Chem. Soc. (Perk II) 1176, 1977

38 Hay, R.W. and R.J. Tretheway
Aust. J. Chem. 22, 109, 1969

39 Hay, R.W. and L. Main Aust. J. Chem. 21, 155, 1968

40 Jencks, W.P. "Catalysis in Chemistry and Enzymology"
McGraw-Hill, New York, 1969

41 Bell, R.P. "The Proton in Chemistry"
Cornell University Press, NY, 1959

42 Betts, R.L. and L.P. Hammett
J. Am. Chem. Soc. 59, 1568, 1937

43 Baltzy, R., I.M. Berger and A.A. Rothstein
J. Am. Chem. Soc. 72, 4149, 1950

44 Jencks, W.P. and J. Carriuolo
J. Am. Chem. Soc. 83, 1743, 1961

45 Butler, A.R. and V. Gold J. Chem. Soc. 1334, 1962

46 Jencks, W.P. and J.F. Kirsch
J. Am. Chem. Soc. 86, 5616, 1964

47 Holland, J.M. and J.G. Miller
J. Phys. Chem. 65, 463, 1961

48 Jencks, W.P. and J. Carriuolo
J. Am. Chem. Soc. 83, 1743, 1961

49 Greenstein, J.P. and M. Winitz
"Chemistry of the Amino Acids"
Vol. 2 Wiley Interscience
New York 1961

50 Butler, A.R. and V. Gold J. Chem. Soc. 4362, 1961

51 Butler, A.R. and T.C. Bruice
J. Am. Chem. Soc. 86, 313, 1964

52 Lewis, E.S. and J.D. Allen
J. Am. Chem. Soc. 86, 2022, 1964

53 Kirby, A.J. and A.R. Fersht
"Progress in Bioorganic Chemistry"
- E.T. Kaiser and F.J. Kezdy (Eds)
Wiley Interscience, 1971, pg 1-82 .

54 Bender, M.L., Y.L. Chow and F. Chlonpek
J. Am. Chem. Soc. 80, 5384, 1958

55 Koshland, D.E. J. Theoret. Biol. 2, 75, 1962

56 Huisgen, R. and H. Ott Tetrahedron 6, 253, 1959

57 Parker, A.J. Quart. Review 163, 1962

58 Tommila, E. and M.L. Murto
Acta. Chem. Scand. 17, 1943, 1963

59 Tommila, E. Suomen. Kemistilehti 378, 117, 1964

60 Ferht, A.R. and A.J. Kirby
J. Am. Chem. Soc. 89, 4857, 1967

61 Matthew, B.W., P.B. Sigler, R. Henderson and D.M. Blow
Nature 214, 652, 1967

62 Shotten, D. and H.W. Watson
Phil. Trans. Roy. Soc. Lon. Ser. B
257, 111, 1970

63 Wright, C.S., R.A. Alden and J. Kraut
Nature 221, 235, 1969

64 Schmir, G.L. and T.C. Bruice
J. Am. Chem. Soc. 80, 1173, 1958

65 Bruice, T.C. and J.M. Sturtevant
J. Am. Chem. Soc. 81, 2860, 1959

66 Schatzle, E., M. Rottenburg and M. Thurkauf
Helv. Chim. Acta. 42, 1708, 1959

67 Bender, M.L. and M.S. Silver
J.Am. Chem. Soc. 84, 4589, 1962

68 Bender, M.L. and H. d'A Heck
(unpublished 1966, see Ref. 2)

69 Bunton, C.A., K. Khaleeludin and D. Whittaker
J. Chem. Soc. 3290, 1965

70 Wolfenden, R. Biochemistry 2, 1090, 1963

71 Hay, R.W., L.J. Porter and P.J. Morris
Aust. J. Chem. 19, 1197, 1966

72 Schmir, G.L. and T.C. Bruice
(see Ref 64)

73 Agren, A.,U. Hedsten and B. Johnson
Acta. Chem. Scand. 15, 1532, 1961

74 Hansen, B. and A. Flormark
Acta Chem. Scand. 17, 1481, 1963

75 Hansen, B. Svensk. Kem. Tidskr. 75, 10, 1963

76 Schafer, J.A. and H. Morawetz
J. Org. Chem. 27, 2269, 1962

77 Larsson, L. Svensk. Kem. Tidskr. 70, 405, 1958

78 Snell E.E. "Vitamins and Hormones" 16, 77 , 1958

79 Braunstein, A.E. "The Enzymes" Vol. 2 Second Ed.
P.D. Boyer, H. Hardy and K. Myrback
(Eds) Academic Press 113, 1960

80 Werle, E. and W. Koch Biochem. Z. 319, 305, 1949

81 Thanassi, J. and J.S. Fruton Biochem. 1, 975, 1962

82 Kalyanker, G.D. and E.E. Snell Biochem. 1, 594, 1962

83 Jencks, W.P. Progr. Phys. Org. Chem. 2, 63, 1964

84 Morino, Y. and E.E. Snell J. Biol. Chem. 242, 2800, 1967

85 Schirch, L. and R.A. Slotter Biochem. 5, 3175, 1966

86 Diebler, H. and R.N.F. Thornely
J. Am. Chem. Soc. 95, 896, 1975

87 Diebler, H. and R.N.F. Thornely
J. Am. Chem. Soc. 96, 1072, 1974

88 Kallen, R.G. and W.P. Jencks
J. Biol. Chem. 241, 24, 5867, 1966

89 French, T.C. and T.C. Bruice Biochem. 3, 1589, 1964

90 Sander, E.G. and W.P. Jencks
J. Am. Chem. Soc. 90, 6155, 1968

91 Capon, B. and R. Capon Chem. Comm. 502, 1965

92 Main, L. M Sc Thesis
Victoria University of Wellington
Wellington, New Zealand, 1966

93 Newman, M.S. and S. Hishida
J. Am. Chem. Soc. 84, 3582, 1962

94 Lee, H.H. M Sc Thesis
University of Waikato
Hamilton, New Zealand, 1975

95 Roughton, F.J.W. and L. Rossi-Bernardi
Proc. Roy. Soc. B164, 381, 1966

96 Chipperfield, J.R. Proc. Roy. Soc. B164, 401, 1966

97 Caplow, M. J. Am. Chem. Soc. 90, 6795, 1968

98 Henrikson, O. and M.B. Jensen
Acta Chem. Scand. 21, 2819, 1967

99 Frahn, J.L. and J.A. Mills
Aust. J. Chem. 2, 256, 1964

100 Wieland, T. and F. Jaenicke Ann. 599, 1956

101 Harned, H.S. and R. Davis

J. Am. Chem. Soc. 65, 2030, 1943

102 Harned, H.S. and Davis, R.

J. Am. Chem. Soc. 67, 1026, 1945

103 Shedlowsky, T. and D.A. M^{c}Innes

Trans. Faraday Soc. 59, 386, 1963

104 Bruice, T.C. and B. Holmquist

J. Am. Chem. Soc. 91, 2986, 1969

105 Frost, A.A. and R.G. Pearson

"Kinetics and Mechanism" pg 205

John Wiley, New York, 1963

106 Jencks, W.P. Progr. Phys. Org. Chem. 2, 63, 1964

107 Mollica, J.A. and K.A. Connors

J. Am. Chem. Soc. 89, 308, 1967

108 Mollica, J.A. and K.A. Connors

J. Am. Chem. Soc. 87, 123, 1965

109 Holmquist, B. and T.C. Bruice

J. Am. Chem. Soc. 91, 2997, 1969

110 Jencks, W.P. and M. Satterthwait

J. Am. Chem. Soc. 22, 7018, 1974

111 Jencks, W.P. and M. Gilchrist

J. Am. Chem. Soc. 90, 2622, 1968

112 "Handbook for Chemistry a d Physics"

55th Edition CRC Press (1974)

113 Anderson, B.M. and W.P. Jencks

J. Am. Chem. Soc. 82, 1773, 1960

114 Kirby, A.J. and W.P. Jencks

J. Am. Chem. Soc. 87, 3209, 1965

115 Vogel, A.I. "A text-book of practical organic chemistry ; Small Scale Preparations" 2nd Edition

Longman, Green and Co London.

116 Bruice, T.C. and G.L. Schmir
J. Am. Chem. Soc. 79, 1663, 1957

117 Brouwer, D.M., M.J. Van der Vlugt and E. Havinga
Koninkl. Nederl. Akad. B60, 275, 1957

118 Bender, M.L. and W.A. Glasson
J. Am. Chem. Soc. 81, 1590, 1959

119 Milstein, J.B. and T.H. Fife
J. Am. Chem. Soc. 90, 2164, 1968

120 Caplow, M. "CO_2: Chemical, Biochemical and Physiological Aspects Symposium" :- Eds R.E. Forster, J.T. Edsall, A.B. Otis and F.J.W. Roughton. pg 48

121 Kirsch J.F. and W.P. Jencks
J. Am. Chem. Soc. 86, 833, 1964

122 Frahn, J.L. and J.A. Mills Aust. J. Chem. 2, 256, 1964

123 Oakenfull, D.G., T. Riley and V. Gold
Chem. Comm. 385, 1966

124 Bender, M.L. and M.C. Neveu
J. Am. Chem. Soc. 80, 5388, 1958

125 Pratt, R.F. and T.C. Bruice
J. Am. Chem. Soc. 92,5956, 1970

126 Holmquist, B. and T.C. Bruice
J. Am. Chem. Soc. 91, 2993, 1969

127 Holmquist, B. and T.C. Bruice
J. Am. Chem. Soc. 91, 3003, 1969

128 Kirby, A.J. and A.R. Fersht
"Progr. in Bioorganic Chemistry" Vol I
Eds. E.T. Kaiser and F.J. Kedzy
Wiley Interscience 1971, pg 1-82.

129 Bender, M.L. "Mechanism of Homogeneous Catalysis from Proton to Proton."
Wiley Interscience 1971

130 Hay, R.W. and P.J. Morris

"Metal Ions in Biological Systems", Vol V, pg 178

Helmut Segel (Ed)

Marcel Dekker (New York) 1976

131 Hay, R.W. and L.J. Porter

J. Chem. Soc. (B) 1261, 1967