HYDROLYSIS OF SOME N-NITROAMIDES

a Thesis submitted by

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ABSTRACT

The chemistry of N-nitroamides and N-nitrosoamides is reviewed with particular reference to hydrolysis reactions. The mechanism of hydrolysis of amides, both in base and acid, is described.

The kinetics of hydrolysis of some N-nitroamides in aqueous solution by a variety of basic (nucleophilic) species are examined. This hydrolysis is shown to occur *via* a nucleophilic catalysed pathway, rather than a general base one and formation of the tetrahedral intermediate is generally the rate limiting step. A large solvent deuterium isotope effect is reported for the hydroxide ion catalysed hydrolysis of N-nitroamides and an explanation of this is given in terms of a rate determining proton transfer to the tetrahedral intermediate.

The kinetics of hydrolysis of some N-nitroamides are also examined in acid and are found to show a monotonical rate increase with increasing acidity and no rate maxima. Evidence is presented for a change in mechanism from an Ac2 to an Ac1 for the case of N-nitro-N-methylbenzamide.

The mechanism of oxidation of p-aminophenols and 5-hydroxyindoles is reviewed with particular regard to oxidation by nitrous acid. The use of phenolic compounds as nitrite traps is outlined.

The oxidation of p-dimethylaminophenol by nitrous acid is studied and it is found to be a two electron oxidation with formation of N,N-dimethyl-p-benzoquinonemonoimine which hydrolyses to p-benzoquinone. The oxidation of some 5-hydroxyindoles by nitrous acid is also examined and it is shown to be a one electron oxidation with formation of polymeric

products. The rate determining step is the formation of nitrosyl acetate which reacts with the substrate in a fast step.

An appendix on the kinetics of hydrolysis of methylnitramine and O-methyl-N-isopropylnitramine in acid is also included.

ACKNOWLEDGEMENTS

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Finally, I would like to thank Maria del Carmen Serrano for typing the manuscript.

Eduarda Rosa,
September, 1978.

CONVENTIONS

To avoid confusion, which may arise as a result of the presence of reactants in dissociated and undissociated forms of acid-base pre-equilibria, the total stoichiometric concentrations of the reactants are denoted by the use of round brackets and the actual concentrations of the active species involved are denoted by the use of square brackets.

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CHAPTER 1

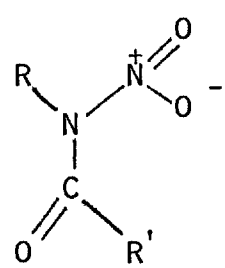
INTRODUCTION

1.1 THE CHEMISTRY OF N-NITROAMIDES AND N-NITROSOAMIDES

1.1,1 Structure

In normal amides, the nitrogen lone pair electrons are known to suffer delocalization into the π bond of the carbonyl group. This gives a partial double bond character to the C-N bond, a planar structure for the amide moiety and allows the existence of configurational isomers[1,2] detectable by spectroscopic methods and dipole moment measurements[3]. These properties are modified by the presence of either N-nitroso or N-nitro[4] substituents for which configurational isomers have not been reported. This implies that the nitrogen lone pair electrons are then delocalized into the nitroso or nitro group rather than the carbonyl function.

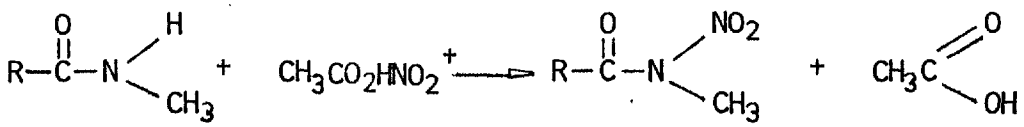
The lowest energy conformation for N-nitroamides[4] and N-nitrosoamides[5] is the trans-trans conformation (1).



(1)

1.1,2 Synthesis

Secondary N-nitroamides are generally prepared by nitration of the corresponding amides. Reagents commonly employed are cupric nitrate - acetic anhydride[6,7] and nitric acid - acetic anhydride[7,8,9], both presumably reacting via the acetylnitrate intermediate (Scheme 1.1,2). N_2O_5 In an organic solvent has also been used to prepare N-nitroamides[4,10].



Scheme 1.1,2

A useful alternative to direct nitration is acylation of nitroamine salts and several N-methyl-N-nitroamides have been prepared in this manner[11,12].

The synthesis of primary N-nitroamides has not been accomplished. A report on the nitration of benzamide identifies benzoic acid as the product of reaction, the half life of its formation being less than five minutes[7].

N-Nitrocarbamates have been prepared by nitration of the corresponding carbamates with fuming nitric acid. At low temperatures (-80°C), the nitroso derivatives are formed which at higher temperature convert rapidly to the N-nitro derivatives. In similar conditions, N-nitroamides give only the nitrate esters[13].

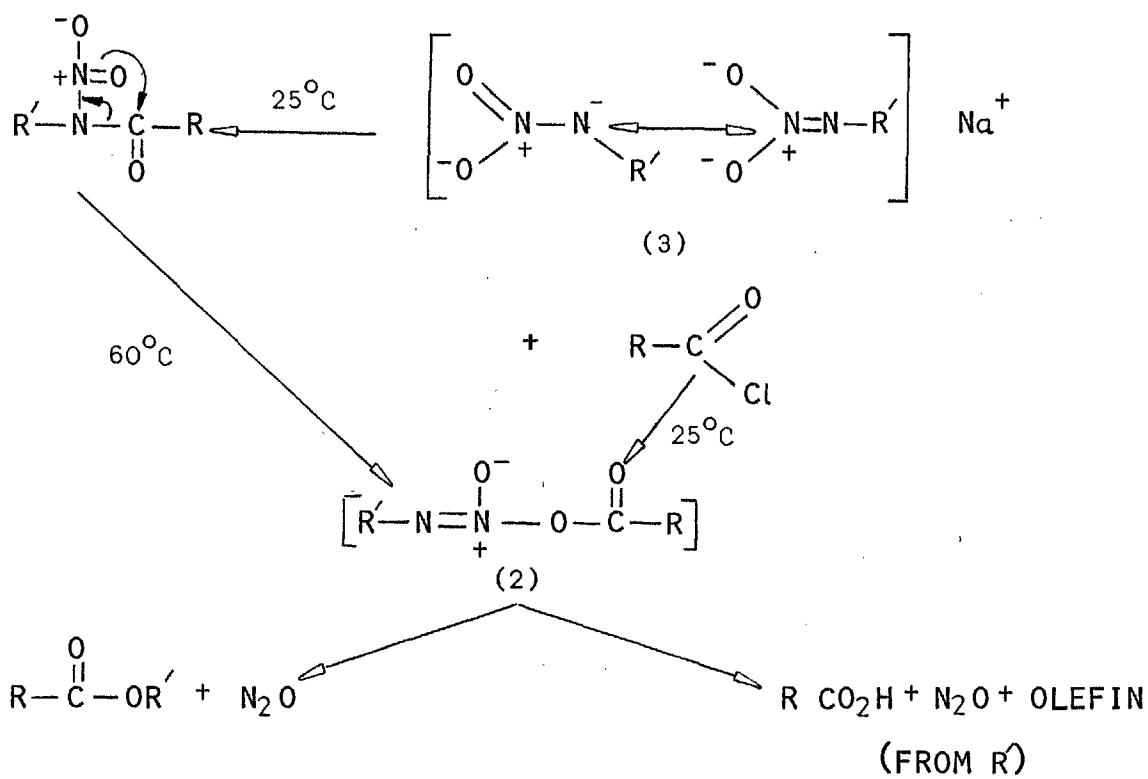
The N-nitrosoamides are prepared by nitrosation of the correspondent N-alkylamides with nitrosating agents as acidified nitrite, nitrous anhydride, nitrosyl chloride and dinitrogen tetroxide. Nitrosation of primary amides, of course, leads only to deaminated products[14].

1.1,3 Reactions

1.1,3a Thermal decomposition

N-Nitroamides are known to decompose thermally by two competing pathways, the products of reaction being either esters or acids and

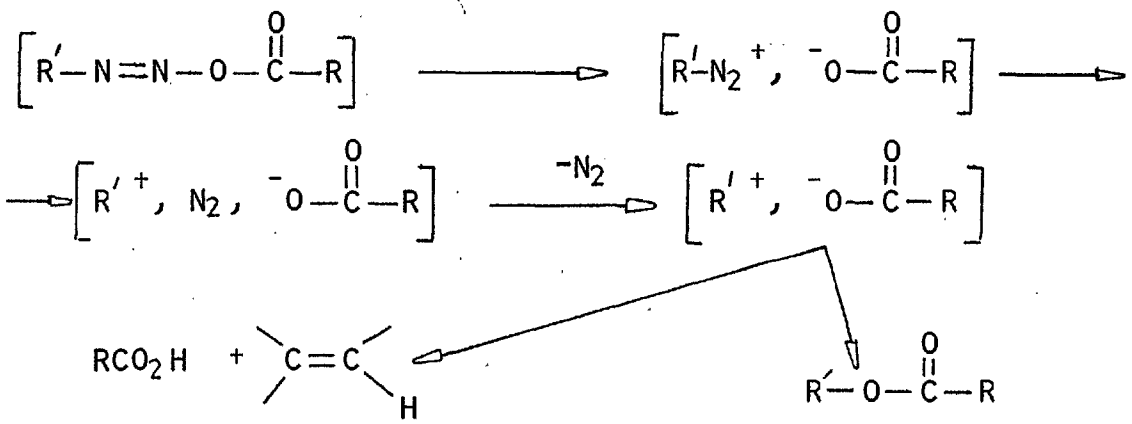
alkenes[15] (scheme 1.1,3a). Both pathways are thought to proceed via a common diazoxyester intermediate (2) shown in Scheme 1.1,3a. The formation of (2) is rate limiting. Under conditions where the N-nitroamide is known to be stable, acylation of the nitroamine salt (3) gives both the N-nitroamide and the same products as those obtained from thermal decomposition. It follows that these decomposition[1] products arise from a diazoxyester formed directly from an interaction of the mesomeric nitroamine anion with acetyl chloride[16,17] (Scheme 1.1,3a).



Scheme 1.1,3a

N-Nitrosoamides thermally decompose in an analogous manner giving similar products as the N-nitroamides in approximately the same yields. The decomposition is thought to proceed via a related mechanism involving diazoester intermediates (R'-N=N-O-COR). On the basis of product

configuration, ^{18}O exchange experiments, medium effects and the presence of a "foreign acid", a mechanism for the decomposition of the diazoester was proposed (Scheme 1.1,3b) in which the first ionization step is followed by loss of N_2 with formation of an ion pair.



Scheme 1.1,3b

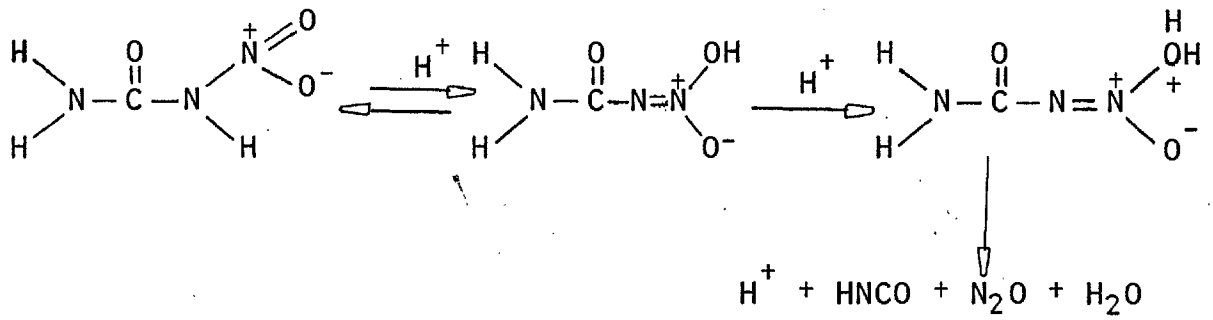
The ion pair collapses to yield different products depending on the structure of the R' alkyl group and on the conditions of reaction[18].

1.1,3b Hydrolysis

1.1,3b1 Hydrolysis of N-nitroamides

These reactions have not been widely investigated but the decomposition of N-methyl-N-nitrobenzamides in 20% aqueous NaOH has been shown to give the corresponding carboxylic acids[7]. Although there is no evidence that simple N-nitroamides lose nitronium ion (NO_2^+) in acid, N-nitrourethanes($ROCON(NO_2)R$) are good nitrating agents when dissolved in concentrated H_2SO_4[19]. The only kinetic measurements concern the hydrolysis of N-nitrourea in H_2SO_4, $HClO_4$, HCl and H_3PO_4,[20] where

decomposition is thought to proceed via the mechanism in Scheme 1.1,3b1.

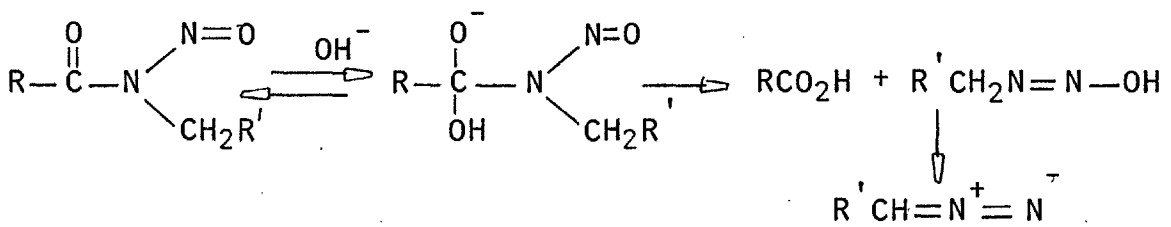


Scheme 1.1,3b1

1.1,3b2 Hydrolysis of N-nitrosoamides

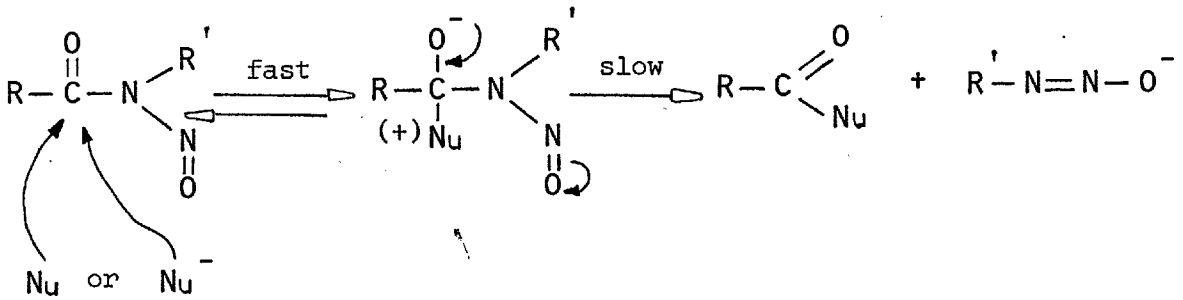
(i) Hydrolysis in base

N-alkylnitrosoamides are used to generate diazoalkanes when hydrolysed in alkali[21,22] as outlined in Scheme 1.1,3b2.



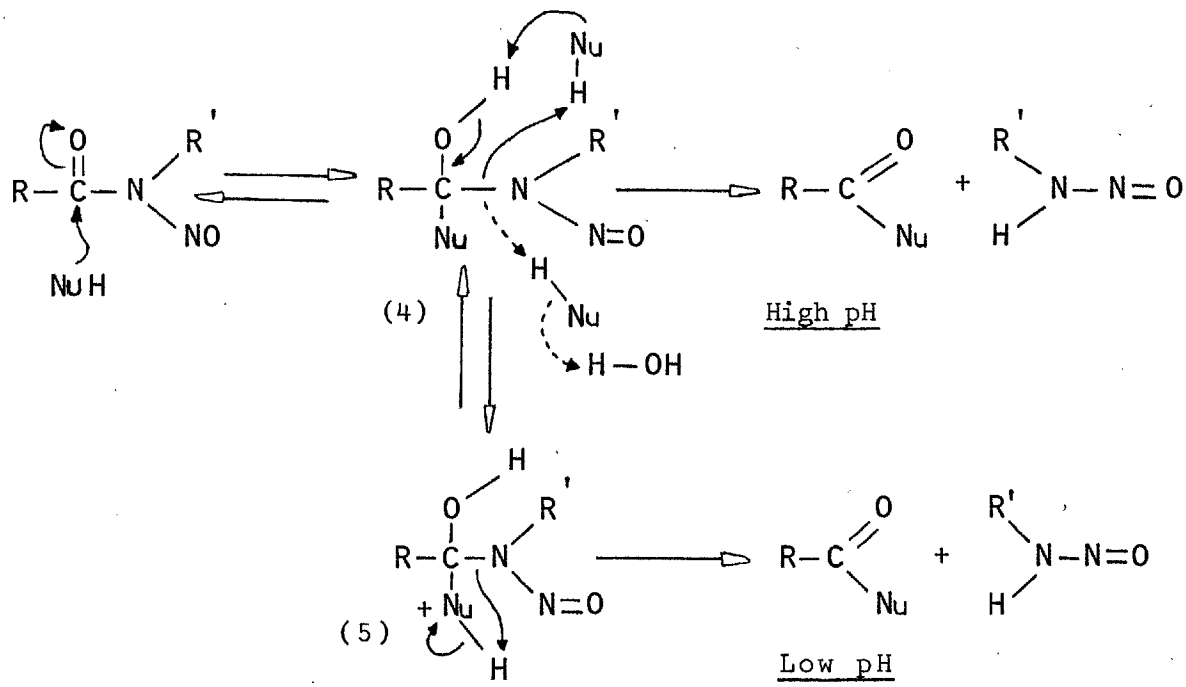
Scheme 1.1,3b2

A recent kinetic study[23,24] shows this process proceeds by a nucleophilic catalysed pathway. A tetrahedral intermediate is formed and then breaks down to products via a concerted process in a rate-limiting step (Scheme 1.1,3b3).



Scheme 1.1,3b3

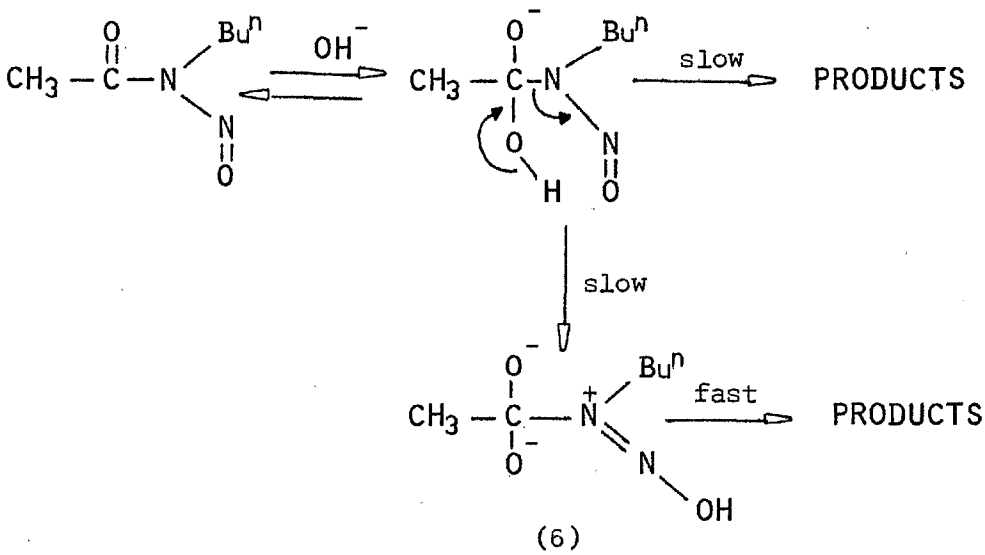
Bifunctional catalysts such as imidazole and HPO_4^{2-} bearing a labile proton (NuH), however, react via the modified process given in Scheme 1.1,3b4.



Scheme 1.1,3b4

At low pH, decomposition occurs via an intramolecular proton transfer as shown, whereas at high pH, where only (4) exists, decomposition occurs via protonation of the amino leaving group by an intermolecular process involving a second molecule of catalyst.

The OH^- catalysed hydrolysis of N-nitrosoamides shows a substantial solvent deuterium isotope effect (K_{OH^-}/K_{OD^-} ca. 5.1) which has been interpreted as evidence for rate limiting proton transfer to the leaving group as shown in Scheme 1.1,3b5. However, it is not certain whether this proton transfer is concerted with the breakdown of the tetrahedral intermediate to products or whether slow proton transfer preceeds fast decomposition of the intermediate (6).



Scheme 1.1,3b5

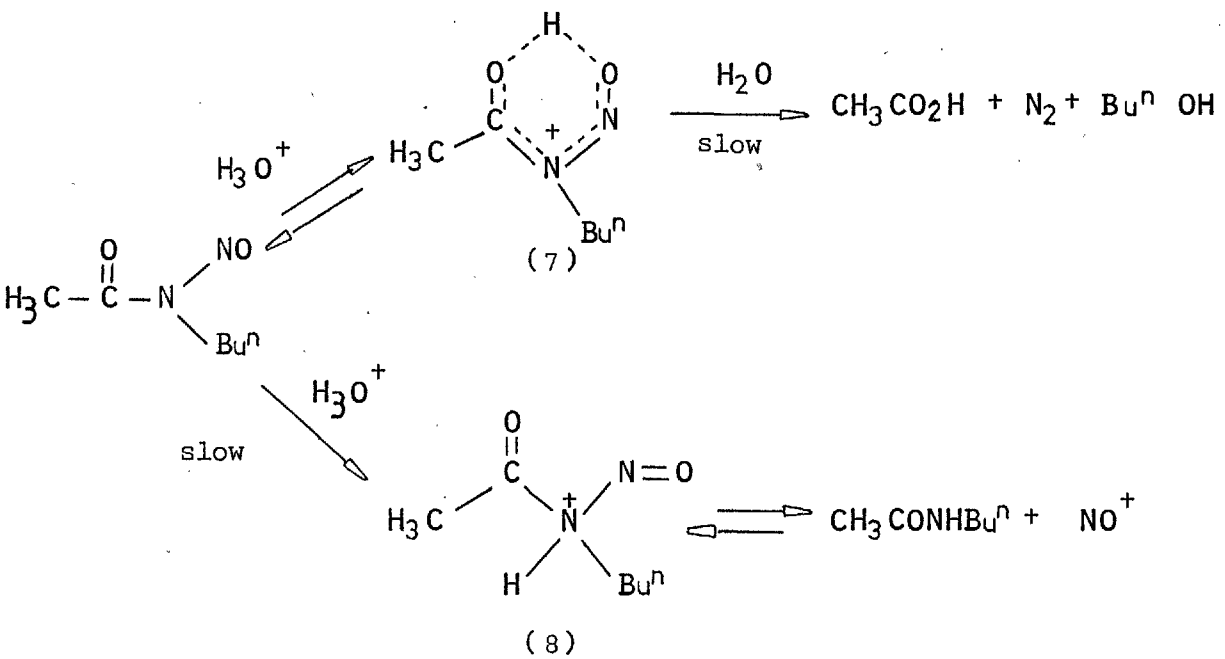
Another unanswered question is whether protonation of the leaving group is on the N-atom of the amino group or on the O-atom of the nitroso group.

(ii) Hydrolysis in acid

Hydrolysis of N-alkyl-N-nitrosoamides in acidic media proceeds via two concurrent pathways[23,25]. One involves release of N_2 with formation of either an ester or an alcohol plus acid (possible with some olefin derived from the amino fragment) whereas the other involves release of

nitrous acid and parent amide (Scheme 1.1,3b6).

The deamination reaction is believed to proceed via the O-conjugated acid (7) formed in a rapid pre-equilibrium step (K_{H_2O}/K_{D_2O} = 0.77), whereas denitrosation involves rate determining protonation of the amino N to give (8) (K_{H_2O}/K_{D_2O} = 1.8 - 2.0) which rapidly decomposes to release NO^+ and the parent amide.



Scheme 1.1,3b6

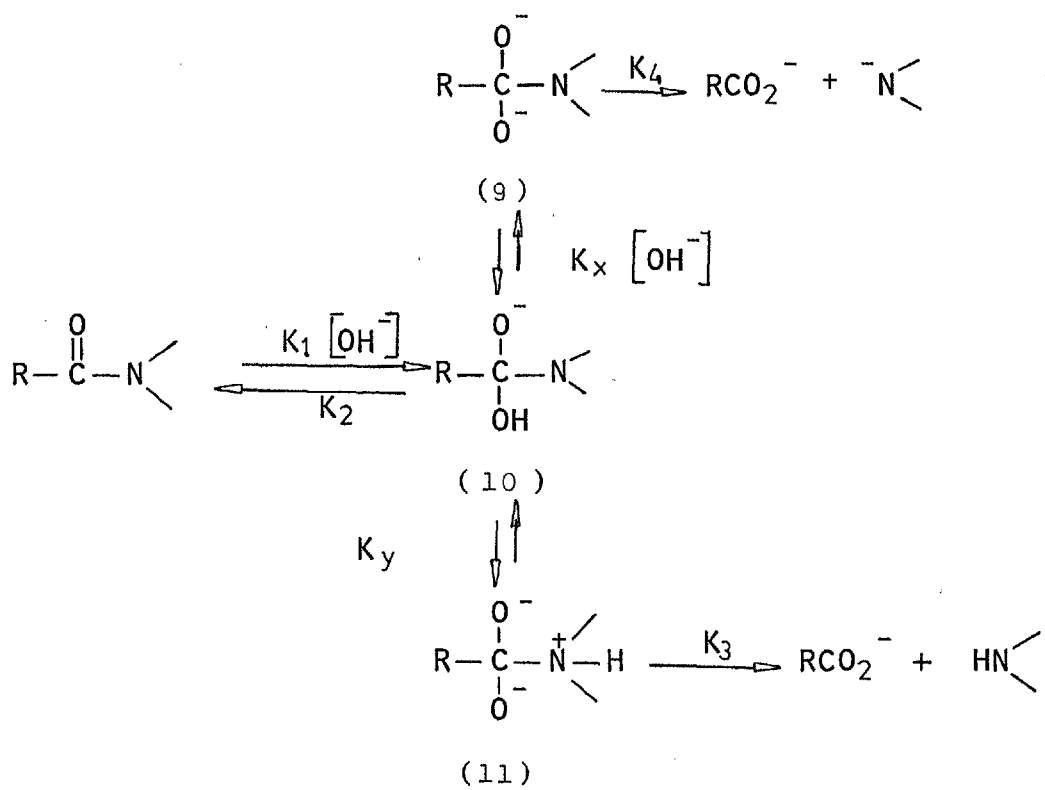
For N-nitroso-2-pyrrolidone[26], however, both the deamination and dinitrosation give similar kinetics. At low acidities general acid catalysis is detected for both pathways giving a common Brönsted plot with α = 0.64. This leads to the conclusion that a unique intermediate is involved in this case, probably the N-conjugated acid species because the rate of dinitrosation is independent of added nucleophiles.

1.2 THE HYDROLYSIS OF AMIDES

1.2,1 Base catalysed hydrolysis

(i) Rate equation and mechanism

It is now generally accepted that amides hydrolyse via attack of a basic entity at the carbonylic carbon atom with the formation of a tetrahedral intermediate (10) which subsequently decomposes to products[22, 27, 28]. A diagramatic representation of the accepted mechanism is given in Scheme 1.2,1a.



Scheme 1.2,1a

Applying the steady state treatment to the above scheme gives equation 1.2,1a.

$$K_{obs} = \frac{K_1 K_3 K_y [OH^-] + K_1 K_4 K_x [OH^-]^2}{K_2 + K_4 K_x [OH^-] + K_3 K_y} \quad \ldots \text{ eq. 1.2,1a}$$

Rate equations described in the literature are normally limiting cases or generalizations of eq. 1.2,1a.

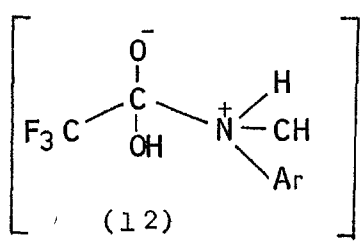
(ii) The rate determining step

The rate expression for the hydrolysis of urea[29], anilides [30], chloroacetamide[31] and N-N-diacylamines[32] in aqueous alkali is usually first order in substrate with both first and second order terms in hydroxide ion. This evidence, together with the fact that extensive ^{18}O carbonyl exchange with the solvent takes place during the reaction, establishes that the rate determining step is the breakdown of the tetrahedral intermediate to products (steps K_3 and/or K_4).

Protonation of the amine leaving group ((11) in Scheme 1.2,1a) is often important. This was first realised by Bender and Thomas[33] from substituent effects on the rates of hydrolysis (ρ = 0.1) and concurrent ^{18}O exchange of acetanilides. They were able to deduce that partitioning of the tetrahedral intermediate gave ρ_{K_2}/ρ_{K_3} = -1. This implies that electron withdrawing substituents favour exchange over hydrolysis ($K_{ex}/K_{hy} = K_2/2K_3$) which is only consistent with the protonation of the amine leaving group before its expulsion (electron withdrawing substituents lower the concentration of the dianionic species and, therefore, promote ^{18}O exchange).

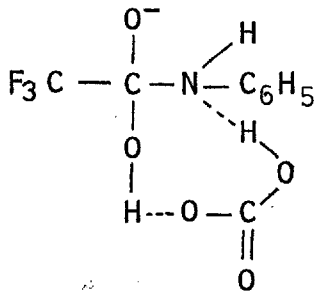
Schowen and his co-workers studied the hydrolysis of both 2,2,2-trifluoro-N-methylacetanilide and ring substituted analogues in aqueous NaOH solutions[34,35] and found that general base catalysis was superimposed upon specific hydroxide ion catalysis. The Brönsted plot for catalysis by water, glycinate ion and OH^- gave β ca. 0.3. This was interpreted as evidence of either general base catalysed decomposition of (10) or general acid catalysed formation of (11) in the rate determining step. They also

observed high solvent deuterium isotope effects[36] which were related to exclusive proton transfer in the rate determining step (Scheme 1.2,1a). In more recent work[37], a change in the rate determining step was apparent when the basicity of the leaving group varied from pK_a <9 to pK_a > 9. This change was also manifested in the solvent deuterium isotope effect reported as K_{H_2O}/K_{D_2O} ca. 1.2-1.5 for leaving groups with pK_a < 9 and K_{H_2O}/K_{D_2O} ca. 0.7-0.9 for those with pK_a > 9. Electron withdrawing substituents were considered to stabilize negative charge on the amidic nitrogen making fission of the C-N bond rather than proton transfer rate limiting. In buffer solutions, however, different results were obtained[38]. The Brönsted coefficient for general acid catalysed hydrolysis of 2,2,2-trifluoro-N-methyl acetanilide (α = 1) suggests that proton transfer occurs before breakdown to products. The authors conclude that intermediate (12) decomposes in the rate determining step with proton transfers to and from N being fast (the dianion (11) in Scheme 1.2,1a decomposes more readily to products because of the two alkoxide centres).



(12)

Other studies of 2,2,2-trifluoro-N-methyl acetanilide[39,40] in buffer solutions have also stressed the importance of general acid catalysed decomposition. In particular, an identical limiting rate with high concentrations of the different general acid catalysts was interpreted as rate determining conversion of the intermediate (10) to (11) via a synchronomous process as in (13).

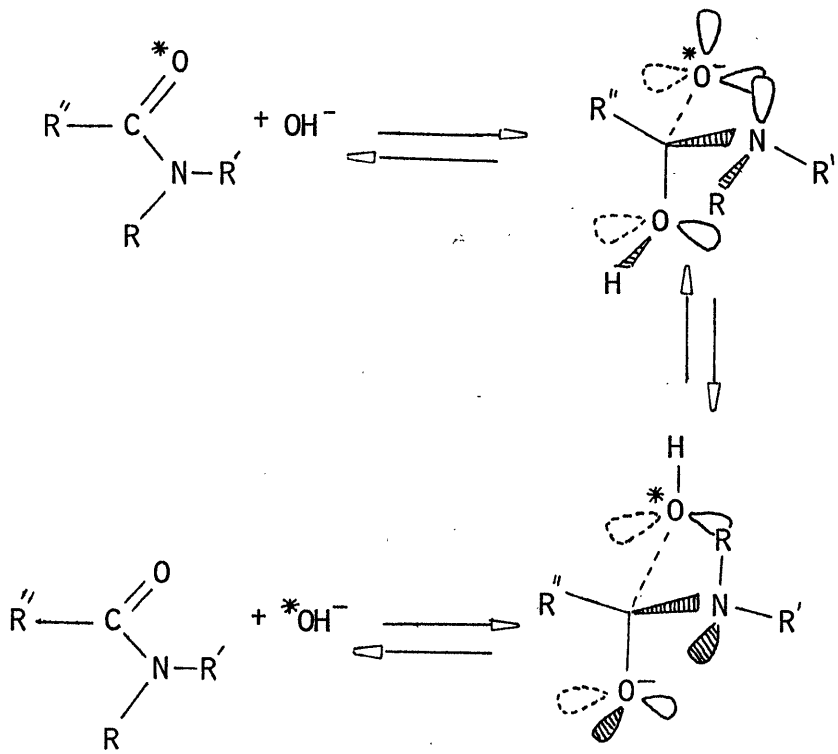


(13)

(iii) ^{18}O Exchange experiments

As noted above, the observation of ^{18}O exchange between the oxygen and solvent water during the alkaline hydrolysis of amides, was one of the first arguments both for the existence of the tetrahedral intermediate, and for its rate determining breakdown to products.

The exchange was observed for benzamide and N-methylbenzamide but, surprisingly, not for N,N-dimethylbenzamide. Recently, Deslongchamps[41,42] has suggested that stereoelectronic factors are relevant to the cleavage of tetrahedral intermediates in the hydrolysis of esters, amides and related compounds. The theory postulates that the conformation of the tetrahedral intermediate is transposed into the product of the reaction and that the cleavage of a C-O or C-N bond is only allowed if two hetero-atoms, in the intermediate, have lone pair orbital oriented antiperiplanar to the bond being broken. It is assumed that proton transfer to the tetrahedral intermediate is a very fast process which occurs before the cleavage of the tetrahedral intermediate takes place. Thus, an O-H bond is considered equivalent to a lone pair orbital. Primary and secondary amides have the correct orbital orientation to permit breakdown to products or reactants with concurrent oxygen exchange. Tertiary amides, however, do not have this property without rotation about the C-N bond. Since breakdown of a properly oriented group is faster than



Scheme 1.2,1b

a conformational change, no ^{18}O-exchange is observed (Scheme 1.2,1b).

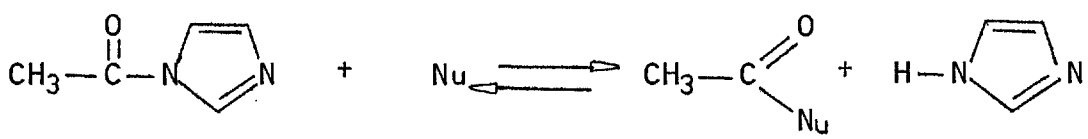
(iv) Structure and reactivity

Generally, rates of hydrolysis of amides are mildly favoured by electron withdrawing substituents and only small positive ρ values have been reported (e.g. ρ = 0.1 acetanilides) for substituent effects as expected for multistep reactions involving opposite electronic interactions[33].

Steric effects, however, are important and rate coefficients for the hydrolysis of alkylamides[30,43], imidazoles and hydroxamic acids[43] correlate with a modified form of the Taft equation. It was found that there is no significant differences between the steric effects on acid and base catalysed hydrolysis of amides and that steric effects are roughly the

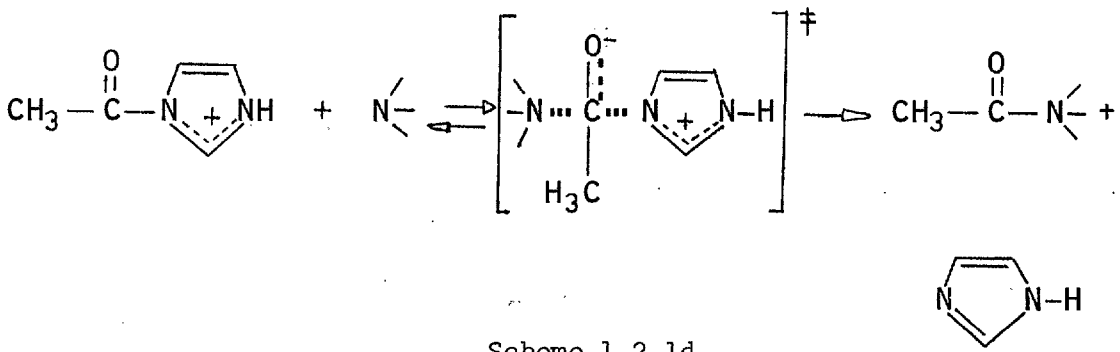
same as those for esters.

Leaving group ability is a very important factor influencing the reactivity of amides. Compounds like acetylimidazole and acetylimidazolium ion (pK_a of leaving groups 14.2 and 7, respectively), therefore, have a greatly enhanced reactivity compared with normal amides. The reaction of acetylimidazole with nucleophilic reagents reflects the fact that expulsion of an amine anion from an amide is difficult or impossible without assistance by general acid-base catalysis[44] (Scheme 1.2,1c). However acetylimidazolium ion



Scheme 1.2,1c

reacts with substituted phenoxides with a change in mechanism from general base to nucleophilic-catalysis at a pK_a value ca. 4.5[45]. Acetylimidazolium ion also reacts with acetate[46] and tertiary amines[44] concurrently by a nucleophilic catalysed pathway, and by a general base catalysed pathway. The existence of the nucleophilic pathway has been demonstrated by the amount of inhibition caused by added imidazole. This reaction is believed to involve a concerted mechanism without the formation of a tetrahedral intermediate because its lifetime must be very short (Scheme 1.2,1d). These reactions are further discussed in Section 2.2,3.



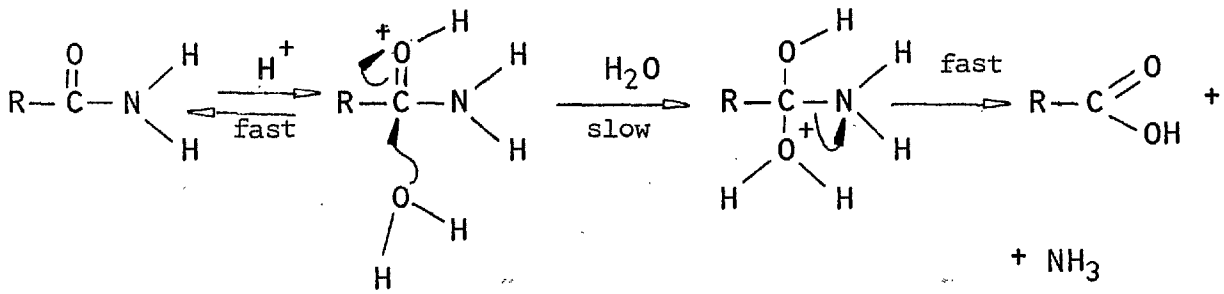
Scheme 1.2,1d

1.2,2 Acid catalysed hydrolysis of amides

The hydrolysis of amides in acid solutions has been thoroughly reviewed[27,28]. In dilute acid, the reaction rates have a first order dependence upon substrate and upon H_3O^+ (eq. 1.2,2a).

$$\text{rate} = \underline{K}_o\,[\text{amide}][H_3O^+] \qquad \ldots \text{ eq. 1.2,2a}$$

As the acidity increases, however, the rate of hydrolysis reaches a maximum and then decreases. The position of this maxima varies with both the structure of the amide and the acid in which the hydrolysis is being studied. This has been interpreted as evidence for a rate determining attack of H_2O on the protonated substrate. The amount of protonated substrate increases with acidity up to the rate maximum, after which, the decrease in rate reflects a corresponding decrease in water activity (Scheme 1.2,2a). Other evidence supports this mechanism. Deuterium solvent isotope effects are consistent with a preequilibrium protonation of the substrate[47] and the fact that no ^{18}O exchange is seen during the acid catalysed hydrolysis of amides (in contrast to base catalysed hydrolysis) confirms that the slow step is the formation of a tetrahedral intermediate which then rapidly breaks down to products. Recently, a very small amount of exchange was found for the hydrolysis of benzamide (rate of exchange 1.30×10^{-5} min^{-1}, 1/320 the rate of hydrolysis) which confirms the previous interpretation[48].



Scheme 1.2,2a

There has been much controversy about the position of protonation in amides. The bulk of the evidence indicates that the O-protonated cation is the dominant species in dilute and concentrated acid solutions existing in equilibrium with a small amount of the N-protonated cation. The N to O protonated ratio has been estimated to be between 10^{-3} and 10^{-7} [49,50,51]. Most of the early workers assumed that it was the thermodynamically favoured O-protonated amide which was involved in the amide hydrolysis (Scheme 1.2,2a) and recent studies on the hydrolysis of N,N-dialkylacetamide and N-acetyltrialkylammonium tetrafluoroborate salts[52], on the hydrolysis of N-acetylpyrolidines and N-acylpiperidines[53] and on the hydrolysis of benzamide and its N-alkyl derivatives[54] support this view. However, other workers have claimed that correlation of hydrolysis rates with a_{H_2O} is more consistent with reaction *via* the N-protonated form[55,56,57]. O'Connor[58], assuming that only the N-conjugated acid is involved, correlates successfully hydrolysis rates with a_{H_2O} for 60 sets of data in the literature. One of the criticisms of these equations is that the activity coefficients for the protonated amide and for the transition state ($f_{BH^+}/f^{\neq}$) are assumed to be medium independent which is not true for all examples[59].

A recent review of the protonation of amides has argued in favour of reaction *via* the N-conjugate acid but this appears to be a divergent view[60].

Acetanilides, however, are found to behave exceptionally at high acidities[61]. Here, rate maxima at intermediate acidities are followed by further increases in the rate at higher acidities. Also different substituent effects are evident at low (ρ = 1) and high acidities (ρ = 5). Both observations suggest a change from bimolecular to unimolecular mechanism as the acidity increases.

Summary

Despite much previous work, questions remain as to the debated mechanism of amide hydrolysis; particularly in regard to the reactive conjugate acid intermediate. The influence of N-substituents, such as nitro groups, on these reactions has not been widely investigated. For example, it is not known whether decomposition of the N-nitroamides in acid produces dinitration as well as hydrolysis. Also, the influence of electron withdrawing substituents must promote hydrolysis *via* a nucleophilic catalysed pathway in alkaline solutions, and by a unimolecular pathway under acid conditions (both effects arising from the enhanced leaving group properties of the N-nitroamine moiety, but again these effects have not been demonstrated.). The present work was intended to investigate some of these questions. Also, information about the behaviour of N-nitroamides towards nucleophilic entities may provide insight into the carcinogenic properties of N-nitroamines, which may be metabolised *in vivo* to the corresponding amides prior to interaction with cellular material.

CHAPTER 2

BASE CATALYSED HYDROLYSIS OF THE N-NITROAMIDES

RESULTS AND DISCUSSION

2.1 RESULTS OF THE BASE CATALYSED HYDROLYSIS OF THE N-NITROAMIDES

Rates of hydrolysis of both N-methyl-N-nitro-acetamide and benzamide were examined in the presence of several base catalysts. All reactions showed a first order dependence on both [substrate] and catalyst and therefore followed:

$$\text{Rate} = K_2 [\text{substrate}] [\text{catalyst}]$$

The observed pseudo first order rate coefficients, however, also contained

$$\text{Rate} = \underline{K}_o [\text{substrate}]$$

a contribution from the spontaneous water catalysed hydrolysis. Thus values of K_2 were obtained from plots of $\underline{K}_o$ versus [catalyst], where [catalyst] refers either to the stoichiometric concentration added or that calculated from the experimental pH of the reaction solution.

The U.V. spectra for the reaction showed good isosbestic points with the exception of the hydrolysis catalysed by imidazole as will be explained in Section 2.1,1d. Usually the reactions were well behaved and $\underline{K}_o$ was constant for at least three half lifes of the reaction as shown in Section 4.4,2.

2.1,1 N-Methyl-N-nitroacetamide

2.1,1a Hydrolysis in HOAc buffers (H_2O)

These reactions were carried out at two pH values in buffers containing $HOAc/OAc^-$ in the ratio of 1:1 and 4:1. The ionic strength of the solutions was kept at 0.75 M by the addition of $NaClO_4$. In determining

the actual $[OAc^-]$, the ionization constant of HOAc was calculated from the following expression[62],

$$pK_a = 4.757 - 1.018\sqrt{\mu} / (1 + 1.597\sqrt{\mu}) + 0.2446\,\mu$$

where μ is the ionic strength. Thus for $\mu = 0.75$ M $pK_a = 4.57$. Values of K_o are listed in Table 2.1,1a. Significantly, plots at each pH are parallel showing that only OAc^- catalysed the reaction.

Table 2.1,1a. Hydrolysis of N-Methyl-N-nitroacetamide in HOAc buffer at 25°C (H_2O). μ = 0.75 M ($NaClO_4$); Initial [substrate] ca. $2 \cdot 10^{-4}$ M

(HOAc)M	(NaOAc)M	pH	$[AcO^-]$M	$10^5 \cdot K_o s^{-1}$
0.052	0.052	4.49	0.047	3.9
0.100	0.100	4.48	0.089	4.8
0.150	0.150	4.54	0.147	6.8
0.152	0.152	4.52	0.161	7.1
0.200	0.200	4.56	0.200	8.8
0.250	0.250	4.60	0.260	11.8
0.300	0.300	4.64	0.320	12.5
0.350	0.350	4.64	0.380	15.9
0.08	0.02	3.94	0.019	2.3
0.16	0.04	3.96	0.039	2.9
0.24	0.06	3.98	0.061	3.9
0.32	0.08	3.98	0.082	4.8
1.00	0.25	3.89	0.210	7.7

A value for the second order rate coefficient $K_2 = 3.7 \cdot 10^{-4}$ l $mol^{-1}s^{-1}$ was calculated from these results.

2.1,1b Hydrolysis in HOAc buffers (D_2O)

HOAc catalysis in D_2O was examined in an analogous manner. Experimental values of pH were converted to the pD values by the expression[63]:

$$pD = pH + 0.4$$

The ionization constant of acetic acid was corrected using the relationship[64]:

$$K_{D_2O}^{HOAc} = \frac{K_{H_2O}^{HOAc}}{3.33} = 0.81 \cdot 10^{-5}$$

Experimental values of K_o are listed in Table 2.1,1b and plotted in Figure 2.1,1a.

Table 2.1,1b. Hydrolysis of N-methyl-N-nitroacetamide in acetate buffers at 25°C (D_2O). μ = 0.75 M ($NaClO_4$); Initial [substrate] ca. $2 \cdot 10^{-4}$ M

(HOAc)M	(NaOAc)M	pD	$[AcO^-]$M	$10^5 \cdot K_o s^{-1}$
0.052	0.052	5.20	0.059	2.1
0.100	0.100	5.19	0.112	3.9
0.152	0.152	5.21	0.173	5.3
0.200	0.200	5.21	0.230	7.4
0.300	0.300	5.26	0.360	11.2
0.350	0.350	5.30	0.430	13.1

A value of $K_2 = 2.9 \cdot 10^{-4}$ l $mol^{-1}s^{-1}$ was calculated. This leads to a value of $(K_2^{OAc^-})_{H_2O} / (K_2^{OAc^-})_{D_2O} = 1.3$. From the intercepts of the plots of K_o versus $[OAc^-]$, a value $\underline{K_o}^{H_2O} / \underline{K_o}^{D_2O} = 2.2$ was calculated.

2.1,1c Hydrolysis catalysed by pyridine

It was not possible to use pyridine buffers for these reactions because pyridine absorbs at the same wavelength as the substrate. Thus an external phosphate buffer was employed to which various amounts of pyridine were added. Table 2.1,1c summarises the first order rate coefficients obtained.

Table 2.1,1c. Hydrolysis of N-methyl-N-nitroacetamide catalysed by pyridine, at 25°C. (KH_2PO_4) - 0.3 M; (NaOH) - 0.225 M; μ = 0.75 M (NaCl); Initial [substrate] ca. $8 \cdot 10^{-4}$ M

10^4.(pyrid)M	pH	10^4.[pyrid]M	$10^4.K_o s^{-1}$
1.0	7.30	0.99	6.2
5.0	7.28	4.9	6.5
10.0	7.27	9.9	6.8
15.0	7.24	14.8	7.5
20.0	7.23	19.8	8.3

The value of the catalytic rate coefficient K_2 for pyridine was calculated to be 0.12 l $mol^{-1}s^{-1}$.

2.1,1d Hydrolysis catalysed by imidazole

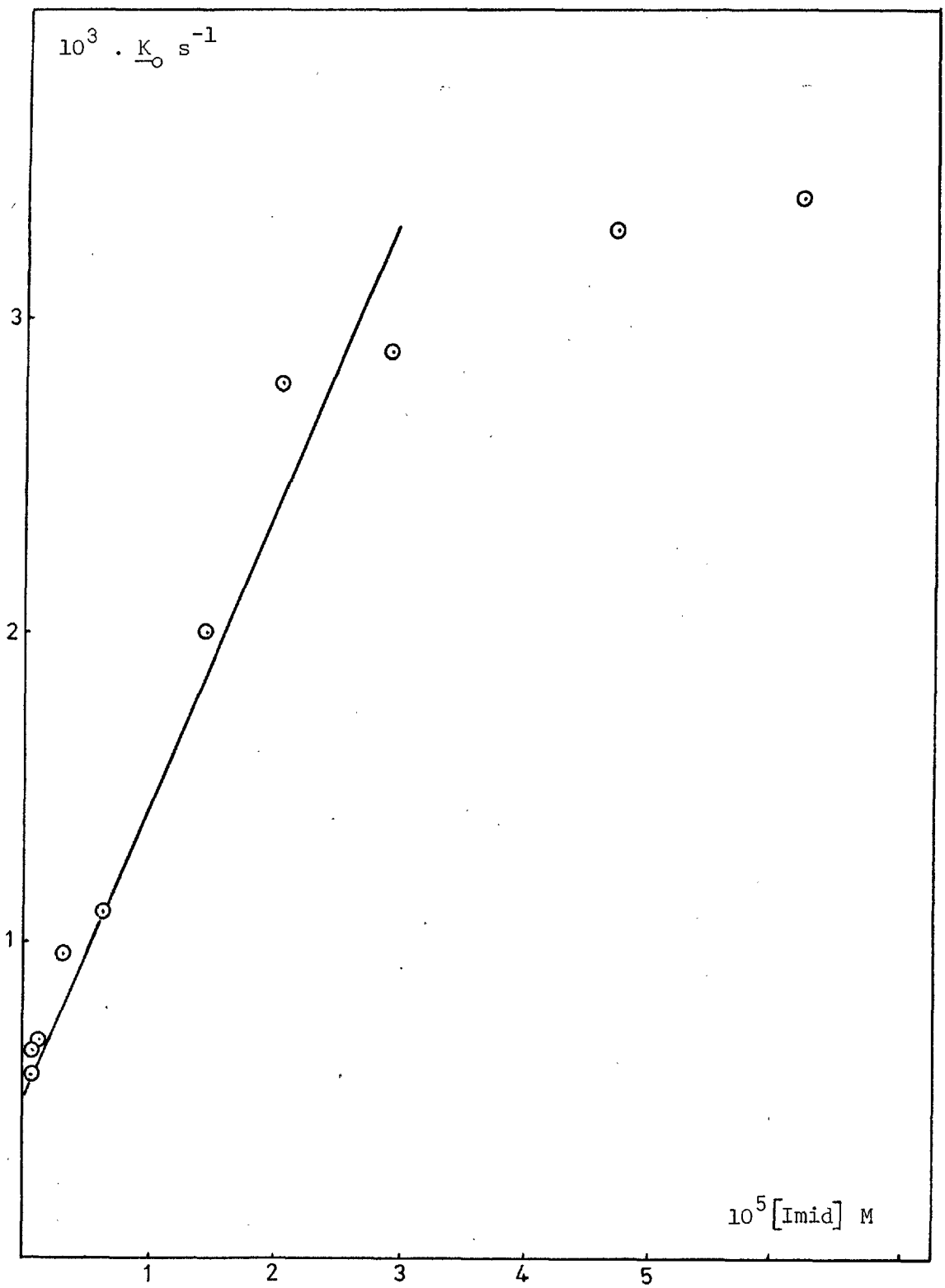
These reactions were also carried out in an external phosphate buffer, as explained in 2.1,1c. Values of K_o are listed in Table 2.1,1d a.

Table 2.1,1da. Hydrolysis of N-methyl-N-nitroacetamide catalysed by imidazole at 25°C. (KH_2PO_4) - 0.3 M; (NaOH) - 0.225 M; μ = 0.75 M (NaCl)

10^4.[substrate]M	10^4.(imid)M	pH	10^4.[Imid]M	$10^3.K_o s^{-1}$
2	0.5	7.20	0.29	0.58
2	1.0	7.19	0.58	0.66
2	2.0	7.20	1.2	0.69
2.	5.0	7.22	3.0	0.97
10	10.0	7.25	6.2	1.1
10	25.0	7.18	14.4	2.0
10	35.0	7.18	20.1	2.8
10	50.0	7.20	29.2	2.9
10	70.0	7.21	40.7	3.3
10	100.0	7.23	60.2	3.4

These results show that K_o becomes independent of [imidazole] at high concentrations (Figure 2.1,1d). Further, it was found that as the

Figure 2.1,1d. Hydrolysis of N-methyl-N-nitro acetamide catalysed by imidazole at 25°C



concentration of imidazole was raised, the U.V. spectra of the reaction solutions did not show the characteristic isosbestic point. This suggests that at high [imidazole] (i.e. when $\underline{K}_o$ is independent of [imidazole]), the acetylimidazole product (λ_{max} = 245 nm) interferes with the hydrolytic rate measurements.

The hydrolysis of an authentic sample of acetylimidazole, carried out under the same conditions as the hydrolysis of N-methyl-N-nitroacetamide gave results shown in Table 2.1,1db.

Table 2.1,1db. Comparison of the rate of hydrolysis of acetylimidazole and N-methyl-N-nitroacetamide catalysed by high [Imidazole]. (KH_2PO_4) - 0.3 M; (NaOH) - 0.225 M; μ = 0.75 M

(Imid)M	(acetamide)M	(acetylimid)M	(Methylnitramine)M	$10^3.K_o s^{-1}$
1.10^{-2}	1.10^{-3}	0	0	3.2
9.10^{-3}	0	1.10^{-3}	1.10^{-3}	3.3

The K_o values obtained for both reactions suggests that the maximum $\underline{K}_o$ in Table 2.1,1da actually refers to hydrolysis of the N-acetyl imidazole product.

Nevertheless, the formation of acetylimidazole suggests that imidazole is acting as a nucleophilic catalyst. From the values of K_o, at low [Imidazole], a value of K_2 = 0.94 l $mol^{-1}s^{-1}$ was calculated. This value may be incorrect since interference may also take place at low [imidazole].

2.1,1e. Hydrolysis catalysed by N-methylimidazole

These reactions were also performed in an external phosphate buffer, as explained in 2.1,1c. Values of $\underline{K}_o$ are reported in Table 2.1,1e.

The reactions showed good isosbestic points, hence the product, N-methyl-acetylimidazolium ion, is not interfering. Acetylimidazolium ions are known to hydrolyse faster than acetylimidazole.

Table 2.1,1e. Hydrolysis of N-methyl-N-nitroacetamide catalysed by N-methylimidazole at 25°C. (KH_2PO_4) - 0.3 M; (NaOH) - 0.225 M; μ = 0.75 M.

10^4[substrate]M	10^4.(N-Meimid)M	pH	10^4.[N-Meimid]M	$10^4.K_o\ s^{-1}$
4	0.52	7.32	0.45	6.1
8	0.72	7.34	0.62	7.1
8	1.0	7.24	0.86	7.7
4	1.5	7.34	1.3	8.3
8	2.0	7.29	1.7	9.2
8	2.5	7.32	2.2	10.1
8	5.0	7.26	4.3	12.3
8	6.8	7.30	5.9	15.0
8	11.0	7.25	9.0	19.7

These results gave a value of K_2 = 1.4 l $mol^{-1}s^{-1}$ for the N-methylimidazole catalysis.

2.1,1f Hydrolysis in phosphate buffers

These reactions were carried out in phosphate buffers prepared by the addition of $HClO_4$ to a solution of Na_2HPO_4. Two buffer ratios were used giving average pH values of 6.30 and 7.36. Their ionic strength was kept constant at 0.75 M by the addition of $NaClO_4$. The values of K_o obtained are listed in Table 2.1,1f.

Table 2.1,1f. Hydrolysis of N-methyl-N-nitroacetamide in phosphate buffer at 25°C. μ = 0.75 M ($NaClO_4$). Initial [substrate] ca.2.10^{-4} M

(a) $[HPO_4^{2-}] / [H_2PO_4^-] = 2$; average pH = 6.30

(Na_2PO_4) M	$(HClO_4)$ M	pH	$10^4.K_o s^{-1}$
0.10	0.050	6.27	0.8
0.15	0.075	6.28	1.3
0.20	0.100	6.30	1.6
0.25	0.125	6.33	2.0
0.30	0.150	6.34	2.3

(b) $[HPO_4^{2-}] / [H_2PO_4^-] = 8$; average pH = 7.36

(Na_2PO_4) M	$(HClO_4)$ M	pH	$10^4.K_o s^{-1}$
0.090	0.010	7.28	1.7
0.135	0.015	7.34	2.4
0.180	0.020	7.39	3.3
0.225	0.025	7.45	4.2

Both sets of results give a value of $K_2 = 1.7 . 10^{-3}$ l $mol^{-1}s^{-1}$. Thus the rate equation cannot contain second order terms in $[HPO_4^{2-}]$ as composite terms dependent on $[HO^-][HPO_4^{2-}]$.

2.1,1g Hydrolysis catalysed by morpholine

These reactions were also performed in an external phosphate buffer as explained in 2.1,1c. Values of K_o are listed in Table 2.1,1g and these lead to a value of K_2 = 44 l $mol^{-1}s^{-1}$.

Table 2.1,1g. Hydrolysis of N-methyl-N-nitroacetamide catalysed by morpholine at 25°C. (KH_2PO_4) - 0.3 M; (NaOH) - 0.225 M; μ = 0.75 M. Initial [substrate] ca. 8.10^{-4}.

10^4.(morph)M	pH	10^5.[morph]M	$10^4.K_o s^{-1}$
0.75	7.41	0.72	8.9
1.00	7.27	0.88	9.6
2.60	7.27	2.20	15.6
4.00	7.27	3.50	20.4
5.00	7.31	4.50	24.3

2.1,1h Hydrolysis catalysed by p-chlorophenol

The hydrolysis of N-methyl-N-nitroacetamide catalysed by p-chlorophenol was examined in external borate buffers at pH 8.30, 8.86 and 9.44. The ionic strength was kept constant at 0.75 with $NaClO_4$. The first order rate coefficients obtained are listed in Table 2.1,1h.

Table 2.1,1h. Hydrolysis of N-methyl-N-nitroacetamide catalysed by p-chlorophenol at 25°C. μ = 0.75 M ($NaClO_4$). Initial substrate ca. 2.10^{-4} M.

(a) ($Na_2B_4O_7$) - $1.25 . 10^{-2}$ M; (HCl) - $1.3 . 10^{-2}$ M; average pH = 8.30

10^4.(p-Clphenol)M	pH	10^5.[p-Clphenol]M	$10^4.K_o s^{-1}$
0.8	8.30	0.93	4.2
1.0	8.24	1.03	4.4
2.0	8.30	2.33	6.4
3.0	8.30	3.49	7.9

(b) ($Na_2B_4O_7$) - $1.25 . 10^{-2}$ M; (NaOH) - $9 . 10^{-4}$ M; average pH = 8.86

10^4.(p-Clphenol)M	pH	10^5.[p-Clphenol]M	$10^3.K_o s^{-1}$
0.5	8.86	1.62	1.1
0.8	8.85	2.55	1.3
1.0	8.86	3.24	1.5
2.0	8.86	6.47	2.1
3.0	8.86	9.71	2.5

(c) $(Na_2B_4O_7)$ - 1.25 . 10^{-2} M; (NaOH) 1.2 . 10^{-2} M; average pH = 9.44

10^4.(p-Clphenol)M	pH	10^5.[p-Clphenol]M	$10^3.K_o s^{-1}$
0.8	9.44	5.2	4.2
1.0	9.45	6.5	4.5
1.5	9.43	9.6	4.7
2.0	9.44	12.9	6.0

The graphical representation of these results in Figure 2.1,1h shows the absence of second order terms in both catalyst and HO^-. The average value of K_2 is 16 l $mol^{-1}s^{-1}$.

2.1,1i Hydrolysis catalysed by piperidine and 2,2',6,6'-tetramethyl piperidine

These reactions were carried out in an external borate buffer of pH ca.8.84 and ionic strength 0.75 M, with varying piperidine concentrations. Values of K_o are listed in Table 2.1,1ia from which K_2 = 1535 l $mol^{-1}s^{-1}$ was calculated.

Table 2.1,1ia. Hydrolysis of N-methyl-N-nitroacetamide catalysed by piperidine at 25°C. $(B_4O_7Na_2)$ - 1.25 . 10^{-2} M; (NaOH) - 2.6 . 10^{-2} M; μ = 0.75 M $(NaClO_4)$. Initial [substrate] ca.1.8 . 10^4

10^5.(piperidine)M	pH	10^7[piperidine]M	$10^3.K_o s^{-1}$
2.5	8.82	1.2	1.1
6.6	8.84	3.4	1.3
16.	8.86	8.9	1.9
40.	8.82	19.8	3.9
60.	8.85	31.8	5.7
75.	8.87	41.9	7.4

Reactions were also performed under similar conditions but using the hindered base 2,2',6,6'-tetramethylpiperidine. The results obtained are shown in Table 2.1,1ib.

Figure 2.1,1h. Hydrolysis of N-methyl-N-nitroacetamide catalysed by p-chlorophenol at 25°C.

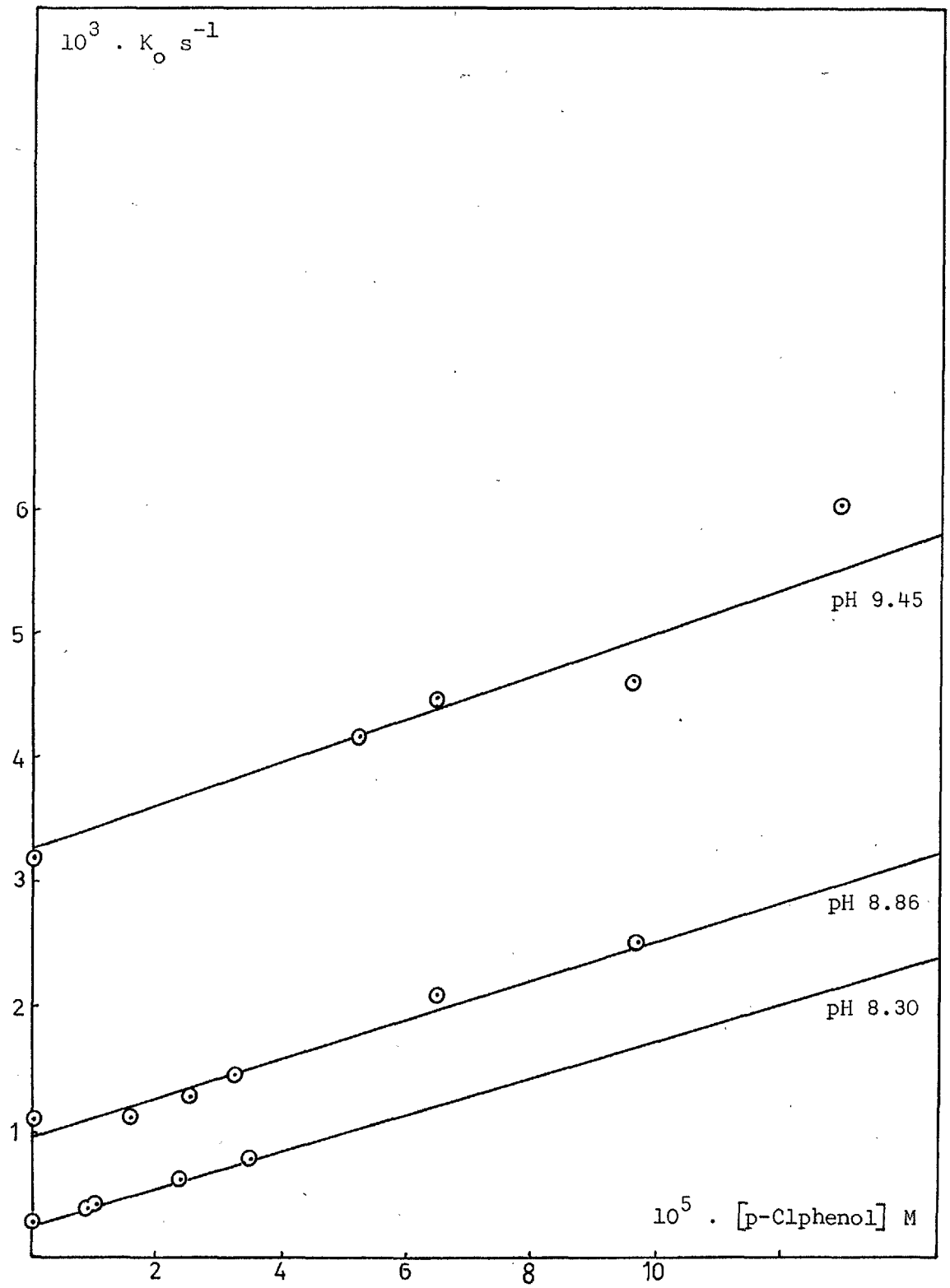


Table 2.1,lib. Hydrolysis of N-methyl-N-nitroacetamide catalysed by 2,2',6,6'-tetramethylpiperidine at 25°C. ($B_4O_7Na_2$) - 1.25 . 10^{-2} M; (NaOH) - 2.6 x 10^{-2} M; μ = 0.75 M ($NaClO_4$). Initial [substrate] ca. 1.8 . 10^{-4} M

10^4.(tetramethylpip)M	pH	10^6.[tetramethylpip]M	$10^3.K_o s^{-1}$
5.0	8.83	2.89	1.08
7.5	8.85	4.87	1.07
10.	8.85*	6.03	1.12
20.	8.85*	12.1	1.18
40.	8.85*	24.0	1.51

* estimated pH value

The low value of K_2 = 21 l $mol^{-1}s^{-1}$ obtained for hindered base confirms catalysis is nucleophilic rather than general base.

2.1,1j Hydrolysis in borate buffers (H_2O)

To investigate catalysis by HO^-, the reaction was examined in borate buffers of different pH. Evidence in Table 2.1,lja shows that the buffer components do not significantly catalyse the reaction. The borate buffers were prepared by addition of NaOH to a solution of $Na_2B_4O_7$. The ionic strength was kept constant at 0.75 M with $NaClO_4$ [65].

Table 2.1,lja. Hydrolysis of N-methyl-N-nitroacetamide in borate buffer at 25°C. μ = 0.75 M ($NaClO_4$). Initial [substrate] ca. 2.10^{-4} M

10^2.($Na_2B_4O_7$)M	10^2.(HCl)M	pH	$10^4.K_o s^{-1}$
1.19	1.2	8.37	3.2
2.39	2.4	8.34	3.5
3.59	3.6	8.29	3.6
4.79	4.8	2.28	3.6

Values of K_o for pH 8.85 to 9.24 are listed in Table 2.1,ljb. This lead to a value of K_2 = 110 l $mol^{-1}s^{-1}$ for HO^-.

Table 2.1,1ja. Hydrolysis of N-methyl-N-nitroacetamide in borate buffer at 25°C (H_2O). ($Na_2B_4O_7$) - 1.2 . 10^{-2} M; μ = 0.75 M. Initial [substrate] ca. 2 . 10^{-4} M.

10^2(NaOH)M	pH	$10^3.K_o s^{-1}$
0	8.83	0.9
0.09	8.85	1.0
0.88	9.13	1.9
1.00	9.24	2.3
1.20	9.40	3.2

2.1,1h Hydrolysis in borate buffers (D_2O)

To determine the solvent deuterium isotope effect reactions were also carried out with borate buffers in D_2O. Experimental values of pH were converted to pD values as described in section 2.1,1b, and the results are summarised in Table 2.1,1h.

Table 2.1,1h. Hydrolysis of N-methyl-N-nitroacetamide in borate buffer at 25°C (D_2O). ($Na_2B_4O_7$) - 1.25 . 10^{-2} M; μ = 0.75 M. Initial [substrate] ca. 2. 10^{-4} M.

10^2(NaOH)M	pH	$10^3.K_o s^{-1}$
0.09	9.40	0.7
0.88	9.69	1.0
1.00	9.83	1.4
1.20	9.89	1.8

A value of K_2 = 21 l $mol^{-1}s^{-1}$ was obtained for catalysis by OD^-. Thus the isotopic rate ratio is $K_2^{HO^-}/K_2^{DO^-}$ = 5.2. Such a value is normally associated with a slow proton transfer in the transition state. The intercepts of the plots of K_o vs $[HO^-]$ or $[DO^-]$ allow calculation of the solvent deuterium isotope effect for the spontaneous reactions. The value of $K_o^{H_2O} / K_o^{D_2O}$ = 2.1 obtained is similar to the value

for reactions in HOAc buffers.

2.1,2 N-methyl-N-nitrobenzamide

2.1,2a Hydrolysis in acetate buffers (H_2O)

The reactions were carried out in acetic acid-sodium acetate (1:1) buffers, the ionic strength being maintained at 0.75 M by addition of $NaClO_4$. The concentration of $[AcO^-]$ was calculated as in section 2.1,1a. Values of K_o listed in Table 2.1,2a lead to $K_2^{OAc^-} = 9.9 \cdot 10^{-4}$ l $mol^{-1} sec^{-1}$.

Table 2.1,2a. Hydrolysis of N-methyl-N-nitrobenzamide in acetate buffer at 25°C. μ = 0.75 M ($NaClO_4$), ethanol 1%. Initial [substrate] ca. $8 \cdot 10^{-5}$ M

(HOAc)M	(NaOAc)M	pH	$[AcO^-]$M	$10^4 . K_o s^{-1}$
0.05	0.05	4.60	0.05	1.2
0.10	0.10	4.62	0.10	1.5
0.15	0.15	4.64	0.16	2.1
0.20	0.20	4.66	0.22	2.9
0.25	0.25	4.67	0.28	3.3
0.35	0.35	4.70	0.40	4.7

2.1,2b Hydrolysis in HOAc buffers (D_2O)

HOAc catalysis was also examined in D_2O solutions. The necessary calculations were performed as in 2.1,1b and values of K_o are listed in Table 2.1,2b.

Table 2.1,2b. Hydrolysis of N-methyl-N-nitrobenzamide in acetate buffer (D_2O). μ = 0.75 M ($NaClO_4$); ethanol 1%. Initial [substrate] ca. $8 \cdot 10^{-5}$ M.

(HOAc) M	(NaOAc) M	pD	$[AcO^-]$ M	$10^4 . K_o s^{-1}$
0.052	0.052	5.03	0.048	0.7
0.100	0.100	5.05	0.095	1.1
0.152	0.152	5.02	0.140	1.7
0.200	0.200	5.04	0.190	2.2
0.250	0.250	5.06	0.240	2.8
0.300	0.300	5.14	0.320	3.6
0.300	0.300	5.08	0.300	3.3

The value of K_2 obtained from these results is the same as for reactions in H_2O. From the intercepts of plots of K_o versus $[OAc^-]$ a value of $K_o^{H_2O} / K_o^{D_2O}$ = 2.06 was calculated.

2.1,2c. Hydrolysis catalysed by pyridine

As explained in 2.1,1c reactions were performed at several [pyridine] with an external phosphate buffer of pH ca.6.9 and μ = 0.75 M. Values of K_o are listed in Table 2.1,2c and these give $K_2 = 4.5 \cdot 10^{-2}$ l mol^{-1} s^{-1}.

Table 2.1,2c. Hydrolysis of N-methyl-N-nitrobenzamide catalysed by pyridine at 25°C. (KH_2PO_4) - 0.1 M; (NaOH) - 0.075 M; μ = 0.75 M (NaCl); ethanol - 1%. Initial [substrate] ca. 10^{-4} M

10^4.(pyridine) M	pH	10^4 [pyridine] M	$10^4 . K_o s^{-1}$
5.0	6.96	4.9	4.3
7.5	6.98	7.4	4.4
10.0	6.88	9.8	4.6
20.0	6.87	19.5	4.9
25.0	6.99	24.0	4.7
40.0	6.89	39.1	5.4
50.0	7.02	49.0	6.0

2.1,2d Hydrolysis catalysed by imidazole

Reaction conditions as in 2.1,1c were used. Values of K_o are given in Table 2.1,2d and plotted in Figure 2.1,2d.

Table 2.1,2d. Hydrolysis of N-methyl-N-nitrobenzamide catalysed by imidazole at 25°C. (KH_2PO_4) - 0.1 M; (NaOH) - 0.075 M; μ = 0.75 (NaCl). Ethanol - 1%.

10^4[substrate]M	10^4(Imidaz)M	pH	10^4[Imidaz]M	$10^4.K_o s^{-1}$
2.	1	6.99	0.52	4.1
2.	4	6.95	2.0	4.7
10.	7	6.99	3.66	5.3
10.	10	6.94	4.97	6.1
10.	10	6.89	4.63	6.1
10.	20	6.92	9.61	6.3

Figure 2.1,2d shows a plateau at high [Imidazole], as in the case of N-methyl-N-nitroacetamide (2.1,1d), and the kinetic runs also do not show good isosbestic points. Thus it seems likely that slow decomposition of benzoylimidazole, the product of the nucleophilic reaction, which is known to absorb in this region (λ_{max} 242 nm [66]), inteferes with these measurements.

2.1,2e Hydrolysis catalysed by N-methylimidazole

Reaction conditions as in 2.1,2c were used. Values of K_o are listed in Table 2.1,2e.

Figure 2.1,2d. Hydrolysis of N-methyl-N-nitrobenzamide catalysed by imidazole at 25°C

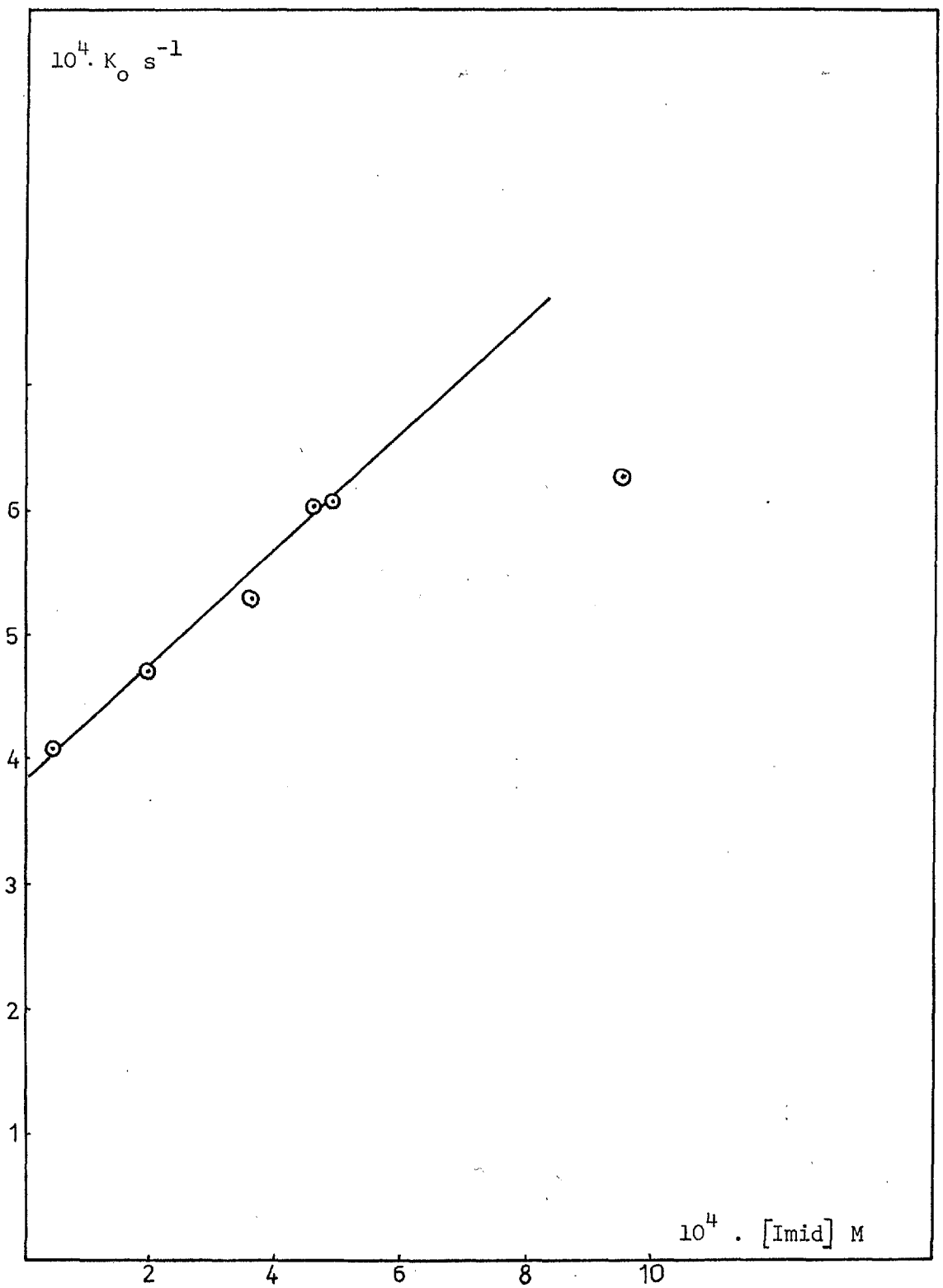


Table 2.1,2e. Hydrolysis of N-methyl-N-nitrobenzamide catalysed by N-methylimidazole at 25°C. (KH_2PO_4) - 0.1 M; (NaOH) - 0.075 M; μ = 0.75 M (NaCl); ethanol 1%. Initial [substrate] ca. $8 \cdot 10^{-5}$ M

10^4(N-Meimid)M	pH	10^4[N-Meimid]M	$10^4 . K_o s^{-1}$
0.5	7.01	0.27	5.9
1.0	7.00	0.53	7.3
1.0	6.88	0.46	6.4
2.0	7.02	1.09	9.8
2.0	6.88	0.92	8.5
3.0	7.03	1.60	12.0

A value for the second order rate coefficient of 5.1 l $mol^{-1}s^{-1}$ was calculated from these data.

2.1,2f Hydrolysis in phosphate buffer

The hydrolysis was studied in phosphate buffers of pH ca. 6.93 and ionic strength 0.75 M. Values of K_o are listed in Table 2.1,2f.

Table 2.1,2f. Hydrolysis of N-methyl-N-nitrobenzamide in phosphate buffer at 25°C. μ = 0.75 M (NaCl); ethanol 1%; Initial [substrate] ca. $8 \cdot 10^{-5}$ M

(KH_2PO_4)M	(NaOH)M	pH	$10^3[HPO_4^{2-}]$M	$10^4 . K_o s^{-1}$
0.01	0.0075	6.83	0.6	1.6
0.05	0.0375	6.87	3.2	2.4
0.10	0.075	6.94	7.4	3.7
0.20	0.15	7.08	18.0	6.7

These data give a value of $K_2 = 9.9 \cdot 10^{-4}$ l $mol^{-1}s^{-1}$.

2.1,2g Hydrolysis catalysed by morpholine

These reactions were performed in phosphate buffers of pH ca. 7.0 and ionic strength 0.75 M. Values of K_o are reported in Table 2.1,2g

from which a value of K_2 = 9.3 l $mol^{-1}s^{-1}$ was obtained.

Table 2.1,2g. Hydrolysis of N-methyl-N-nitrobenzamide catalysed by morpholine at 25°C. (KH_2PO_4) - 0.1 M; (NaOH) - 0.075 M; μ = 0.75 (NaCl). Initial [substrate] ca. 8 . 10^{-5} M

10^4.(morph)M	pH	10^6.[morph]M	$10^4.K_o s^{-1}$
1.	6.95	4.2	3.6
2.	6.95	8.3	4.1
4.	7.02	19.0	5.2
6.	7.02	28.0	6.0

2.1,2h Hydrolysis in borate buffers (H_2O)

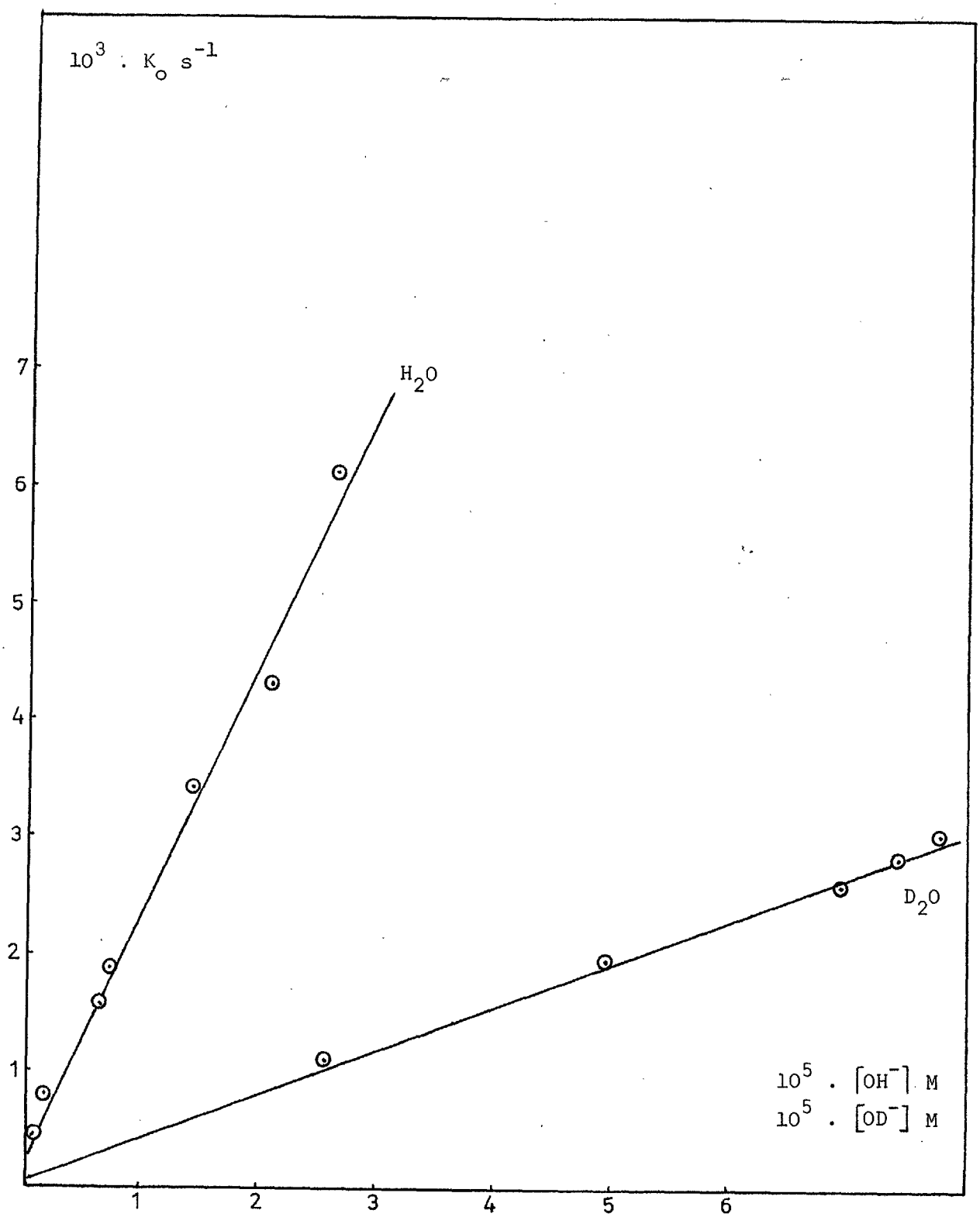
The reaction was studied over the pH range 7.89 to 9.24 in aqueous borate buffers prepared by the addition of HCl or NaOH to a solution of $Na_2B_4O_7$. The ionic strength was kept constant at 0.75 M with $NaClO_4$. Values of K_o are listed in Table 2.1,2ha.

Table 2.1,2ha. Hydrolysis of N-methyl-N-nitrobenzamide in borate buffers at 25°C. $(Na_2B_4O_7)$ - 1.25 . 10^{-2} M; μ = 0.75 M ($NaClO_4$). Initial [substrate] ca. 8 . 10^{-5} M.

(HCl)M	pH	$10^3.K_o s^{-1}$
0.020	7.89	0.5
0.015	8.23	0.8
(NaOH)M		
-	8.82	1.5
0.001	8.86	1.8
0.009	9.15	3.4
0.010	9.24	4.3
0.012	9.42	6.1

The plot of K_o versus HO^- (Figure 2.1,2h) gives $K_2^{OH^-}$ = 206 l $mol^{-1}s^{-1}$.

Figure 2.1,2h. Hydrolysis of N-methyl-N-nitrobenzamide in borate buffers at 25°C



These reactions are not significantly catalysed by borate as shown in Table 2.1,2hb.

Table 2.1,2hb. Hydrolysis of N-methyl-N-nitrobenzamide in borate buffers at 25°C. μ = 0.75 M ($NaClO_4$); Initial [substrate] ca. 8.10^{-5} M

$10^2(Na_2B_4O_7)M$	$10^2.(NaOH)M$	pH	$10^3.K_o s^{-1}$
1.25	0.88	9.16	3.67
2.50	1.76	9.22	3.54
3.75	2.64	9.28	4.55

2.1,2i Hydrolysis in borate buffers (D_2O)

Solvent deuterium isotope effects were determined by similar reactions in D_2O as explained in section 2.1,1h. Table 2.1,2i shows K_o values for the hydrolysis in D_2O.

Table 2.1,2i. Hydrolysis of N-methyl-N-nitrobenzamide in borate buffers (D_2O) at 25°C. ($Na_2B_4O_7$) - 1.25 . 10^{-2} M; μ = 0.75 M ($NaClO_4$). Initial [substrate] ca. 8 . 10^{-5} M

$10^2(NaOH)M$	pD	$10^3.K_o s^{-1}$
0.09	9.40	1.1
0.88	9.69	1.9
1.00	9.84	2.6
1.20	9.89	3.1

A value of K_2 = 36 l $mol^{-1}s^{-1}$ was obtained from Figure 2.1,2h from which $K_2^{HO^-} / K_2^{DO^-}$ = 5.7 was calculated. From the intercepts of the same Figure $K_o^{H_2O} / K_o^{D_2O}$ = 2.0 was obtained. This value is similar to the value obtained in HOAc buffers.

2.1,2j O^{18} Exchange experiments

A large scale hydrolysis of O^{18} carbonyl labelled N-methyl-N-nitrobenzamide was performed in phosphate buffer in which samples of starting material were extracted at timed intervals and analysed for the loss of ^{18}O by mass spectrometry. No loss of the ^{18}O was seen, as shown in Table 2.1,2j. Another experiment was undertaken in which the reaction was allowed to go to completion. Here, the product being extracted was the O^{18} carbonyl labelled benzoic acid in which no loss of the O^{18} was also found. Details of these experiments are reported in Section 4.6.

Table 2.1,2j. O^{18} Percentages of incorporation of N-methyl-N-nitrobenzamide

	(M+2) · 100/M M = 180, 122	(M+2) · 100/M M = 105
Starting material	11.6	8.3
Sample 1 (10 min)	12.1	8.7
Sample 2 (20 min)	12.1	9.1
Sample 3 (30 min)	12.8	11.0
Benzoic acid	11.4	5.9

2.1,3 N-methyl-N-nitro-p-chlorobenzamide

2.1,3a Hydrolysis in phosphate buffer

The reaction was studied in phosphate buffers made by addition of a solution of $HClO_4$ to a solution of Na_2HPO_4. The ionic strength was kept constant at 0.75 M by addition of $NaClO_4$. Values of K_o listed in Table 2.1,3a, gave a value of $K_2 = 4.9 \cdot 10^{-3}$ l $mol^{-1}s^{-1}$.

Table 2.1,3a. Hydrolysis of N-methyl-N-nitro-p-chlorobenzamide in phosphate buffers at 25°C. μ = 0.75 M ($NaClO_4$); dioxane 1%. Initial [substrate] ca. $9.9 \cdot 10^{-5}$ M

(Na_2HPO_4)M	$(HClO_4)$M	pH	$10^4.K_o\ s^{-1}$
0.05	0,025	6.35	2.7
0.10	0.05	6.38	3.8
0.20	0.10	6.42	6.5
0.30	0.15	6.48	8.7

2.1,3b Hydrolysis in borate buffer

The hydrolysis was performed in borate buffers prepared by the addition of HCl to a solution of $Na_2B_4O_7$. The ionic strength was kept constant at 0.75 M by the addition of $NaClO_4$. Values of K_o are reported in Table 2.1,3b.

Table 2.1,3b. Hydrolysis of N-methyl-N-nitro-p-chlorobenzamide in borate buffer at 25°C. $(B_4O_7Na_2)$ - $1.25 \cdot 10^{-2}$ M; Initial [substrate] ca. $9.9 \cdot 10^{-5}$ M

$10^2.(HCl)$M	pH	$10^3.K_o\ s^{-1}$
2.48	7.36	0.6
2.23	7.73	1.0
1.98	7.89	1.2
1.49	8.20	2.1
0.99	8.42	3.6
0.99	8.42	3.8
0.49	8.60	5.1

These gave a value of $K_2^{HO^-}$ = 1246 l $mol^{-1}s^{-1}$ for HO^- catalysis.

2.1,4 N-methyl-N-nitro-p-nitrobenzamide

2.1,4a Hydrolysis in borate buffer

The hydrolysis was performed in borate buffers as in 2.1,3b. Values of K_o are reported in Table 2.1,4a.

Table 2.1,4a: Hydrolysis of N-methyl-N-nitro-p-nitrobenzamide in borate buffer at 25°C. ($B_4O_7Na_2$) - $1.25 \cdot 10^{-2}$ M; μ = 0.75 M ($NaClO_4$); dioxane - 1%. Initial [substrate] ca.$9.9 \cdot 10^{-5}$ M.

10^2(HCl)M	pH	$10^3.K_o$ s^{-1}
2.48	7.38	3.3
2.23	7.74	5.4
1.98	7.89	6.35
1.98	7.89	6.36
1.49	8.19	10.9
1.49	8.20	9.9

These data give K_2 = 5204 l $mol^{-1}s^{-1}$ for HO^- catalysis.

2.2 DISCUSSION OF THE BASE CATALYSED HYDROLYSIS OF N-NITROAMIDES

The results reported in Section 2.1 show that N-methyl-N-nitroamides hydrolyse readily in the presence of various base catalysts with the formation of the corresponding carboxylic acids and methylnitramine. Only first order dependences upon substrate and catalyst are observed (eq. 2.2) and the absence of higher order catalytic terms is significant.

$$\text{Rate} = K_2 \text{ [N-nitroamide] [catalyst]} \quad \text{... eq. 2.2}$$

Values of K_2 obtained for several catalysts are summarised in Table 2.2

Table 2.2 Second order rate coefficients at 25°C for the hydrolysis of N-nitroamides

N-Methyl-N-nitroacetamide

Catalyst	pK_a	Average pH	K_2 l $mol^{-1}s^{-1}$
H_2O	-1.7	-	0.00001
acetate (H_2O)	4.57	4.55; 3.95	0.00037
acetate (D_2O)	5.09	5.23	0.00029
pyridine	5.2	7.6	0.12
imidazole	7.1	7.21	0.94
N-methylimid.	7.0	7.29	1.4
phosphate	6.9	6.30; 7.36	0.0018
morpholine	8.7	7.31	44.5
p-chlorophenol	9.28	8.30; 8.86; 9.44	16.4
piperidine	11.2	8.84	1533.
tetramethylpiper.	11.07	8.85	20.
hydroxide	15.75	8.83 - 9.40	110.
deuteroxide	16.61	9.40 - 9.89	21.

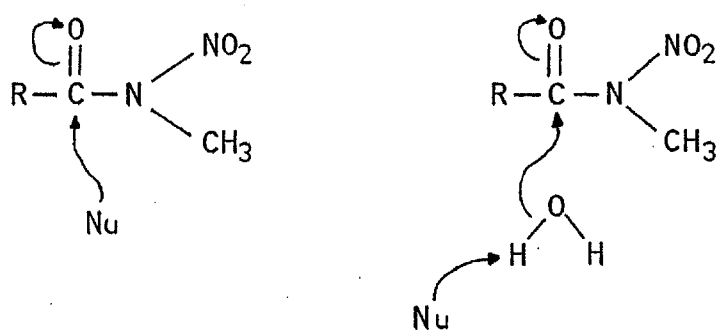
N-Methyl-N-nitrobenzamide

Catalyst	pK_a	Average pH	K_2 l $mol^{-1}s^{-1}$
H_2O	-1.7	-	0.000025
acetate (H_2O)	4.57	4.65	0.00099
acetate (D_2O)	5.09	5.06	0.00099
pyridine	5.2	6.94	0.044
imidazole	7.1	6.95	0.46
N-methylimid.	7.0	6.97	5.1
phosphate	6.9	6.93	0.0036
morpholine	8.7	6.98	9.3
hydroxide	15.75	7.89 - 9.42	206.
deuteroxide	16.61	9.40 - 9.89	36.

2.2, 1 Nucleophilic versus base catalysis

(i) Product and kinetic evidence

The first mechanistic question of interest is whether hydrolysis involves nucleophilic or general base catalysis (Figure 2.2,1)



Nucleophilic catalysis General base catalysis

Figure 2.2,1

For both N-metyl-N-nitroacetamide and N-methyl-N-nitrobenzamide, in the presence of imidazole, the formation of N-acetylimidazole and N-benzoyl-

imidazole, products of the nucleophilic pathway, were detected by u.v. assay of the reaction solutions. Further, several kinetic tests are consistent with a nucleophilic catalysed reaction. Thus, rate ratios (Table 2.2,1a) for N-methylimidazole *versus* HPO_4^{2-}, N-methylimidazole *versus* HO^-, pyridine *versus* OAc^- and piperidine *versus* 2,2'-6,6'-tetramethylpiperidine are of the order expected for nucleophilic catalysis[67].

Table 2.2,1a. Nucleophilic versus general base catalysis

	$CH_3CON(NO_2)CH_3$	$C_6H_5CON(NO_2)CH_3$
$\underline{K}_2$ MeIm / $\underline{K}_2$ HPO_4^{2-}	794.	1416.
$\underline{K}_2$ pyrid / $\underline{K}_2$ OAc^-	310.	44.
$\underline{K}_2$ HO^- / $\underline{K}_2$ MeIm	77.	40.
$\underline{K}_2$ pip / $\underline{K}_2$ tetraMepip	76.	-

(ii) Solvent deuterium isotope effect

A general base catalysed mechanism is usually associated with a solvent deuterium isotope effect ($\underline{K}_{2H}$ / $\underline{K}_{2D}$) greater than 2, whereas the nucleophilic reaction normally has a lower isotope effect[67]. Values of the solvent deuterium isotope effect for the acetate catalysed reaction, for the water reaction and for HO^- catalysed reaction are listed in Table 2.2,1b.

Table 2.2,1b. Solvent deuterium isotope effects at 25°C

	$CH_3CON(NO_2)CH_3$	$C_6H_5CON(NO_2)CH_3$
$(\underline{K}_2^{OAc^-})_{H_2O} / (\underline{K}_2^{OAc^-})_{D_2O}$	1.3	1.0
$(\underline{K}_o)_{H_2O} / (\underline{K}_o)_{D_2O}$	2.2	2.1
K_{HO^-} / K_{DO^-}	5.2	5.7

The isotope effect for the acetate reaction is that expected for a nucleophilic pathway. However, the value for the water reaction is within the range usually found for general base catalysis. It is conceivable that this reflects general base catalysis by water of the water reaction (Figure 2.2,2) and similar differences for other nucleophilic catalysed reactions are known. Thus examples in Table 2.2,1c

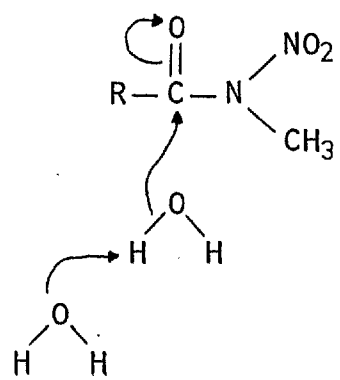


Figure 2.2,2

show that compounds reacting via a nucleophilic pathway have higher solvent isotope effects for the water catalysed reaction than for the nucleophilic catalysed.

Table 2.2,1c. Solvent deuterium isotope effects at 25°C

	catalysed reaction $\underline{K}_H / \underline{K}_D$	water reaction $\underline{K}_{H_2O} / \underline{K}_{D_2O}$
acetic anhydride + formate	1.07[68]	2.9[69]
benzoic anhydride + OAc^-	1.48[70]	3.9[71]
acetylimidazolium ion + NH_3	1.3[72]	2.5[72]

The large solvent deuterium isotope effect observed for the hydroxide catalyst remains (K_{HO^-}/K_{DO^-} ca. 5) and this appears to be too large for simple nucleophilic catalysis by this entity. It suggests that HO^- catalyses the reaction in a different way from other catalysts as is discussed further below.

2.2,2 Correlation of the rates of reaction with the basicity of catalyst

Variation of the rate coefficients ($\underline{K}_2$) for both N-nitroamides studied with catalyst pK_a are shown in Figures 2.2,2a and 2.2,2b. Both compounds give similar plots suggesting a common raction mechanism. It can be seen that the nitrogen bases lie on a line from which β values of 0.64 and 0.60 are obtained for N-methyl-N-nitroacetamide and N-nitro-N-methyl-benzamide, respectively. These values are not far away from the values obtained from other nucleophilic reactions, e.g. for p-nitrophenyl acetate β ca. 0.8. Imidazole does not show the usually enhanced reactivity attributed to an α-effect. This may reflect steric congestion in the transition state, or alternatively the value of K_2 for imidazole may be incorrect due to interference of acetylimidazole (Section 2.1). Both

Figure 2.2,1a. Brönsted plot for N-methyl-N-nitroacetamide

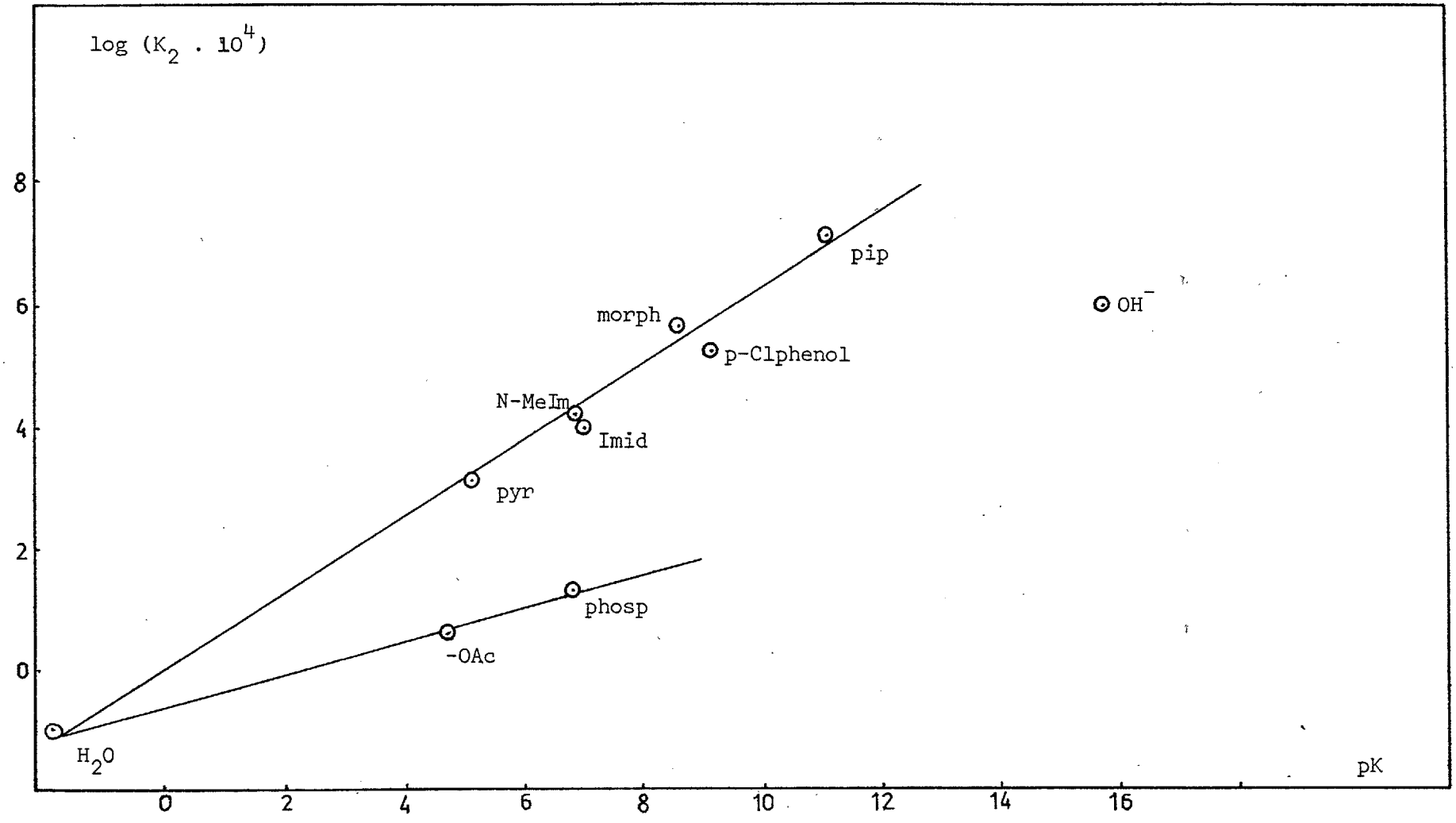
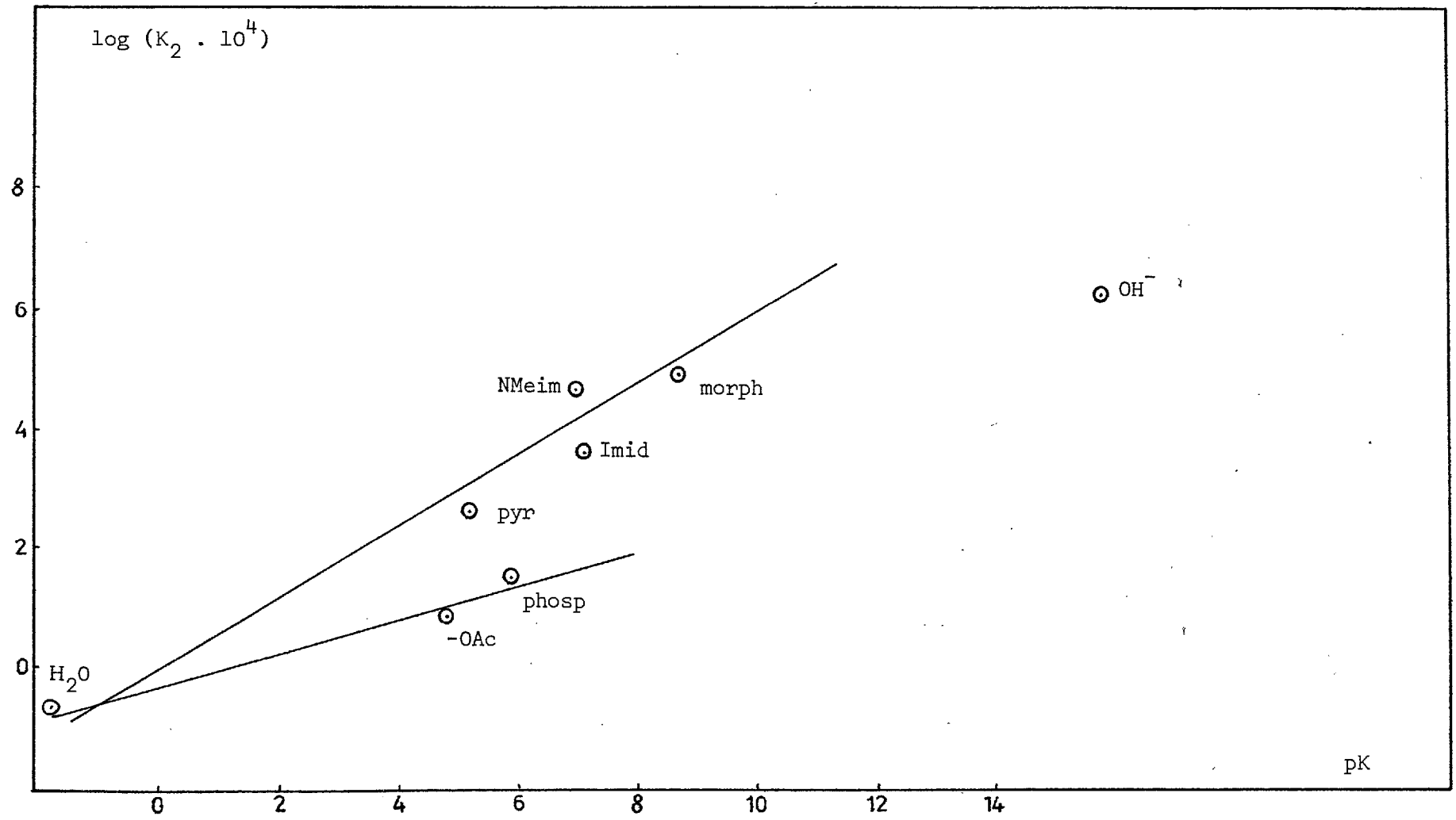


Figure 2.2,2b. Brönsted plot for N-methyl-N-nitrobenzamide



OAc^- and HPO_4^{2-} show a much lower reactivity than the other bases studied. This can be explained by their higher steric requirements. This is not consistent, however, with the higher K_2 values obtained for ^-OAc and HPO_4^{2-} with benzamide compared to acetamide. The benzoyl moiety should be more sensitive to the steric effect than the acetyl function.

An alternative explanation is that OAc^- and HPO_4^{2-} react *via* a general base catalysed pathway, but this is inconsistent with the solvent deuterium isotope effects obtained for the reaction in OAc^- (Section 2.2,1, (ii)).

2.2,3 Correlation between rates of reaction of N-nitroamides with rates of reaction of p-nitrophenylacetate, acetylimidazolium ion and acetylpiridinium ion

Comparison of the second order rate coefficients for N-nitro-N-methylacetamide with those for p-nitrophenyl acetate[73], acetyl 4-methyl pyridinium[74] ion and acetylimidazolium ion[75], (Figures 2.2,3a, 2.2,3b and 2.2,3c) gives linear correlations with slopes of 1.07, 0.76 and 1.1, respectively. Hence, the reactivity of these compounds affords a good model for the reactivity of the N-nitroamides. This again confirms that hydrolysis of the N-nitroamides proceeds *via* the nucleophilic catalysed pathway. Jencks and coworkers extensively studied the hydrolysis of esters with very good leaving groups (eg. p-nitrophenylacetate[73]) and very reactive amides (acetyl 4-methylpiridinium[74] ion and acetylimidazolium ion[75]).

On the basis of the β values obtained for both variation of nucleophile and leaving group, Jencks classifies these reactions into three main groups corresponding to three different transition states.

Figure 2.2,3a. Correlation of the rate of reaction of N-methyl-N-nitroacetamide with the rate of reaction of p-nitrophenylacetate

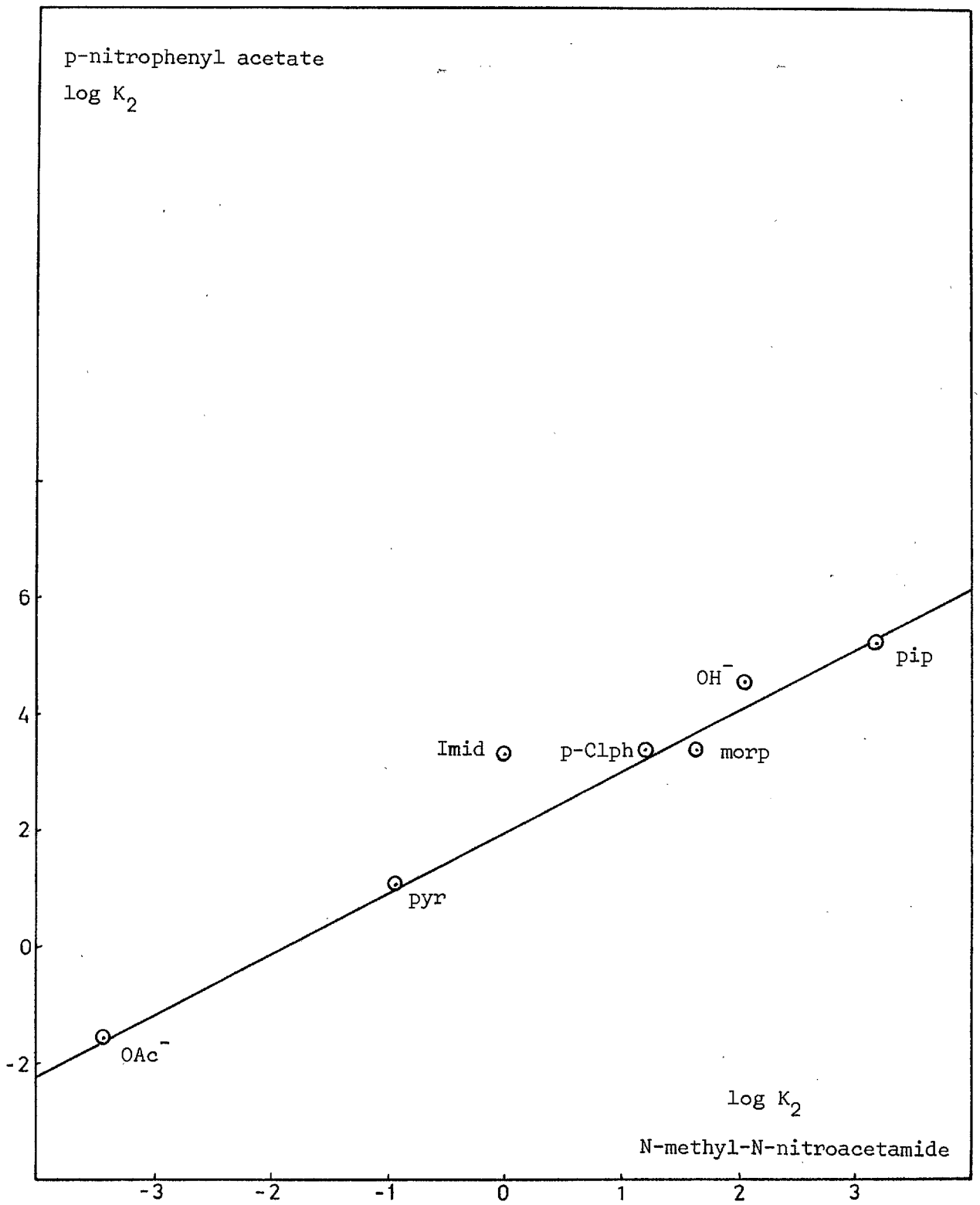


Figure 2.2,3b. Correlation of the rate of reaction of N-methyl-N-nitroacetamide with the rate of reaction of acetyl-4-methylpyridinium ion

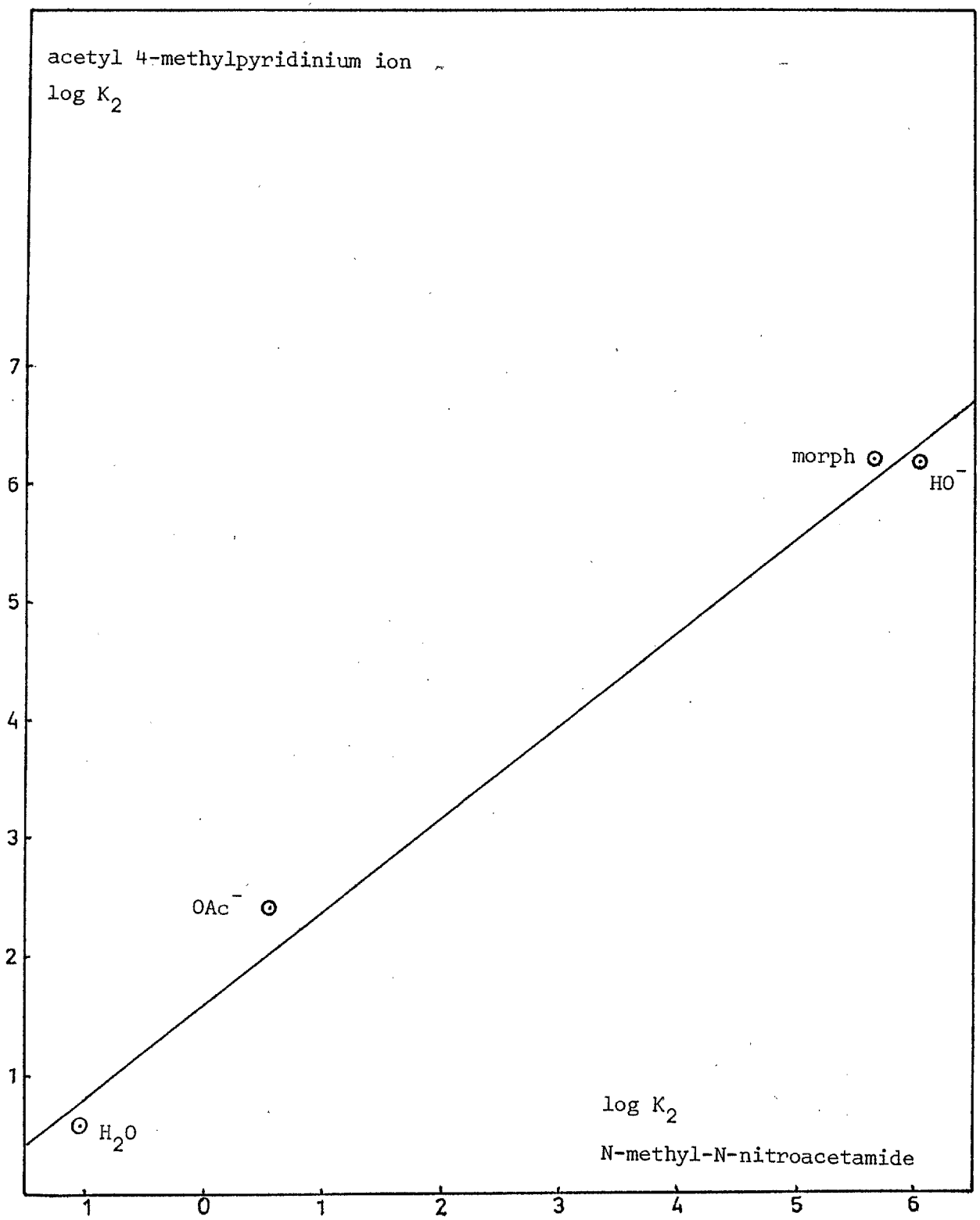
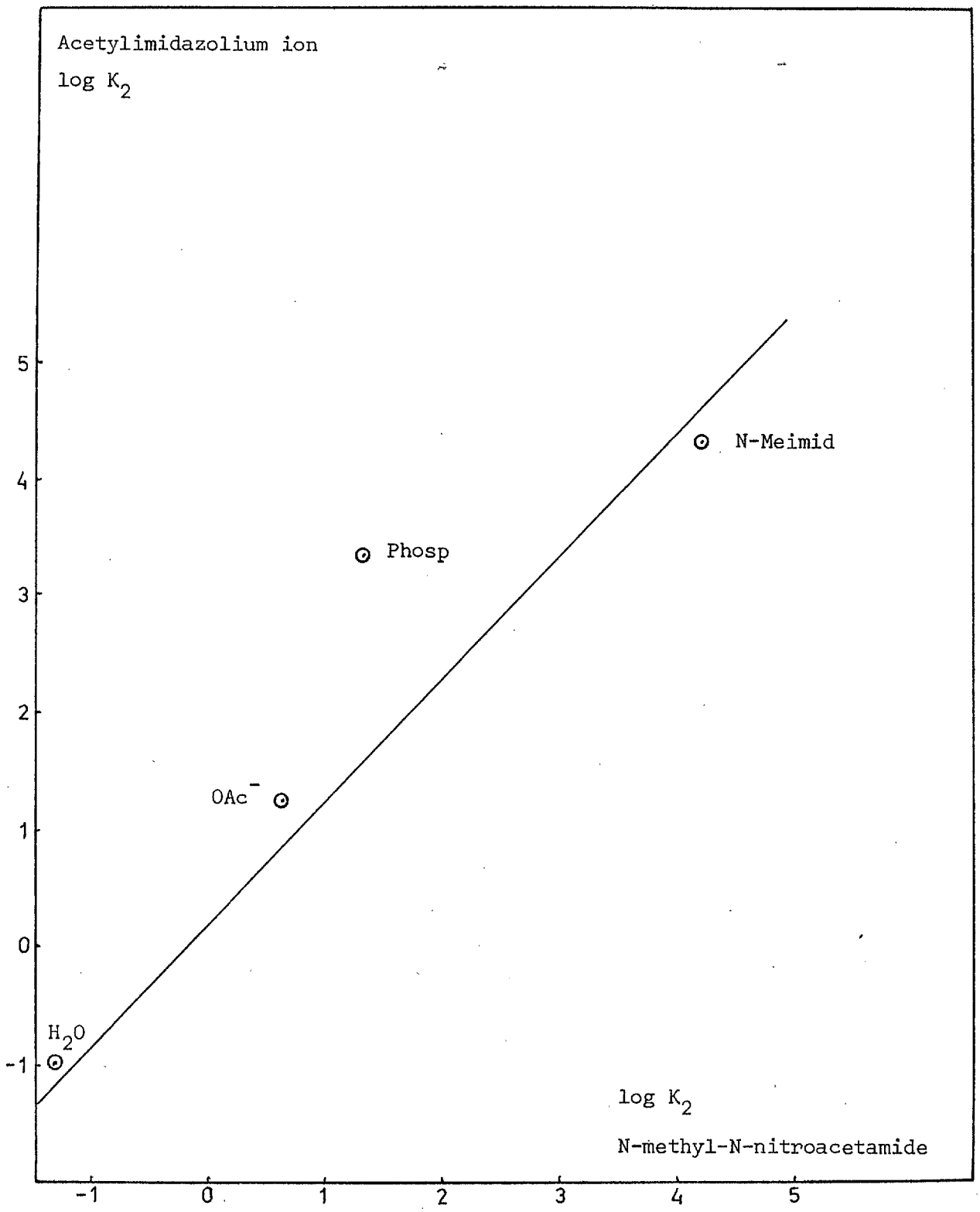


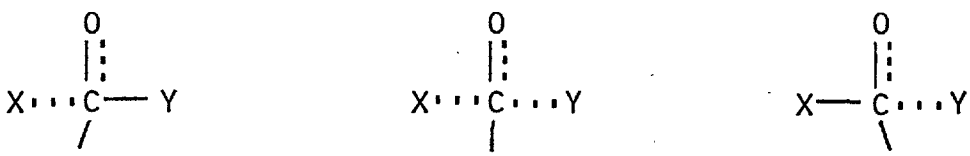
Figure 2.2,3c. Correlation of the rate of reaction of N-methyl-N-nitroacetamide with the rate of reaction of acetylimidazolium ion



This classification is summarized in Table 2.2,3a and the corresponding transition states are shown in Scheme 2.2,3a.

Table 2.2,3a. Classification of Acyl transfer reactions according to Jenks[74]

Class		β nucleophil	-β leaving group
I	Basic alkoxide (OH^-) and most esters Basic amines - reactive esters (pK leaving group < 5) Basic amines - acetylpiridinium ions Basic alkoxide - acetyl-$\overset{+}{N}$<	0 - 0.4	0 - 0.4
II	RO^- and esters (similar pKs attacking and leaving groups) Most amines - phenyl esters Most amines - acetyl-$\overset{+}{N}$ RO^- - acetyl-$\overset{+}{N}$<	0.7-1.0	0.7-1.0
III	Weakly basic RO^- - alkyl esters Most amines - alkyl esters Weakly basic RO^-(acetate)acetylimidazolium ion	1.2-1.6	1.2-1.6

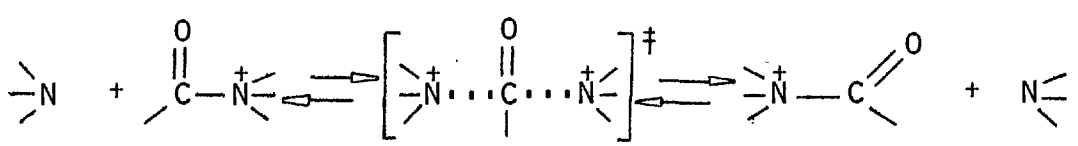


class I class II class III

Scheme 2.2,3a

Class I applies to reactions of strongly basic nucleophiles with acyl substrates bearing good leaving groups: if a tetrahedral intermediate is involved then the transition state for its formation is as shown.

Class III is the converse of class I and the transition state shown applies to rate determining breakdown of the intermediate. Class II comprises reactions where the transition state appears to be more or less symmetrical with respect to both entering and leaving groups. The Brönsted β values usually obtained for these reactions are difficult to explain when obtained from catalysts of wide reactivity as, for example, in the reaction of amines with esters and acetylimidazolium ion. Bearing in mind that the range of catalysts for which both formation and decomposition of the tetrahedral intermediate are both partially rate determining should be small, it suggests that the transition state intermediate to class I and III spans a wide range of structures. The conclusion is that either no tetrahedral intermediate is involved (Scheme 2.2,3c) or this intermediate is so unstable that it closely resembles the transition states for its formation and breakdown which must also be very similar in structure to each other.



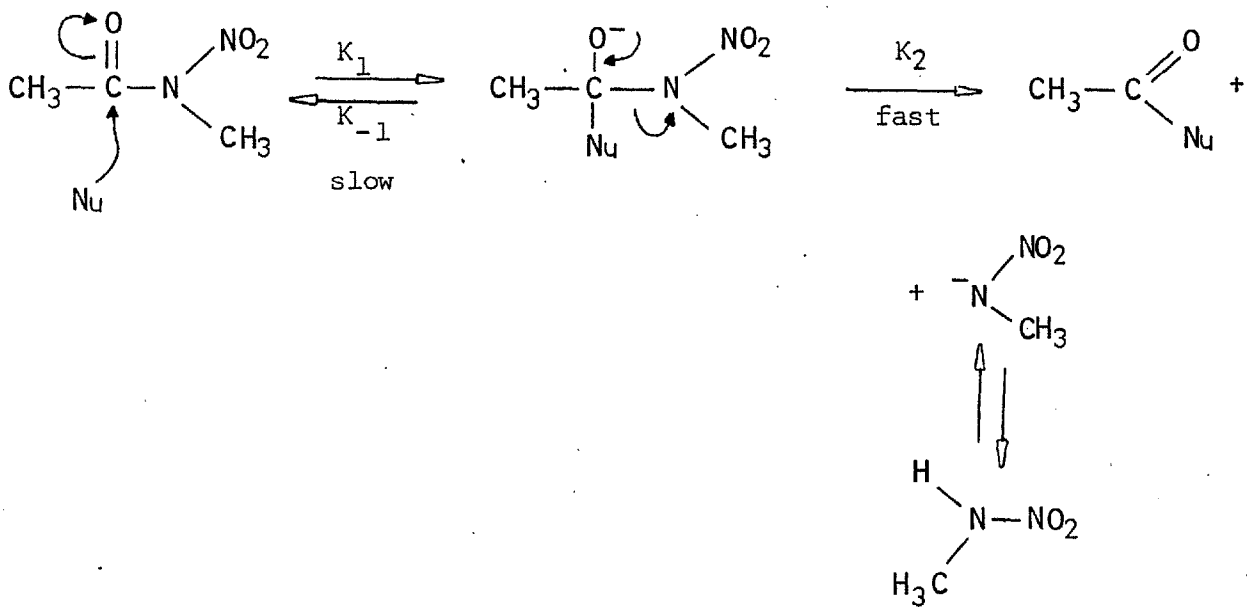
Scheme 2.2,3c

2.2,4 Mechanism of reaction

2.2,4a Reaction of N-nitroamides with nucleophiles in general

The reaction of N-nitroamides with nucleophiles afford a Brönsted coefficient of β ca. 0.62. This together with the correlations obtained with p-nitrophenylacetate, acetylimidazolium ion and acetylpiridinium ion suggests that we are dealing with a process that can be fitted into Jencks

classification (Section 2.2,3) between class I and II. The best mechanism for the data must be rate determining attack of the nucleophile (Scheme 2.2,4a1) with the formation of a tetrahedral intermediate which breaks down to products in a fast step.



Scheme 2.2,4a1

Applying the steady state treatment to Scheme 2.2,4a1, the rate equation 2.2,4a1 is obtained.

$$\text{rate} = \frac{K_1 K_2}{K_{-1} + K_2} \text{ [N-nitroamide][nucleophile] .. eq. 2.2,4a1}$$

When K_{-1} is very small, the rate equation becomes equation 2.2,4a2, which is the observed rate equation

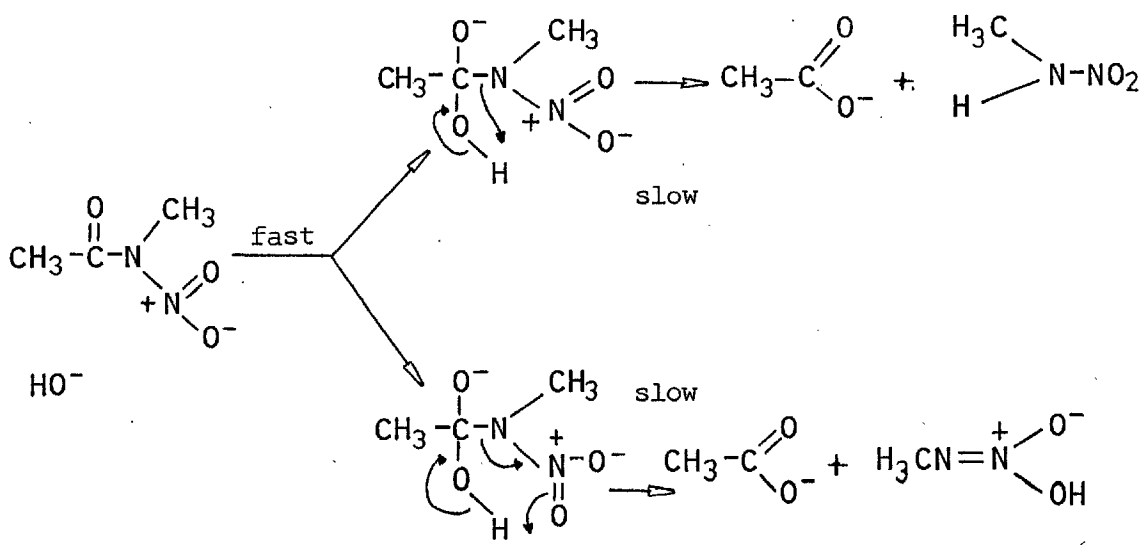
$$\text{rate} = K_1 \text{ [N-nitroamide] [nucleophile] ... eq. 2.2,4a2}$$

Proton transfer to the leaving group is not usually important because it occurs in a fast step after the transition state (c.f. $(K_2^{OAc^-})_{H_2O}/(K_2^{OAc^-})_{D_2O}$ ca. 1, Section 2.2,1)

2.2,4b Reaction of N-nitroamides with hydroxide ion

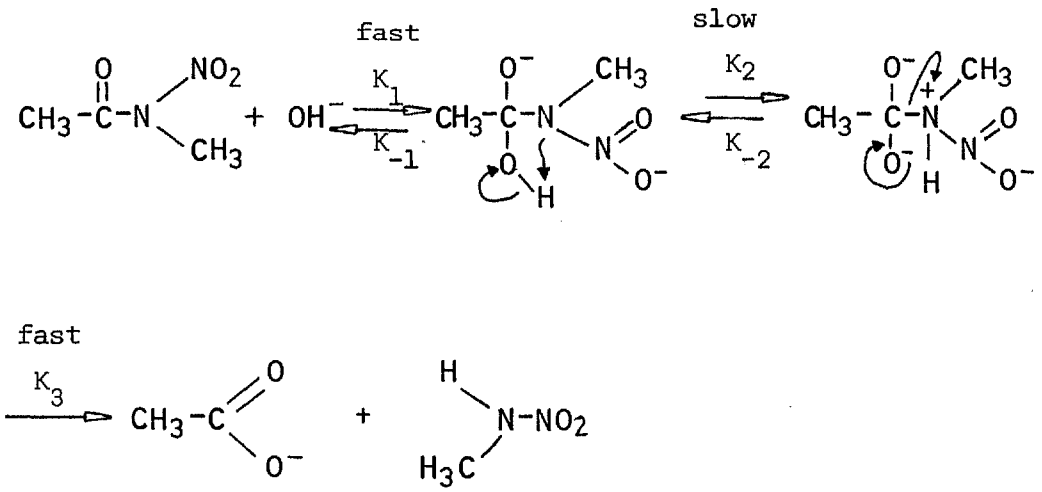
The hydroxide catalysed hydrolysis of N-nitroamides may not proceed via the same mechanism. The value of K_{HO^-}/K_{DO^-} ca. 5 found for N-nitroamides (and the value K_{HO^-}/K_{DO^-} ca. 5.1 for N-nitrosoamides, Section 1.1,3c2) suggests that a change in the rate determining step must have taken place. This value of the solvent deuterium isotope effect is difficult to accommodate with a rate determining nucleophilic attack by HO^- because DO^- is known to be a better nucleophile than HO^-. Also, for phenyl acetate which is known to suffer rate limiting HO^- attack, a solvent deuterium isotope effect has been reported $K_{HO^-}/K_{DO^-} = 0.75$[76].

A possible interpretation of these results is depicted in Scheme 2.2,4b1 where the tetrahedral intermediate breaks down to products in a rate determining step with concerted proton transfer to the leaving group.



Scheme 2.2,4b1

However, in the light of Swain, Kuhn and Schowen's solvation rule[77], a concerted process such as Scheme 2.2,4b1, should not give a large solvent deuterium isotope effect. This suggests that the proton transfer process itself may be rate limiting as in Scheme 2.2,4b2. Significantly, slow formation of the dianionic species is considered to be rate-limiting for the hydrolysis of trifluoroacetanilides (Section 1.2,1).



Scheme 2.2,4b2

The reaction of N-nitroamides with nucleophiles in general proceeds via rate determining attack of the nucleophile and proton transfer to the leaving group is not detected. In the reaction of N-nitroamides with HO^-, however, the first step becomes so fast that the rate determining step is now, probably, proton transfer to the leaving group.

2.2,4c Substituent effects

Values for the hydroxide catalysed hydrolysis of N-methyl-N-nitrobenzamide and its p-chloro and p-nitro derivatives were given in Section 2.1. These values were found to correlate with σp as shown in Figure 2.2,4c, from which a value of ρ ca. 1.9 was calculated. In rate processes the sign of ρ is positive for nucleophilic reactions. With reactions of complex mechanism, the observed ρ value is composed of ρ values for individual steps. However, if one elementary reactions is rate determining the observed ρ value usually corresponds to this step.

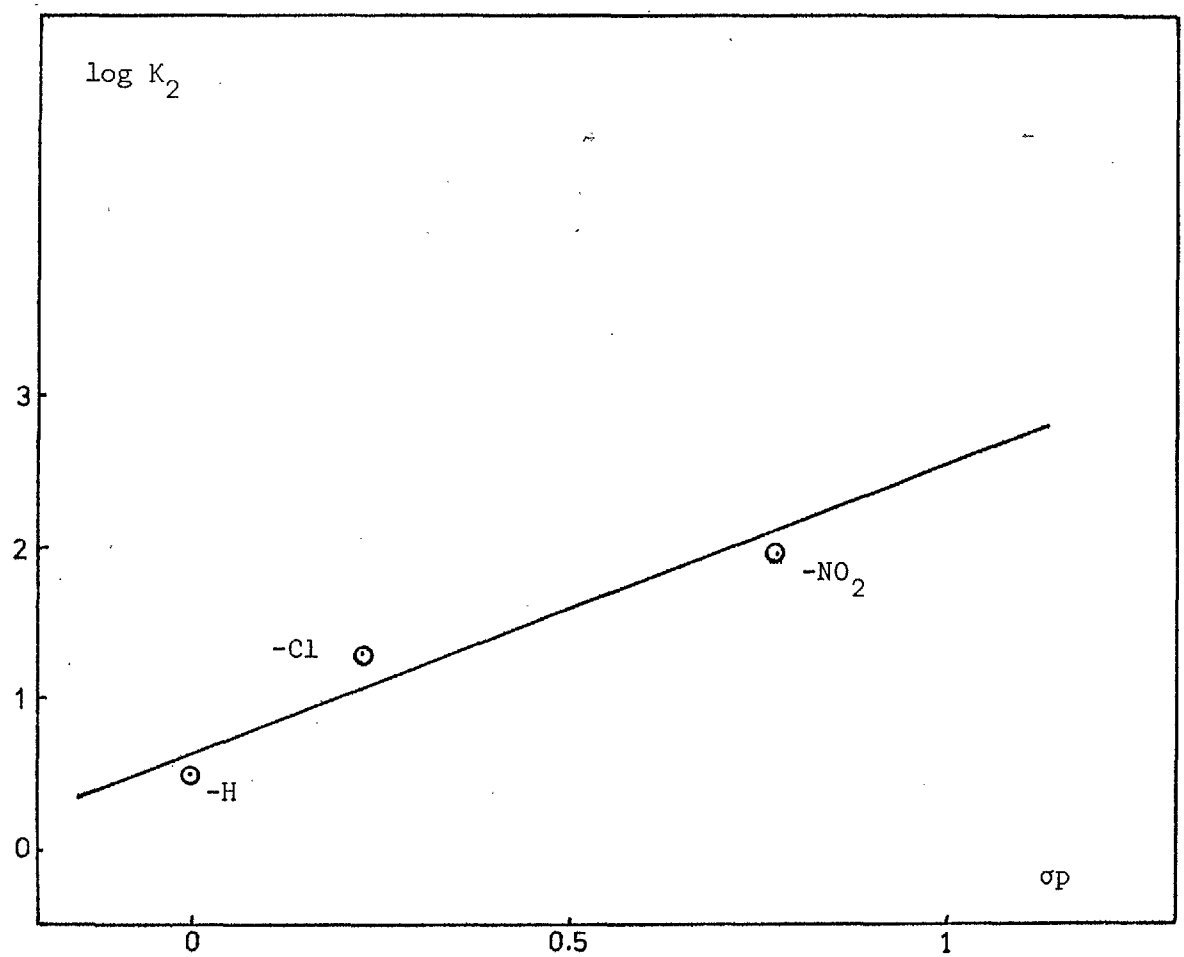
The mechanism proposed for the hydroxide catalysed hydrolysis of N-nitroamides (Scheme 2.2,4b2) is a multiple step mechanism, hence the observed ρ value is composite. Table 2.2,4c lists ρ values for some esters and amides.

Table 2.2,4c. ρ Values for esters and amides

	ρ (alkaline hydrolysis)	Ref
phenylbenzoates		78
$X\text{-}C_6H_4COOC_6H_5$	1.9	
$X\text{-}C_6H_4COOC_6H_4NO_2$	2.0	
$C_6H_5COOC_6H_4\text{-}X$	1.27	
$O_2N\text{-}C_6H_4COOC_6H_4\text{-}X$	1.25	
Acetanilides	0.1 (+1, -1)	33
Benzoylimidazoles	1.4	79

The ρ value obtained for N-nitrobenzamides (1.9) compares with ρ values obtained for phenyl benzoates (1.9, 2.0).

Figure 2.2,4c. Substituent effects N-methyl-N-nitrobenzamide



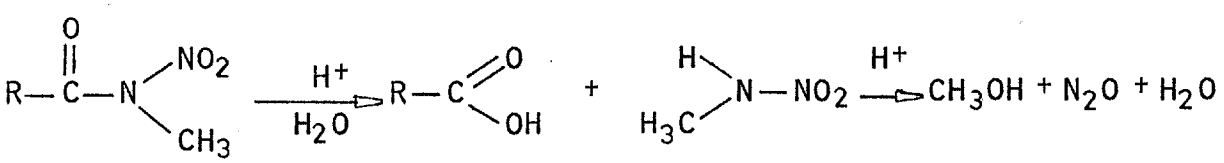
CHAPTER 3

ACID CATALYSED HYDROLYSES OF N-NITROAMIDES

RESULTS AND DISCUSSION

3.1 RESULTS OF THE ACID CATALYSED HYDROLYSIS OF THE N-NITROAMIDES

Rates of hydrolysis of the N-nitroamides were also measured in H_2SO_4 and D_2SO_4, following the reaction by the decrease in UV absorption of the reactant. The products formed initially were shown to be the corresponding carboxylic acid and methylnitramine (Scheme 3.1). Methylnitramine, however, also hydrolyses under the reaction conditions to give methanol, N_2O and H_2O.



Scheme 3.1

The reaction gave a first order dependence in [substrate] (eq. 3.1) and $\underline{K}_o$ was found to be acidity dependent.

$$\text{rate} = \underline{K}_o\,[\text{substrate}] \qquad \ldots \text{ eq. } 3.1$$

For N-methyl-N-nitroacetamide, it was necessary to correct $\underline{K}_o$ for the decomposition of methylnitramine using a computer programme as explained in Section 4.4,2. In the case of N-methyl-N-nitrobenzamide and N-methyl-N-nitro-p-chlorobenzamide, a theoretical infinity (the sum of absorptions due to the corresponding acid and methylnitramine) was used to calculate $\underline{K}_o$. The values obtained for $\underline{K}_o$ were constant for at least 2 half lifes.

3.1,1 N-Methyl-N-nitroacetamide

3.1,1a Hydrolysis in H_2SO_4 and D_2SO_4

Tables 3.1,1a1 and 3.1,1a2 summarise K_o values obtained in H_2SO_4 and D_2SO_4 and these results were plotted in Figure 3.1,1a. The reaction is strongly acid catalysed but rates are similar in both H_2SO_4 and D_2SO_4.

Table 3.1,1a1. Hydrolysis of N-methyl-N-nitroacetamide in H_2SO_4 at 25°C

Initial [substrate] ca. $2 \cdot 10^{-4}$ M

$[H_2SO_4]$ M	$10^4 \cdot K_o s^{-1}$
1.00	2.2
2.07	4.6
4.07	9.4
6.07	18.9
7.65	37.3
8.95	80.0

Table 3.1,1a2. Hydrolysis of N-methyl-N-nitroacetamide in D_2SO_4 at 25°C

Initial [substrate] ca. $2 \cdot 10^{-4}$ M

$[D_2SO_4]$ M	$10^4 \cdot K_o s^{-1}$
1.8	4.3
3.5	9.1
5.5	17.9
6.8	29.7
8.2	53.5

The solvent isotope effect calculated at several acidities is listed in Table 3.1,1a3. The $K_{H_2SO_4} / K_{D_2SO_4}$ ratio is virtually independent of acidity and consistent with a fast pre-equilibrium protonation of the substrate.

Figure 3.1,1a. Hydrolysis of N-methyl-N-nitroacetamide in H_2SO_4 and D_2SO_4 at 25°C

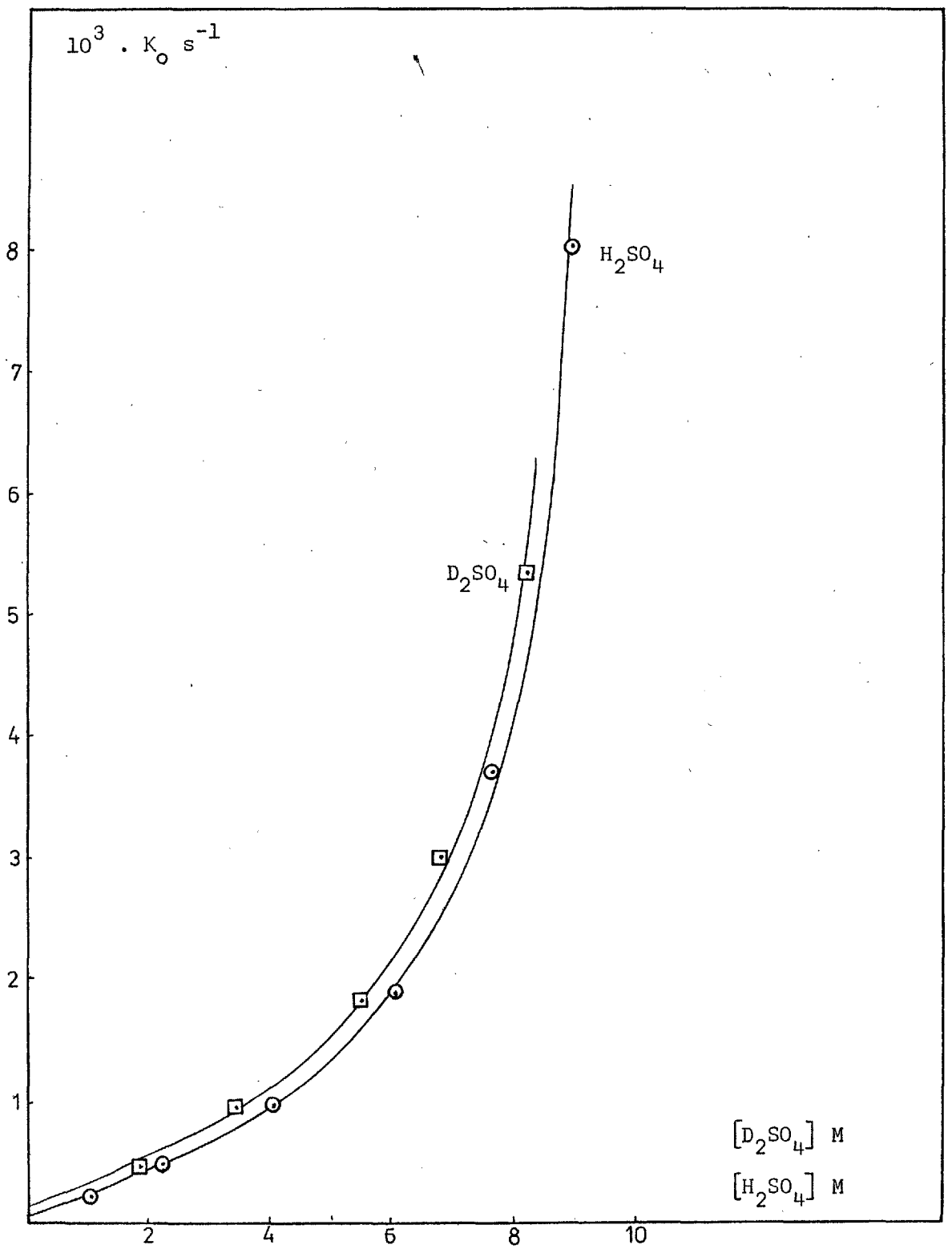


Table 3.1,1a3. Deuterium solvent isotope effect for the hydrolysis of N-methyl-N-nitroacetamide at 25°C

$[L_2SO_4]M$	$K_{H_2SO_4} / K_{D_2SO_4}$
3	0.84
4	0.85
5	0.87
6	0.88
7	0.90
8	0.88

3.1,1b Effect of temperature

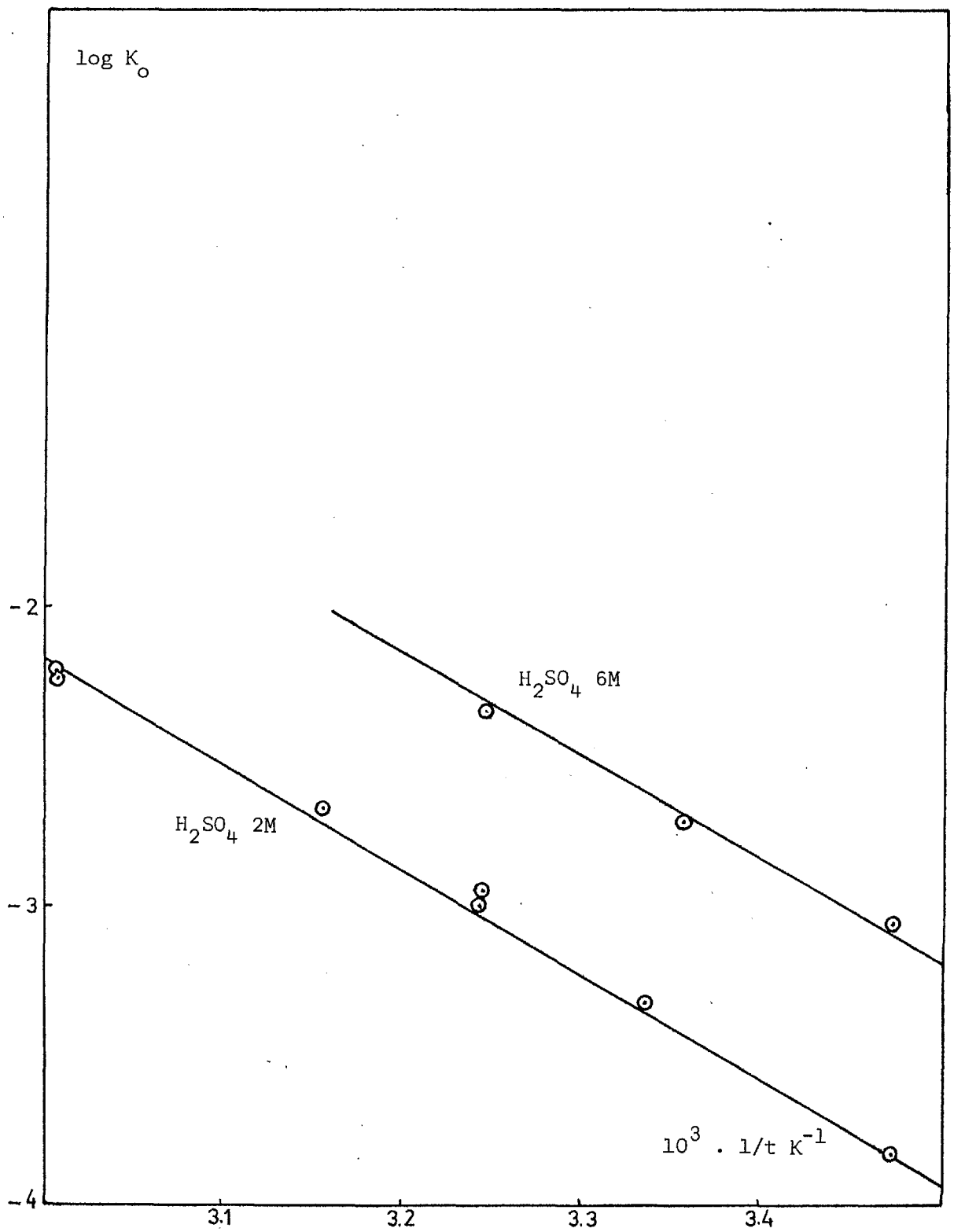
The effect of temperature upon the reaction rate was studied in H_2SO_4 2M and 6M. The results are listed in Table 3.1,1b

Table 3.1,1b. Effect of temperature on the hydrolysis of N-methyl-N-nitroacetamide in H_2SO_4

$[H_2SO_4]M$	Temp °C	$10^4 . K_o s^{-1}$
2	15	1.4
2	25	4.6
2	35	10.6 (9.7)
2	44	21.1
2	60	57.6 (61.0)
6	15	8.5
6	25	18.9
6	35	43.8

An Arrhenius plot of these data (Figure 3.1,1b) gives E_a (2M) = 67.4 k J mol^{-1} and E_a (6M) = 66.9 k J mol^{-1}. Values of the entropy of activation calculated from the relationship [80] (eq. 3.1,1b) at 298° K, were $\Delta S^{\neq}$ (2M) = -92 J mol^{-1} $°K^{-1}$ and $\Delta S^{\neq}$ (6M) = -79 J mol^{-1} $°K^{-1}$.

Figure 3.1,1b. Arrhenius plot for N-methyl-N-nitroacetamide in H_2SO_4 2M and 6M.



$$\log \underline{K} = \log \left(\frac{KT}{h}\right) + \frac{\Delta S^{\neq}}{2.303R} - \frac{E_a}{2.303\ RT} \quad \ldots \text{ eq. } 3.1,1b$$

where $\underline{K}$ is the experimental rate constant
$\underline{R}$ the gas constant
K the Boltzmann constant
h the Planck constant

3.1,2 N-Methyl-N-nitrobenzamide

3.1,2a Hydrolysis in H_2SO_4 and D_2SO_4

The variation of the pseudo first-order rate constant $\underline{K}_o$ (eq. 3.1) with $[H_2SO_4]$ and $[D_2SO_4]$ is given in Tables 3.1,2a1 and 3.1,2a2.

Table 3.1,2a1. Hydrolysis of N-methyl-N-nitrobenzamide in H_2SO_4 at 25°C. Initial [Substrate] ca. $1 \cdot 10^{-4}$ M

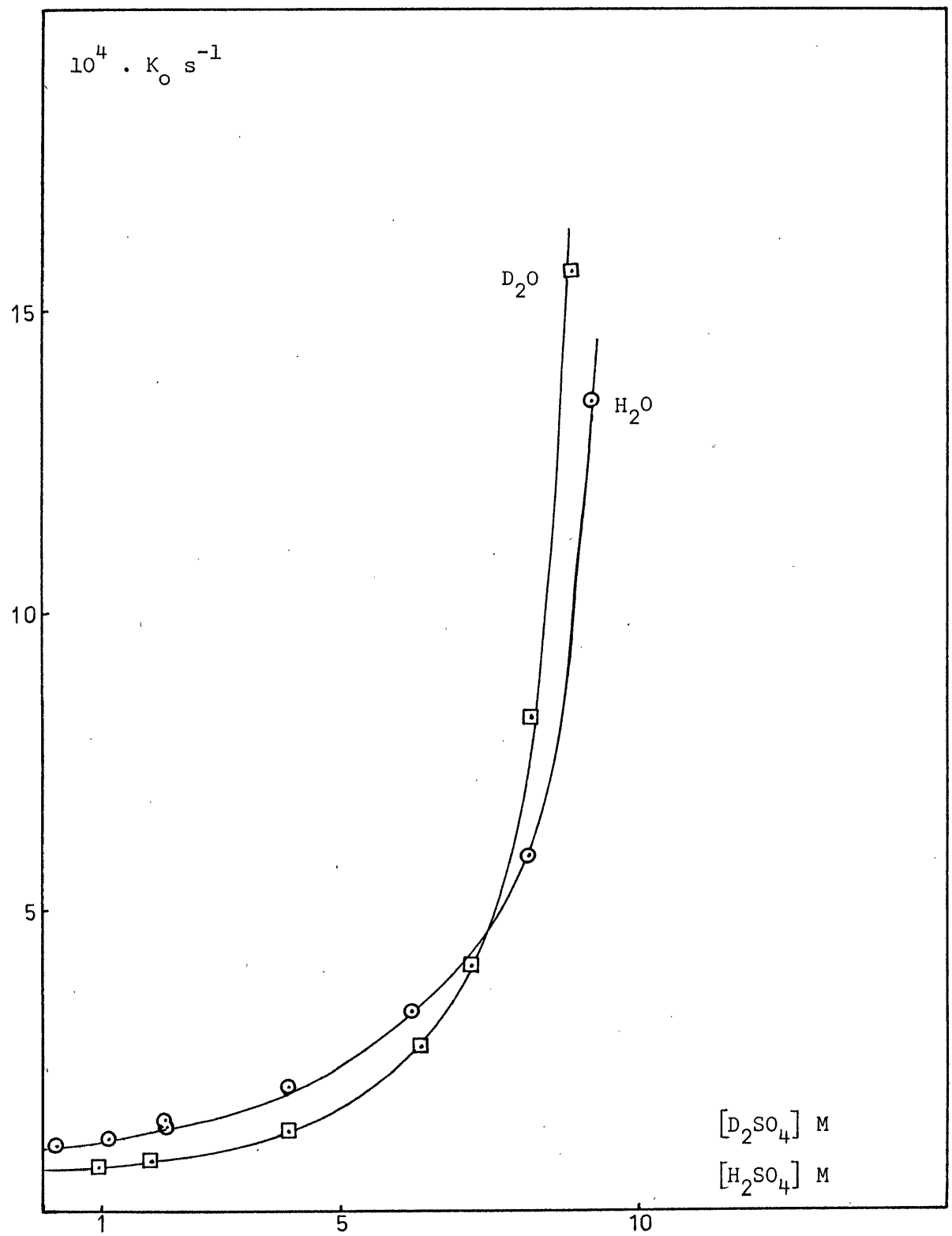
$[H_2SO_4]$ M	pH	$10^4 \cdot K_o\ s^{-1}$
0.005	2.18	1.04
0.01	1.89	1.02
0.05	1.26	1.06
0.1	0.99	1.04
1.10	-	1.3
2.05	-	1.4
4.05	-	2.0
6.10	-	3.3
8.06	-	5.9
9.16	-	13.5
9.60	-	26.5

Table 3.1,2a2. Hydrolysis of N-methyl-N-nitrobenzamide in D_2SO_4 at 25°C. Initial [substrate] ca. $1 \cdot 10^{-4}$ M

$[H_2SO_4]$ M	$10^4 \cdot K_o\ s^{-1}$
0.90	0.74
1.77	0.87
4.07	1.3
6.25	2.7
7.10	4.1
8.10	8.2
8.90	15.8

These results are plotted in Figure 3.1,2a. Values of the solvent isotope effect were calculated at several acidities and are listed in Table 3.1,2a3. The reaction appears to be virtually independent of

Figure 3.1,2a. Hydrolysis of N-methyl-N-nitrobenzamide in H_2SO_4 at 25 °C



acidity at low acid concentrations and significantly, the $K_{H_2SO_4}/K_{D_2SO_4}$ ratio is acidity dependent.

Table 3.1,2a3. Deuterium solvent isotope effect for the hydrolysis of N-methyl-N-nitrobenzamide at 25°C

$[L_2SO_4]$ M	$K_{H_2SO_4}/K_{D_2SO_4}$
2	1.5
4	1.5
6	1.3
7	1.1
8	0.98
9	0.58

Both factors suggest that the mechanism of the reaction changes from an uncatalysed pathway at low acidity to an acid dependent reaction ca 5M H_2SO_4. The value of $K_{H_2SO_4}/K_{D_2SO_4}$ ca 1.5 bellow 5 M H_2SO_4 probably reflects the difference in nucleophilic activity of H_2O and D_2O. Above 8M H_2SO_4, $K_{H_2SO_4}/K_{D_2SO_4} < 1$ is consistent with rapid pre-equilibrium protonation of the substrate.

3.1,2b Effect of temperature

The effect of temperature upon the reaction rate was studied in H_2SO_4 0.1M, 2M and 9M. These results are reported in Table 3.1,2b.

Table 3.1,2b. Effect of temperature on the hydrolysis of N-methyl-N-nitrobenzamide in H_2SO_4. Initial [substrate] ca. 1.10^{-4}M

H_2SO_4 M	Temp °C	$10^4 . K_o$ s^{-1}
0.1	25	1.04
0.1	35	2.1
0.1	60	9.5

/continued...

Table 3.1,2b/continued...

H_2SO_4 M	Temp °C	$10^4 . K_o$ s^{-1}
2	15	0.5
2	25	1.5
2	35	3.3
2	45	7.2
2	60	16.1 (15.0)
9	11	2.1
9	16	3.7
9	25	13.6
9	35	52.5

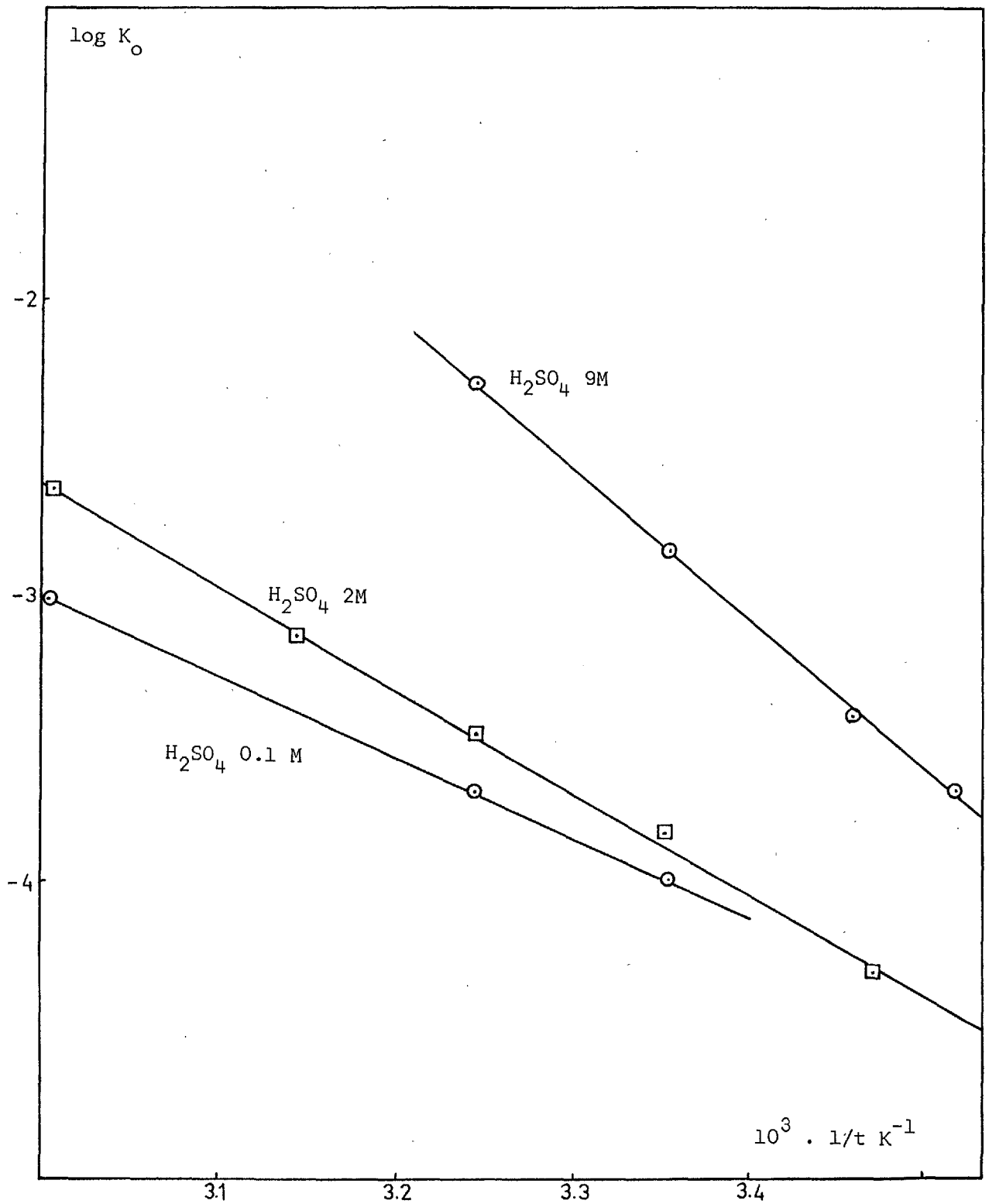
Figure 3.1,2b shows the Arrhenius plots for these results. The activation energies calculated from the slopes are E_a (0.1M) = 53 k J mol^{-1} E_a (2M) = 67 k J mol^{-1} and E_a (9M) = 96 k J mol^{-1}. Using eq. 3,1,1b values for the entropy of activation were calculated at 298 °K as $\Delta S^{\neq}$(0.1M) = -150 J $mol^{-1} {}^{o}K^{-1}$, $\Delta S^{\neq}$ (2M) = -100 J mol^{-1} $^{o}K^{-1}$ and $\Delta S^{\neq}$ (9M) = + 14.6 J mol^{-1} $^{o}K^{-1}$. The change in activation parameter on going from low to high acidities is also indicative of a change in reaction mechanism.

Although the Arrhenius plots are linear, the possibility of a thermal rearrangement (Section 1.1,3) at higher temperature was considered. A solution of N-methyl-N-nitrobenzamide in THF at 25°C shows no measureable decomposition over 48 h and at 60°C the reaction rate gives K_o = $5 . 10^{-5}$ s^{-1}. This is low compared to the value K_o = $9.5 . 10^{-4}$ s^{-1} in H_2SO_4 0.1 M at 60°C. This nucleophilic attack by the solvent on the neutral substrate must be the only significant reaction in dilute H_2SO_4.

3.1,2c ^{18}O Exchange experiments

A relatively large scale hydrolysis of carbonyl ^{18}O labelled N-methyl-N-nitrobenzamide was performed in 1M and 9M H_2SO_4. Samples of

Figure 3.1,2b. Arrhenius plot for N-methyl-N-nitrobenzamide in H_2SO_4 0.1 M, 2M and 9M



the reaction mixture were extracted at timed intervals and analysed by mass spectrometry for ^{18}O content of either substrate or benzoic acid product (Table 3.1,2c).

Table 3.1,2c. ^{18}O Percentages of incorporation of N-methyl-N-nitrobenzamide

	$[H_2SO_4]$M	Extracted sample	(M+2)·100/M M = 180, 122	(M+2)·100/M M = 105
Starting material	-	substrate	11.6	8.3
Sample 1 (42 min)	1	product	11.3	6.0
Sample 2 (85 min)	1	product	11.6	5.5
Sample 3 (127 min)	1	product	10.9	5.8
Sample 1 (5 min)	9	Substrate	11.6	10.8
Sample 2 (10 min)	9	Product	11.4	5.6

It is clear that no ^{18}O exchange of either substrate or product occurs during reaction.

3.1,3 Other substrates

3.1,3a N-Methyl-N-nitro-p-chlorobenzamide and N-methyl-N-nitro-p-nitrobenzamide

Rates of hydrolysis in H_2SO_4 at 25°C for N-methyl-N-nitro-p-chlorobenzamide are given in Table 3.1,3a.

Table 3.1,3a. Hydrolysis of N-methyl-N-nitro-p-chlorobenzamide in H_2SO_4 at 25 °C

Initial [substrate] ca $1 . 10^{-4}$ M

$[H_2SO_4]$ M	$10^4 . K_o$ s^{-1}
1.01	2.2
2.00	2.2
3.95	2.4
4.95	2.5
5.93	3.6

Hydrolysis is apparently weakly catalysed by acid at these acidities.

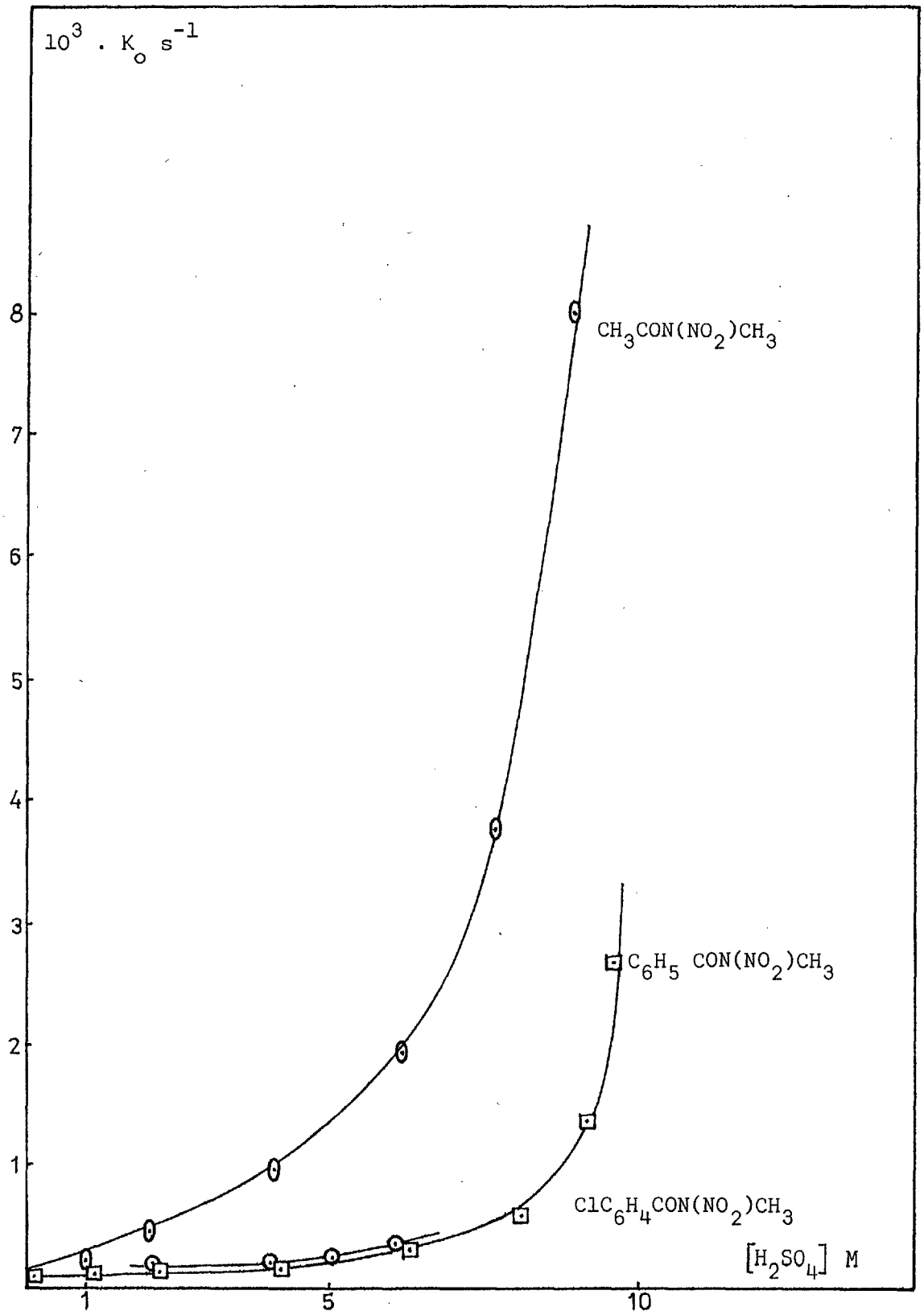
It was not possible to study the reaction at higher acidities because of U.V. absorption by the p-chlorobenzoic acid product. The same problem was encountered with N-methyl-N-nitro-p-nitrobenzamide and even the results at low acidity were considered unreliable.

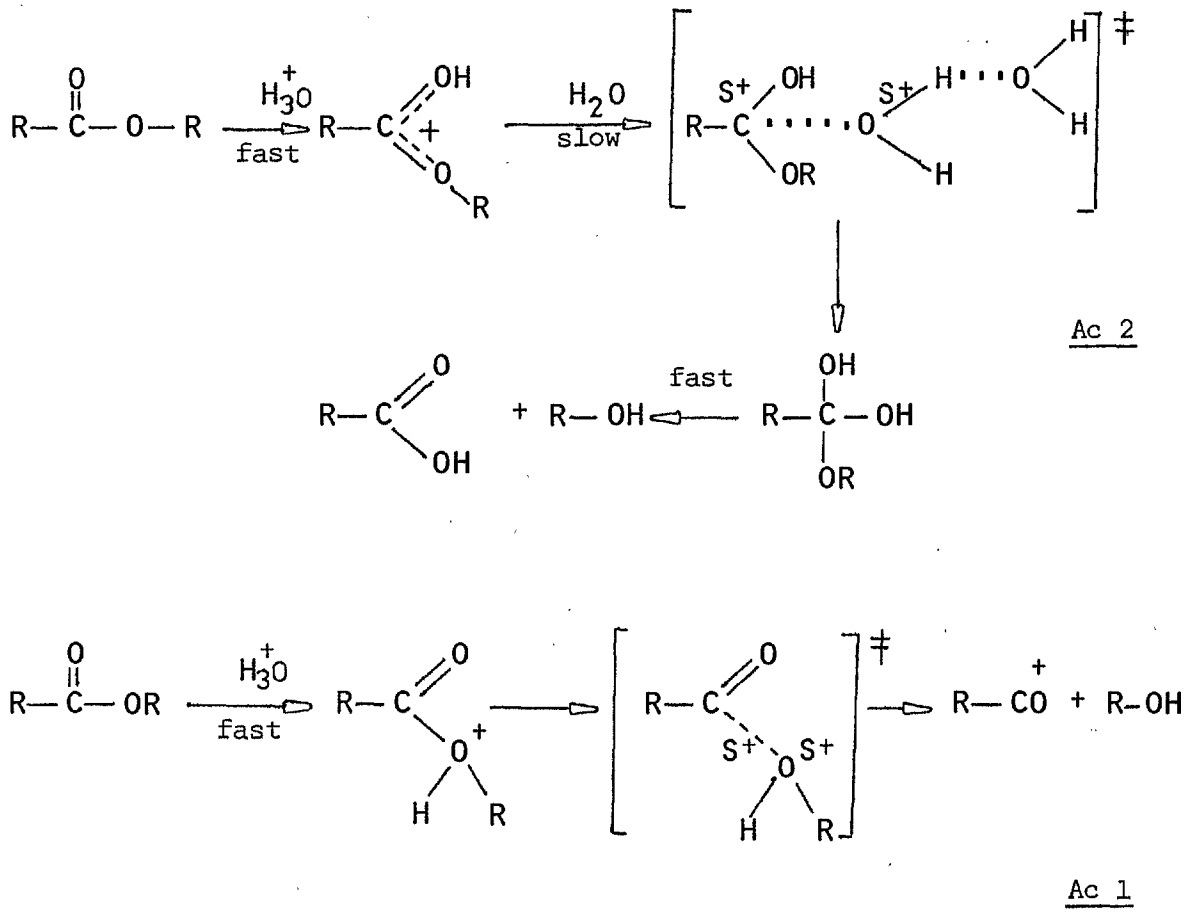
3.2 DISCUSSION OF THE ACID CATALYSED HYDROLYSIS OF N-NITROAMIDES

Figure 3.2 shows the variation of the pseudo first order rate constants (Rate = k_o [Substrate]) with $[H_2SO_4]$, for the three N-nitroamides studied. It is clear that N-methyl-N-nitroacetamide and N-methyl-N-nitrobenzamides have different acidity dependences. However, both show a monotonical rate increase with increasing acidity and no rate maxima. This behaviour differs from ordinary amides in which a rate maxima associated with complete substrate protonation is usually observed. Even compounds with good leaving groups like acetanilides show a rate maxima followed by further rate increases above 75% H_2SO_4. These latter, which have been interpreted as evidence for a change in reaction mechanism from bimolecular (Ac 2) to unimolecular (Ac 1), are supported by the different substituent effects observed at low acidity (ρ ca. 1) and at high acidity (ρ ca. 5) [61].

Esters with good leaving groups, e.g. p-nitrophenylacetate[81], show a similar behaviour to N-nitroamides in that the rate increases continuously as the acidity is raised without any maximum rate. This difference from the rate maxima observed with single esters has also been interpreted as evidence for the incursion of an Ac 1 mechanism rather than the usual Ac 2 pathway, at high acidities, (Scheme 3.2). Correlations with the activity of water were found to support this change of mechanism, the number of molecules involved in the rate determining step going from 2 at low acidities to 0 at high acidities.

Figure 3.2. Hydrolysis of N-nitroamines in H_2SO_4 at 25°C.





Ac 2

Ac 1

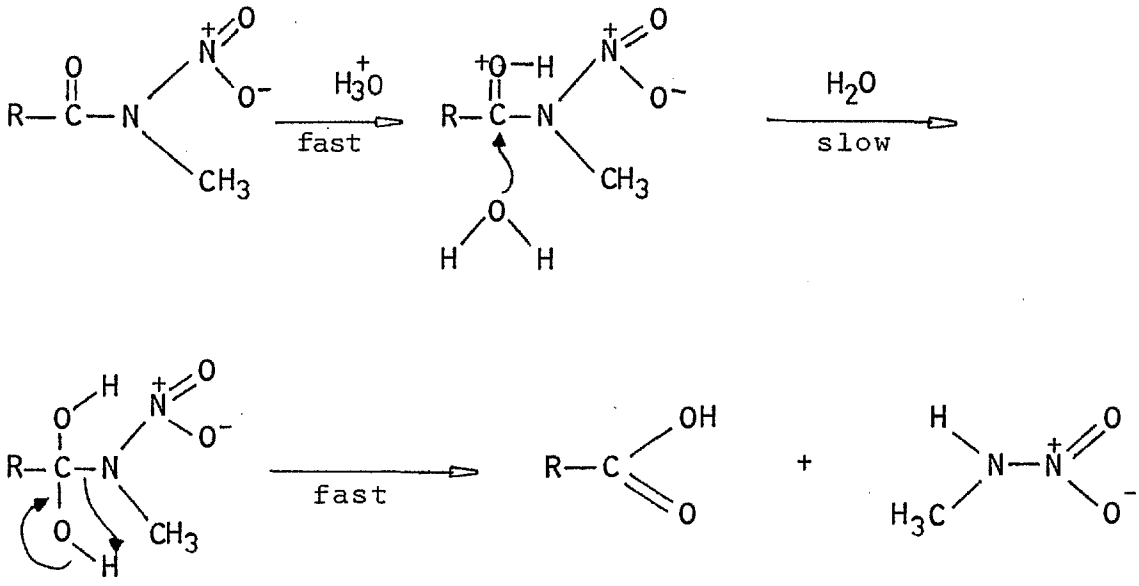
Scheme 3.2

3.2,1 N-Methyl-N-nitroacetamide

The deuterium solvent isotope effect for the hydrolysis of N-methyl-N-nitroacetamide was found to be independent of acidity and its mean value is 0.87 (Section 3.1,1a). This value is consistent with a fast pre-equilibrium protonation of the substrate with a higher concentration of the conjugate acid in D_2O than H_2O.

Entropies of activation can be used to distinguish between bimolecular and unimolecular mechanisms, based on the fact that the loss of translational and rotational freedom of a water molecule associated with the bimolecular mechanism should lead to a lower entropy of activation relative to the unimolecular case. This prediction is amply borne

out by entropies of activation for Acl and Ac2 ester hydrolysis, typical values of $\Delta S^{\neq}$ being 0 to 41 J $mol^{-1}\,{}^{o}K^{-1}$ for Acl and -84 to -125 J $mol^{-1}\,{}^{o}K^{-1}$ for Ac2 . Entropies of activation for N-methyl-N-nitroacetamide calculated in H_2SO_4 2M and 6M, respectively -92 J $mol^{-1}\,{}^{o}K^{-1}$ and -79 J $mol^{-1}\,{}^{o}K^{-1}$ are not substantially different from those for Ac 2 ester hydrolysis.[82] Hence reaction must proceed <u>via</u> the rate determining attack of H_2O on the protonated substrate probably with formation of a tetrahedral intermediate that breaks down to products in a fast step (Scheme 3.2,1).



Scheme 3.2,1

Rates of reaction of N-methyl-N-nitroacetamide correlate with the acidity function H_o and H''' , with slopes of 0.38 and 0.3 respectively but not with H_A (Figure 3.2,1a). For esters, it has been found[83] that the relationship between measured ionization ratios and some suitable function H_S is given by equation 3.2,1a

$$\log \left[SH^+\right]/\left[S\right] = -\,H_S + pK_{SH^+} \quad \ldots \text{ eq. } 3.2,1a$$

Ionization ratios were measured spectrophotometrically for several acetates and it was found that these closely obey the linear relationship (equation 3.2,1b)

$$\log [SH^+] / [S] = - m H_o + cte \qquad \text{... eq. 3.2,1b}$$

For acetate esters m = 0.62. Hence, when the Bunnett treatment was modified by substitution of H_o by mH_o (eq. 3.2,1c) good correlations were obtained.

$$\log K_1 + mH_o = R \log a_{H_2O} + (\text{constant}) \qquad \text{... eq. 3.2,1c}$$

Since we were not able to determine the pK_{SH^+} for N-nitroamides due to the instability of the compounds at higher acidities, we are not able to apply this type of relationship rigorously. However, correlation of the pseudo first order rate coefficients of N-methyl-N-nitroacetamide with p-nitrophenylacetate (Figure 3.2,1b) gives a straight line of slope 1.0, suggesting that both compounds have very similar acidity dependences. Hence, it seem reasonable to use the m value determined for esters and apply eq. 3.2,1c to our data. Figure 3.2.1c presents this correlation, both for N-methyl-N-nitroacetamide and p-nitrophenylacetate, over the same range of acidity. The fact that the rates of hydrolysis correlate with the activity of water is another argument in favour of the bimolecular mechanism.

No evidence of a unimolecular pathway was seen for the hydrolysis of N-methyl-N-nitroacetamide in the acidity range studied. The fact that no rate maximum is observed is probably due to the fact that the compound is not completely protonated since the nitro group directly attached to the amide function must lower the basicity of the compound very much. Also, unimolecular mechanism would not be favoured due to the instability

Figure 3.2,1a. Correlation of hydrolysis rates of N-methyl-N-nitro-acetamide with acidity functions

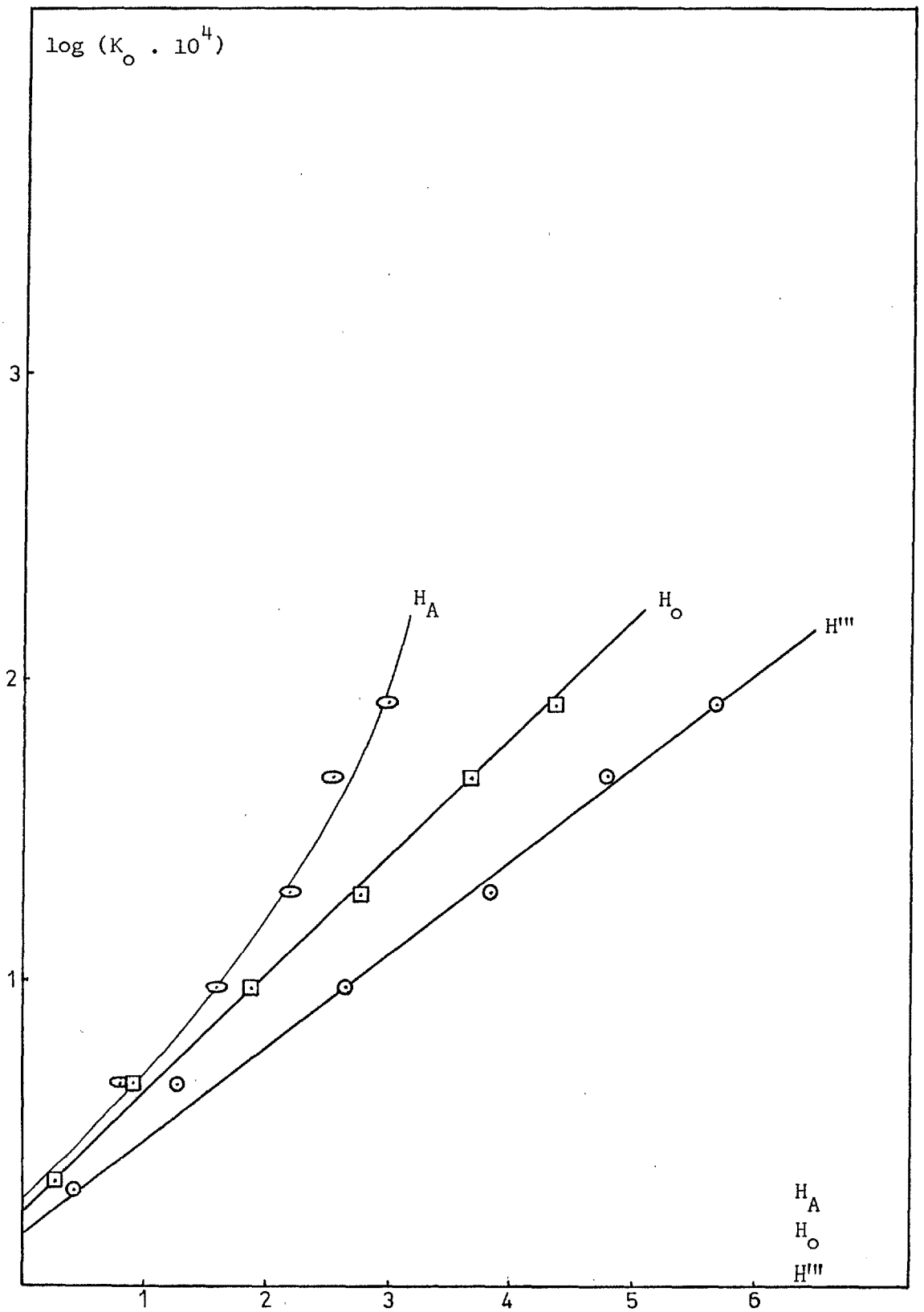


Figure 3.2,1b. Correlation of hydrolysis rates of N-methyl-N-nitro-acetamide and p-nitrophenylacetate

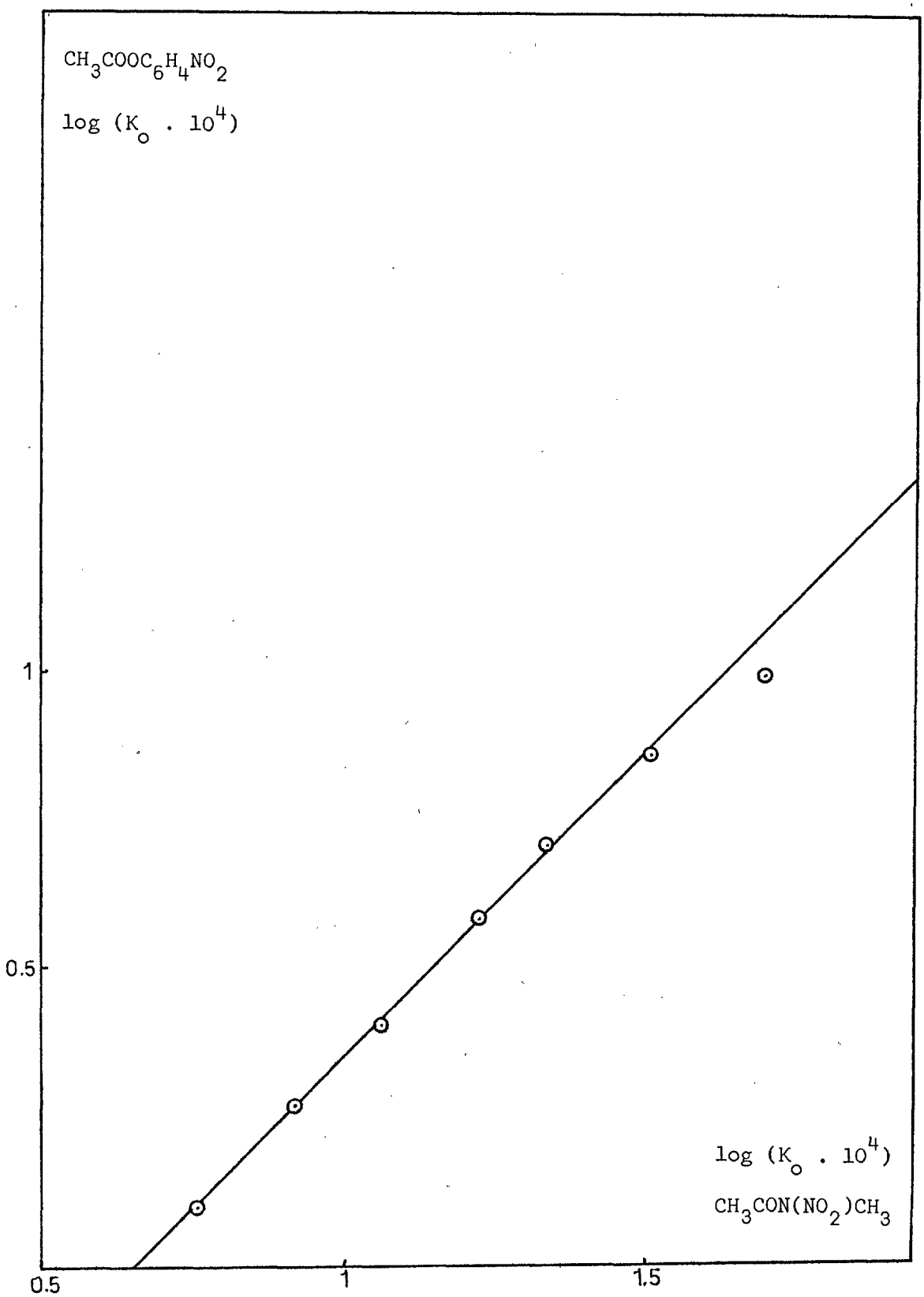
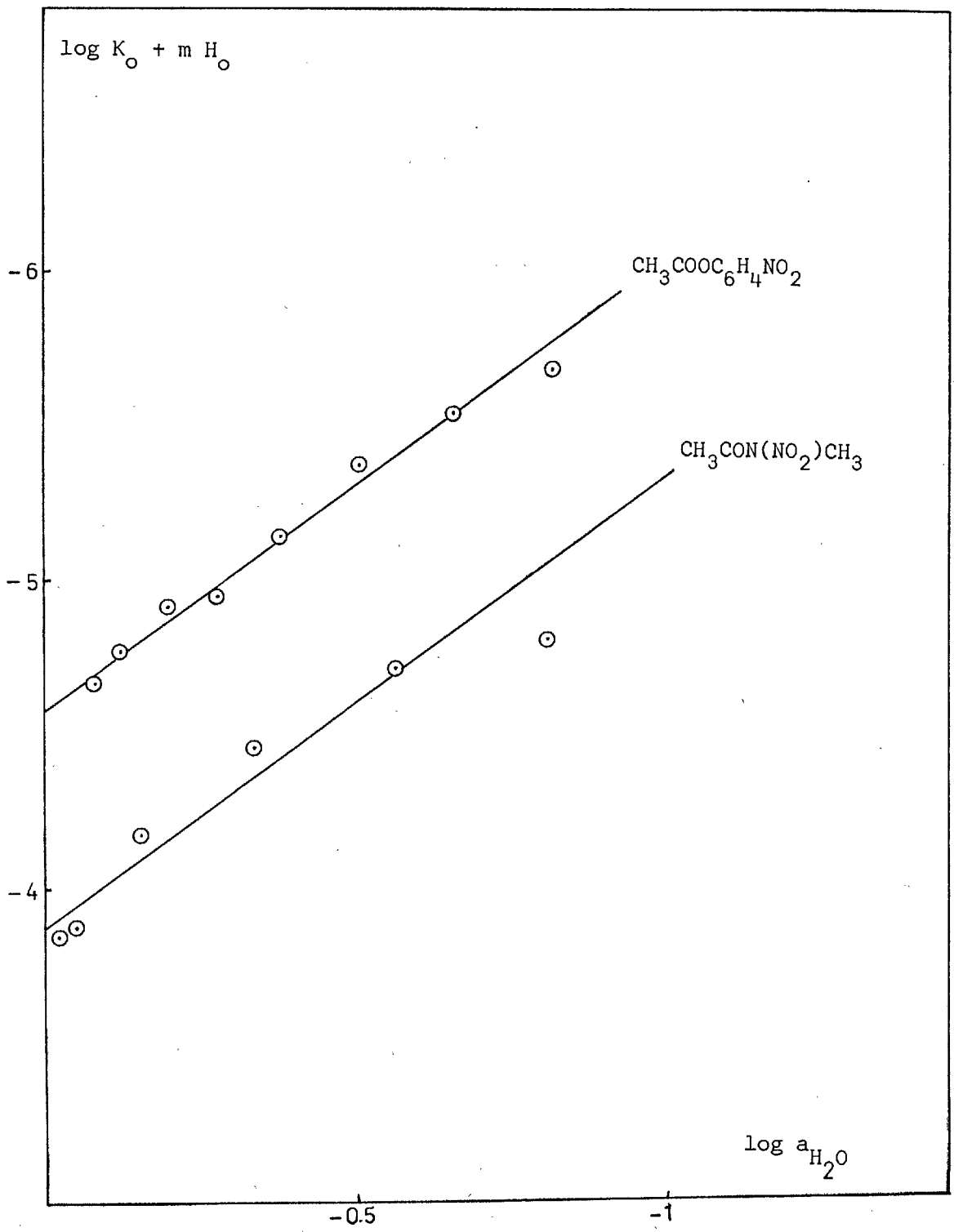


Figure 3.2,1c. Correlation of hydrolysis rates of N-methyl-N-nitro-acetamide and p-nitrophenylacetate with the activity of water



of the acylcarbonium ion intermediate on this reaction pathway.

3.2,2 N-Methyl-N-nitrobenzamide

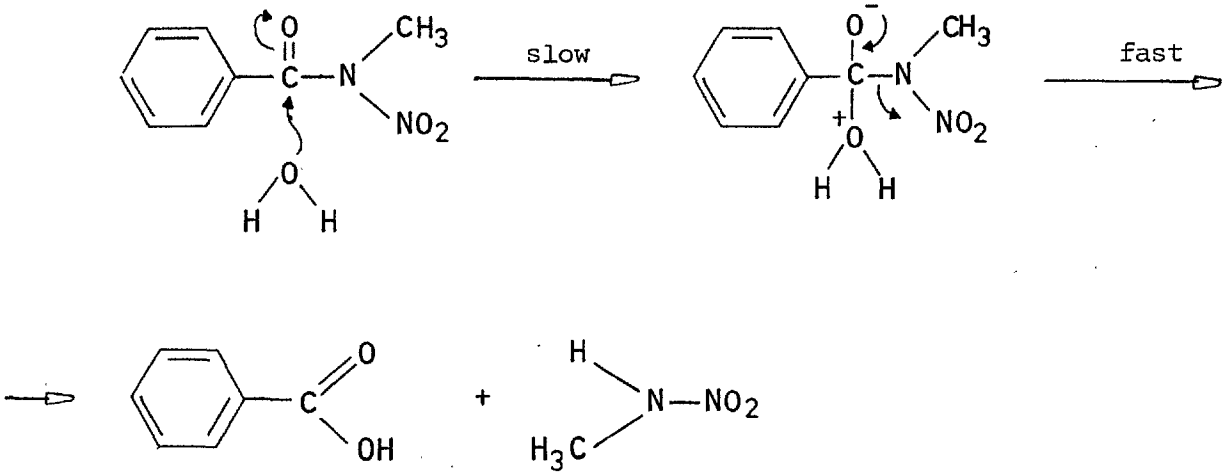
At low acidities, the rate of hydrolysis of N-methyl-N-nitrobenzamide is virtually acidity independent but the reaction is strongly acid catalysed above 6M H_2SO_4. Also, the solvent deuterium isotope effect was found to change from $K_{H_2SO_4}/K_{D_2SO_4}$ ca. 1.5 at low acidities to $K_{H_2SO_4}/K_{D_2SO_4}$ ca. 0.6 after 7.5 M H_2SO_4. Further, the energy of activation and the entropy of activation was found to change with acidity as shown in Table 3.2,2.

Table 3.2,2. Variation with acidity of $E_a^{\neq}$ and $\Delta S^{\neq}$ for N-methyl-N-nitrobenzamide

$[H_2SO_4]$M	$E_a^{\neq}$ K J mol^{-1}	$\Delta S^{\neq}$ J mol^{-1} $^oK^{-1}$
0.1	53.	-150.
2.	67.	-100.
9.	96.	+ 14.

Thus, the kinetic acidity dependence, the solvent deuterium isotope effect and the energy and entropy of activation, all point to a change of reaction mechanism with increasing solvent acidity.

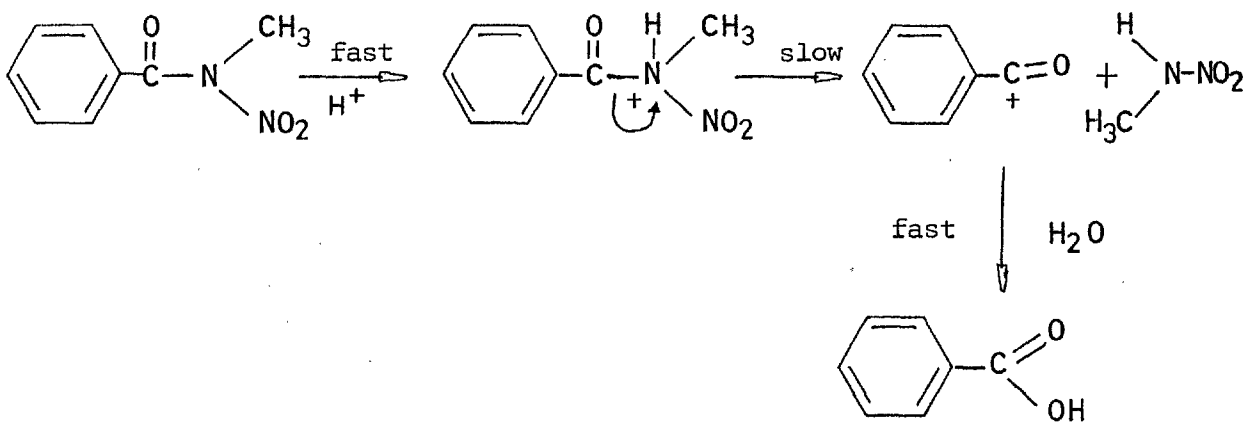
At low acidities, the predominant reaction is the hydrolysis of neutral substrate by solvent H_2O (Scheme 3.2,2a). This mechanism agrees with the solvent deuterium isotope effect ($K_{H_2SO_4}/K_{D_2SO_4}$ ca. 1.5). At low acidity not much of the substrate is protonated, so the overriding factor is that D_2O is a poorer nucleophile than H_2O.



Scheme 3.2,2a

The negative entropy of activation is also consistent with a bimolecular mechanism (cf. Section 3.2,1).

At very high acidities, however, it is clear that the conjugate acid of the substrate is involved. The positive value for $\Delta S^{\neq}$ at 9 M H_2SO_4 implies an Ac 1 mechanism (Scheme 3.2,2b) although it is possible that at intermediate acidities there is a contribution of an Ac 2 pathway involving the conjugated acid of the substrate.



Scheme 3.2,2b

The solvent deuterium isotope effect is also consistent with the Ac 1 pathway. The rate of reaction is faster in D_2O than H_2O because concentration of the conjugate acid intermediate is higher as expected for fast pre-equilibrium protonation of the substrate.

The pseudo first order rate coefficients for this reaction do not correlate with the acidity functions H_o, H''' or H_A (Figure 3.2,2) and this is expected because there is a change in the mechanism of reaction.

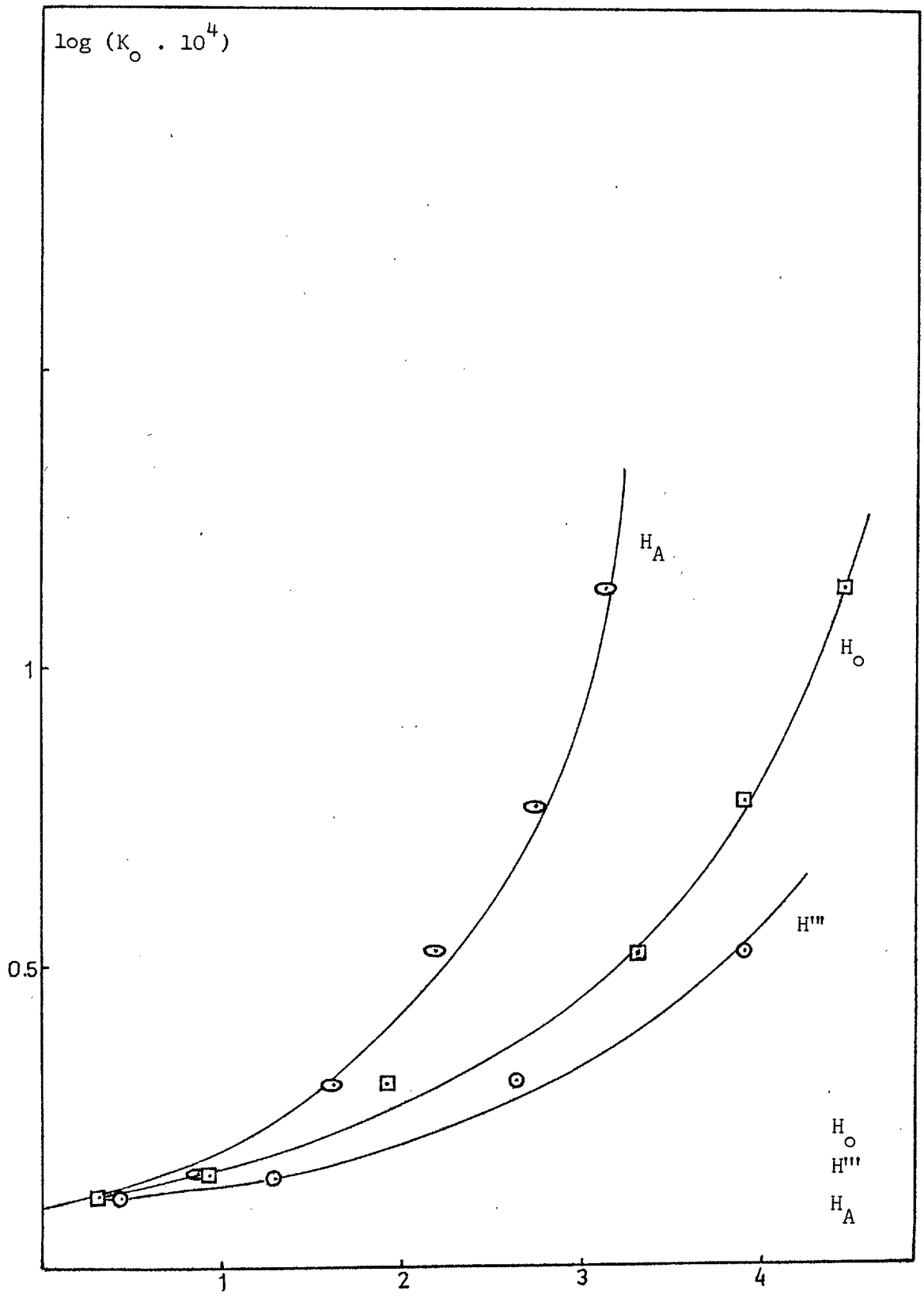
Another piece of evidence is the lack of ^{18}O exchange for the hydrolysis of N-methyl-N-nitrobenzamide both at 2M and 9M H_2SO_4 and this is in agreement with the mechanisms proposed.

The detection of an Ac 1 mechanism in the case of N-methyl-N-nitrobenzamide and not in the N-methyl-N-nitroacetamide is probably a consequence of the greater stability of the benzyloxocarbonium ion in relation to the methyloxocarbonium ion. N-Methyl-N-nitro-p-chloro-benzamide seems to hydrolyse via a similar mechanism of N-methyl-N-nitrobenzamide. In the range of acidities studied, (1 to 6 M H_2SO_4) the reaction rate is almost independent of acidity. K_o Values are nevertheless higher than the ones for N-methyl-N-nitrobenzamide (in H_2SO_4 1 M, K_o values are $2.2 \cdot 10^{-4}$ s^{-1} and $1.3 \cdot 10^{-4}$ s^{-1} respectively) and this is expected since the Cl group increases the positive character of the carbonyl group and facilitates nucleophilic attack by water.

3.2,3 O And N protonation

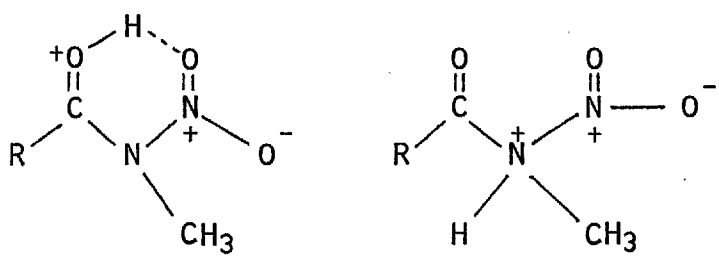
The site of protonation of amides has been the subject of controversy (cf. Section 1.2,2). This problem is manifested with N-nitroamides

Figure 3.2,2. Correlation of hydrolysis rates of N-methyl-N-nitrobenzamide with acidity functions



although delocalisation of nitrogen lone pair electrons to the carbonyl group is diminished by the presence of the N-nitro group.

Considering the two possible conjugated acids I and II (Figure 3.2,3), it is possible to argue that I will not favour the existence of an Ac 1 mechanism. The Ac 1 pathway probably goes via intermediate II. However, the Ac 2 pathway can go as well by either of the two intermediates and it is not possible to draw conclusions about the site of protonation at the moment.



I II

Figure 3.2,3

The deamination of N-nitrosoamides has been argued to proceed via the O-protonated conjugated acid for N-alkyl-N-nitrosoamides. However, this has been deduced indirectly. N-nitrosoamides can either deaminate or denitrosate in acid via two independent pathways involving unique conjugated acid intermediates and since denitrosation goes most probably via the N-conjugate acid, deamination must go via the O-conjugate acid. Since N-nitroamides hydrolyse exclusively via deamination, it is not possible to draw a parallel between the two reactions. The reason why N-nitroamides do not denitrosate must lie in the different leaving group ability of the $-NO_2$ and -NO groups. The basicity of NO^+ is known to

be $pK_A = -6.12$[84]. An equivalent figure is not known for NO_2^+ but it only forms at higher acidities[85] and must, therefore, be a stronger acid (hence poorer leaving group).

CHAPTER 4

EXPERIMENTAL DETAILS

4. EXPERIMENTAL DETAILS

Melting points were measured using a Köffler hot stage and are uncorrected. Ultraviolet spectra were recorded on a Unicam SP 1800 or a Unicam SP 800. Infrared spectra were recorded on a Perkin-Elmer 157G. Proton NMR spectra were recorded on a Varian T60 spectrometer, using TMS as an internal or external reference. Mass spectra were recorded on an AEI MS9 spectrometer. pH Measurements were carried out on a Radiometer model 26 and an EIL 7050 pH meter. Gas liquid chromatography was performed on a Perkin Elmer F11 gas chromatograph.

4.1 Purification of reagents and solvents

4.1,1 Reagents

Acetic acid, sodium acetate, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, disodium tetraborate, sodium chloride, sodium perchlorate, sulphuric acid and perchloric acid were all Analar reagents and were used without further purification.

Pyridine, N-methylimidazole, morpholine, piperidine and 2,2',6,6'-tetramethylpiperidine were vacuum distilled from KOH and then kept at $-20^{\circ}C$.

Imidazole and acetylimidazole were recrystallised from benzene, p-chlorophenol from petroleum ether ($60-80^{\circ}C$) and all were vacuum dried. Sodium hydroxide and hydrochloric acid were BDH volumetric solutions.

4.1,2 Solvents

The distilled water used in preparing reaction solutions was

passed through an Elgastat ion exchange resin. Dimethyl-sulphoxide was purified by distillation from calcium hydride. Analar grade methanol was used without further purification. Other solvents (chloroform, ether, petroleum-ether, benzene) were of GPR grade.

4.2 Synthesis of Substrates

4.2,1 N-Nitroamides

The N-nitroamides were prepared by nitration of the corresponding amides. N-methylacetamide was obtained from Aldrich, and the N-methyl-benzamides were prepared by the Schotten-Bauman reaction [7].

4.2,1a N-Methyl-N-nitroacetamide

Dinitrogen pentoxide [12] (5.6 g) was dissolved in chloroform (60 ml) and N-methylacetamide (3.6 g) was added at -60°C. The stirred mixture was warmed to room temperature, washed with sodium bicarbonate solution (5%) until neutral and dried over sodium sulphate. The chloroform was then evaporated under reduced pressure and the resultant oil distilled at 9.5 mmHg. The fraction boiling at 58°C was collected and fractionally distilled, giving a colourless liquid, b.p. 58-59/9.5 mmHg (lit.[4] 58-59/12 mmHg) Yield 30%.

R.I. n_D^{23} - 1.4662 (lit.,[4] n_D^{26} - 1.4656; [12] n_D^{20} - 1.4657)

UV. λ_{max} (H_2O) 244 nm (log ε 3.93)

IR. ν_{max} (liquid film) 3000, 2940, 1700, 1560, 1415, 1370, 1320, 1230, 1120, 1040, 940, 760 cm^{-1}

NMR. δ ($CDCl_3$) 2.65 (3H, s), 3.6 (3H, s)

Analysis. Found: C, 30.89 ; H, 5.31 ; N, 23.77%; $C_3H_6N_2O_3$ requires C, 30.51 ; H, 5.12 ; N, 23.73%.

4.2,1b N-Methyl-N-nitrobenzamides

General procedure[7] :

The N-methyl-N-nitrobenzamides were prepared by nitration of the corresponding benzamides with nitric acid in acetic anhydride. A typical synthesis is given.

N-Methyl-N-nitrobenzamide:

Acetic anhydride (2 ml) was cooled to -15°C and fumming nitric acid (1 ml) was added. N-methylbenzamide (0.25 g) was dissolved in acetic anhydride (4 ml) and then added to the mixture while maintaining the temperature at -15°C. The temperature was allowed to rise to 0°C and so maintained for 1 hour with continuous stirring. The reaction solution was then poured onto ice, the precipitate filtered off, washed with water and dried. Recrystallisation from petroleum ether (40-60°C) gave white needles, m.p. 58-59.5°C (lit.[7] 63°C). Yield 57%.

UV. λ_{max} (water) 251 nm (log ε 4.03) (lit.[7] λ_{max} (isooctane) 248 nm, log ε 4.88)

IR. ν_{max} (nujol) 1700, 1550, 1330, 1240, 970 cm^{-1}, (lit.[7] ν_{max} (CCl_4) 1711, 1570, 1323 cm^{-1})

NMR. δ ($CDCl_3$) 3.64 (3H, s), 7.6 (5H, m)

Mass Spec. M/e 180 (M^+), 122, 105, 77, 51

Analysis. Found C 53.30 ; H, 4.39 ; N, 15.43%. $C_8H_8N_2O_3$ requires C, 53.33 ; H, 4.44 ; N, 15.55%

Other compounds prepared in this manner were:-

N-Methyl-N-nitro-p-chlorobenzamide:

Yield. 63%

m.p. 59-60°C (Lit.[7] 65°C) from petroleum ether (60-80)

UV. λ_{max} (water) 256 nm, log ε 4.06 (Lit.[7] λ_{max} (isooctane) 258 nm, log ε 4.07)

IR. ν_{max} (nujol) 1700, 1590, 1550, 1415, 1325, 1240 cm^{-1} (Lit.[7] ν_{max} (CCl_4) 1713, 1579, 1323 cm^{-1})

NMR. δ ($CDCl_3$) 3.95 (3H, s), 7.8 (4H, m)

Mass Spec. M/e 214 (M^+), 139, 111, 75, 50

Analysis. Found: C, 44.55; H, 3.16; Cl, 16.16; N, 12.90%. $C_8H_7ClN_2O_3$ requires C, 44.86; H, 3.27; Cl, 16.35; N, 13.08%.

N-Methyl-N-nitro-p-nitrobenzamide:

Yield. 30%

m.p. 58°C (Lit.[7] 58°C) from petroleum ether (60-80°C)

UV. λ_{max} (water) 262 nm, log ε 4.17 (Lit.[7] λ_{max} (isooctane) 256 nm, log ε 4.64)

IR. ν_{max} (nujol) 1700, 1550, 1510, 1350, 980 cm^{-1} (Lit.[7] ν_{max} (CCl_4) 1710, 1580, 1532, 1345 cm^{-1})

NMR. δ ($CDCl_3$) 3.9 (3H, s), 8.4 (4H, ABq)

Mass Spec. M/e 225 (M^+), 179, 164, 150, 120, 104, 76, 50

Analysis. Found: C, 42.86 ; H, 3.15 ; N, 18.47 % $C_8H_7N_3O_5$ requires C, 42.67 ; H, 3.11 ; N, 18.67%.

N-Methyl-N-nitro-p-methoxybenzamide:

Nitration of N-methyl-p-methoxybenzamide with nitric acid has been reported [7] to give the N-methyl-N-nitro-4-methoxy-3-nitrobenzamide. Attempts at preparing this compound by reaction of p-methoxybenzoyl chloride with the methylnitramine sodium salt were unsuccessful. The only product obtained for this reaction was the p-methoxybenzoylanhydride.

4.2,1c Labelled N-methyl-N-nitrobenzamide[131]

N-methylbenzimidoylchloride (1g) and $^{18}H_2O$ (0.2 g, 10% incorporation) were slowly mixed together and stirred until a solid was formed. This solid, without purification, was treated with nitric acid in acetic anhydride as described before. The product showed the incorporation of 11.5% ^{18}O by mass spectrometry.

4.2,2 N-Methylnitramine

N-Methyl-N-nitro-p-toluenosulphonamide (2 g) was heated under reflux with sodium hydroxide (20 ml, M) until dissolved. The solution was then acidified, saturated with sodium chloride, cooled in dry ice, and extracted with ether (6 x 20 ml). The ethereal solution was dried over anhydrous magnesium sulphate and evaporated to give an oil which on crystallization from dichloromethane-petroleum ether (40-60°C) gave white needles, m.p. 32-34°C (Lit.[86] 32-36°C). Yield 30%.

UV. λ_{max} (H_2O) 229 nm, log ε 3.87. (Lit.[87] λ_{max} (5 . 10^{-3} HCl N) 232.5, log ε 3.85; λ_{max} (N/4 KOH) 228, log ε 3.88)

IR. ν_{max} (CCl_4) 3410, 3300, 2960, 1580, 1475, 1440, 1395, 1335, 1185, 1110, 870 cm^{-1}. (Lit.[88] ν_{max} (CCl_4), 3412, 3290, 3158, 2979, 2947, 2912, 2852, 1582, 1471, 1439, 1399, 1326, 1178, 1099)

Mass Spec. M/e 76 (M^+), 59, 46.

4.3 Product Analysis

4.3,1 N-Methyl-N-nitroacetamide

4.3,1a Hydrolysis in acetic acid-sodium acetate buffer

N-Methyl-N-nitroacetamide (3.4 g), acetic acid (1.7 g), sodium acetate (2.4 g) and water (40 ml) were mixed together and maintained at 0°C for one week. When the N-methyl-N-nitroacetamide had dissolved completely in the aqueous solution, the solution was acidified to pH ca.2.5, saturated with sodium chloride, cooled and extracted with ether (5 x 50 ml). The ethereal solution was dried over magnesium sulphate and evaporated under reduced pressure. The resultant oil was found by NMR to be a mixture of methylnitramine and acetic acid. Using a silica gel chromatographic column, a pure sample of methylnitramine was obtained. Its physical properties were the same as authentic methylnitramine.

4.3,1b Spectra of the reaction solutions

The ultraviolet spectra of the reaction solutions at infinity showed the characteristic peaks for the products, acetic acid and

and methylnitramine. Similar spectra were obtained when authentic samples of acetic acid and methylnitramine were mixed in similar concentrations (Figure 4.3,1b). In strong acidic solutions, methylnitramine undergoes hydrolysis to give methanol, N_2O and H_2O. The NMR spectra of N-methyl-N-nitroacetamide in concentrated D_2SO_4, shows resonances due to protonated acetic acid (δ 2.72. Lit.[89] δ 2.67) and to methanol (δ 4.18).

4.3,2 N-Methyl-N-nitrobenzamide

4.3,2a Hydrolysis in phosphate buffer

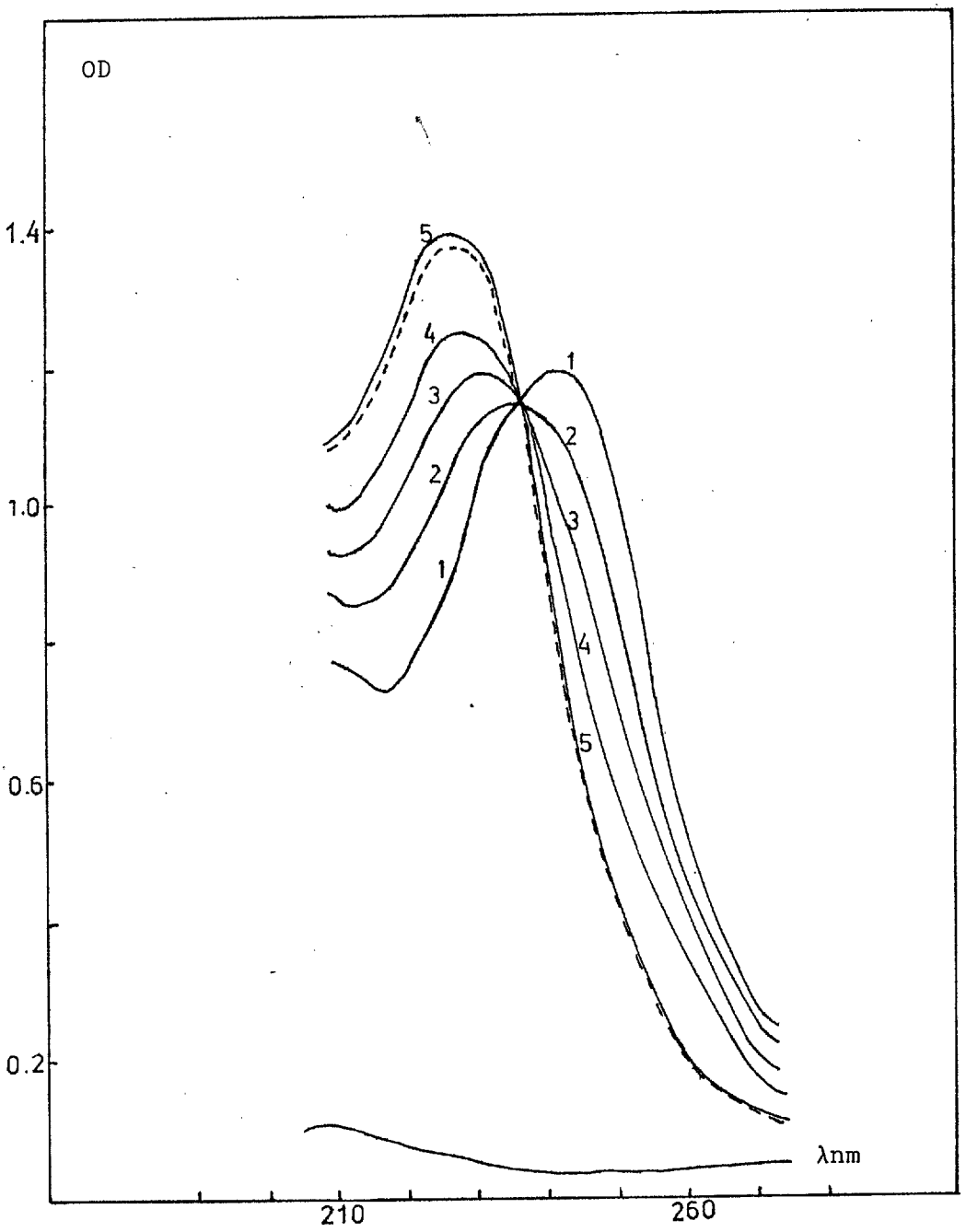
N-Methyl-N-nitrobenzamide (0.09 g), dioxane (15 ml) and phosphate buffer (20 ml, KH_2PO_4 0.5 M, NaOH 0.375 M, NaCl 2.5 M) were mixed together and diluted to 100 ml with water. The mixture was left stirring until hydrolysis was complete. The solution was then acidified to pH ca. 1 (H_2SO_4 M) and extracted with ether (3 x 50 ml). The ether was dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The resultant solid was recrystallised from petroleum ether (80-100°C) and gave white plates, m.p. 115-122°C (Lit.[90] 122°C). A mixed melting point with a sample of benzoic acid shows no depression.

4.3,2b Hydrolysis in sulphuric acid

H_2SO_4 1 M and 9 M

N-Methyl-N-nitrobenzamide (0.095 g), dioxane (10 ml) and sulphuric acid (10 ml, 10 M) were mixed and diluted to 100 ml with water. After 2 hours, the solution was extracted with ether (3 x 25 ml), the ether dried over anhydrous magnesium sulphate and evaporated. The resultant solid was recrystallised from petroleum ether (80-100) and gave white plates,

Figure 4.3,1b. U.v. spectra of the products of hydrolysis of N-methyl-N-nitro acetamide



——— $2 . 10^{-4}$ M N-methyl-N-nitroacetamide in 0.1 M borate buffer

------ $2 . 10^{-4}$ M methylnitramine + $2 . 10^{-4}$ M acetic acid

m.p. 118-122°C. The mass spectrum of this compound was identical to benzoic acid (M^+ 122). A similar result was obtained when the hydrolysis was carried out in 9 M H_2SO_4.

4.3,2c Spectra of the reaction solutions

The UV spectra of the reaction solutions showed absorbances due to benzoic acid and methylnitramine. Similar spectra were obtained when authentic samples of benzoic acid and methylnitramine were mixed in similar concentrations (Figure 4.3,2c). The NMR spectra of the reaction solution in c D_2SO_4 shows only resonances due to benzoic acid and methanol (δ 4.18 (3H, s), 8.3 (5H, m)).

4.3,3 N-Methyl-N-nitro-p-nitrobenzamide

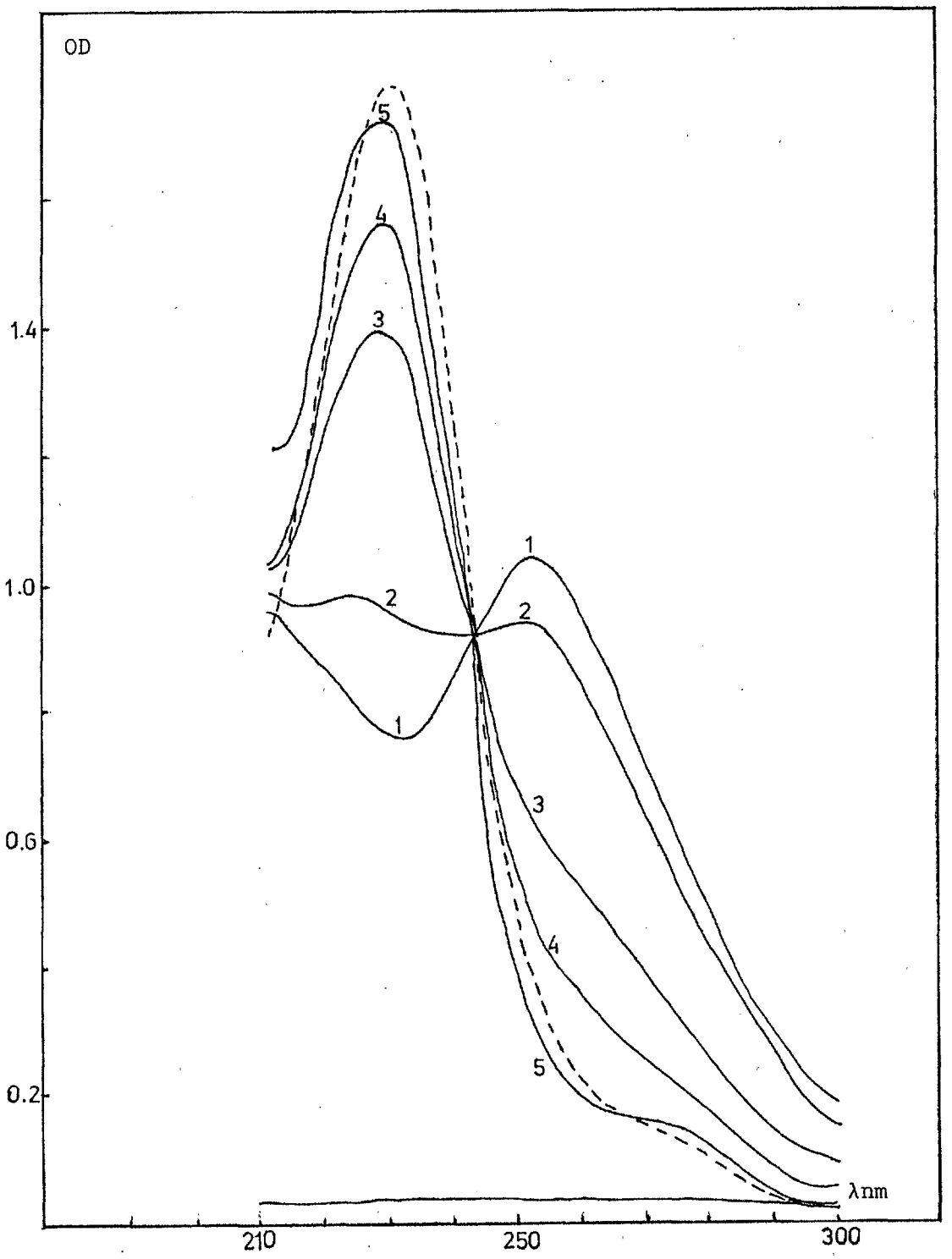
4.3,3a Hydrolysis in sulphuric acid

N-Methyl-N-nitro-p-nitrobenzamide (225 mg) was dissolved in methanol (30 ml) and sulphuric acid (20 ml, 2M) added. The solution was made up to 100 ml with water, and left stirring overnight. A solid precipitated out which was filtered and recrystallised from water, giving crystals (0.05 g), m.p. 220-232 (p-nitrobenzoic acid m.p. 242°C).

IR. ν_{max} (Nujol) 1690, 1600, 1520, 1355, 1295, 1285, 720
(Lit.[91] (Nujol) 1689, 1433, 1357, 1317, 1300, 1290, 717.)

The filtrate was extracted with ether (3 x 50 ml), the extract dried over magnesium sulphate and evaporated under reduced pressure. The resultant solid was purified by silica gel preparative layer chromatography using chloroform as eluent. Two products were isolated and identified as methyl-p-nitrobenzoate ester, m.p. 90-94°C (Lit.[90] 96°C),

Figure 4.3,2c. U.v. spectra of the products of hydrolysis of N-methyl-N-nitrobenzamide



——— 1 . 10^{-4} M N-methyl-N-nitrobenzamide in 0.01 M NaOH

- - - - - 1 . 10^{-4} M methylnitramine + 1 . 10^{14} M benzoic acid

Mass spec. M/e 181 (M^+), and p-nitrobenzoic acid.

4.4 Details of kinetic experiments

4.4,1 Kinetic method

The hydrolysis of the N-nitroamides was followed by monitoring the decrease in UV absorbance of the substrate, using a Unicam SP 1800 spectrophotometer. The reaction was followed continuously in the spectrophotometer cuvette which was thermostated at the appropriate temperature. The measurements were made against a reference cell containing all components of the reaction solution except the substrate.

In a typical experiment, the reaction solution was prepared in a 25 ml or 10 ml volumetric flask and placed in a thermostated bath. After equlibration, the reaction was started, in the case of N-methyl-N-nitroacetamide, by addition of microlitre quantities using a syringe and in the case of the other N-nitroamides by the addition of 1 ml of an aqueous solution. The reaction solution was then made up to the appropriate volume, shaken vigorously and an aliquot transferred to the UV cell. The absorbance of this solution was then monitored at timed intervals, using either the spectral scan or fixed wavelength mode. The reactions were followed for ca. 10 half-lives to obtain an infinity reading. At the conclusion of the experiment, either the pH of the reaction solution was measured or the acidity of the solution was determined by titration against standard alkali.

The temperature of the runs was checked regularly, within the UV cell, using a calibrated thermometer and was found to be within $\pm$ 0.1°C.

Reactions in solvent D_2O were made in an analogous manner to those in H_2O, but on a smaller scale. The pHs of the D_2O solutions were measured in the same way, but pD values were calculated from the expression $pD = pH + 0.4$ [63].

4.4,2 Computation of rate coefficients

The observed rate has a first order dependance upon [substrate] (eq. 4.4,2a) and usually a pseudo first order rate coefficient, $\underline{K}_o$ was calculated from the experimental data by means of equation 4.4,2b where- a_o is the initial [substrate] and x is the amount reacted at time t.

$$\frac{-d}{dt}[\text{substrate}] = \underline{K}_o [\text{substrate}] \qquad \ldots \text{ eq. } 4.4,2a$$

$$\ln \frac{a_o}{(a_o - x)} = K_o t \qquad \ldots \text{ eq. } 4.4,2b$$

In terms of absorbance, eq. 4.4,2b becomes:

$$\ln \frac{(At - A_\infty)}{(A_o - A_\infty)} = K_o t \qquad \ldots \text{ eq. } 4.4,2c$$

where At, A_o and A_∞ are values of the absorbances of the substrate at times t, o and ∞. For the base catalysed reactions plots of ln $(At-A_\infty)$ against time were linear and the rate coefficients could be calculated accurately from the slope. In basic media, especially at the higher pHs studied (ca 9.5), K values are accurate to about $\pm$ 13%.

Typical kinetic runs are given in Tables 4.4,2a; 4.4,2b; 4.4,2c and 4.4,2d.

Table 4.4,2a. 6.75 ml, 0.5 M Na_2HPO_4 + 0.38 ml 1M $HClO_4$ + 4.7 ml 2M $NaClO_4$, diluted to 25 ml; T = 25°C; μ = 0.75 M; pH = 7.34; λ_{max} 244 nm; Initial $[CH_3CON(NO_2)CH_3]$ ca. 2.10^{-4} M

t min	A_t	A_t-A_∞	% Reaction	$10^4.K_o s^{-1}$
2	1.500	0.800	-	-
7	1.440	0.740	7.5	2.6
20	1.320	0.620	22.5	2.3
25	1.280	0.580	27.5	2.2
35	1.200	0.500	37.5	2.5
40	1.165	0.465	41.9	2.4
50	1.100	0.400	50.0	2.5
60	1.050	0.350	56.2	2.2
80	0.960	0.260	67.5	2.5
100	0.895	0.195	75.6	2.4
200	0.750	0.050	93.7	2.4
∞	0.70	-		-
			Ave. =	2.34

Table 4.4,2b. 3.1 ml borate buffer ($Na_2B_4O_7$ 0.125 M, NaOH 9 . 10^{-3}M) + 9.2 ml 2M $NaClO_4$ + 2.5 ml 10^{-3}M p-chlorophenol diluted to 25 ml; T = 25°C; μ = 0.75 M; pH = 8.6; λ_{max} = 224 nm; Initial $[CH_3CON(NO_2)CH_3]$ ca. 2 . 10^{-4} M

t min	A_t	A_t-A_∞	% Reaction	$10^4.K_o s^{-1}$
0.75	1.250	0.830	-	-
1.75	1.180	0.760	8.4	14.7
2.25	1.140	0.720	13.2	13.5
4.25	1.025	0.605	27.1	14.5
6.25	0.930	0.510	38.5	14.3
8.25	0.845	0.425	48.9	15.2
10.25	0.775	0.355	57.2	14.9
12.25	0.720	0.300	63.8	14.1
14.25	0.670	0.250	69.9	15.2
16.25	0.635	0.215	74.1	12.6
17.25	0.615	0.195	76.5	16.6
∞	0.42	-	-	-
			Ave. =	14.6

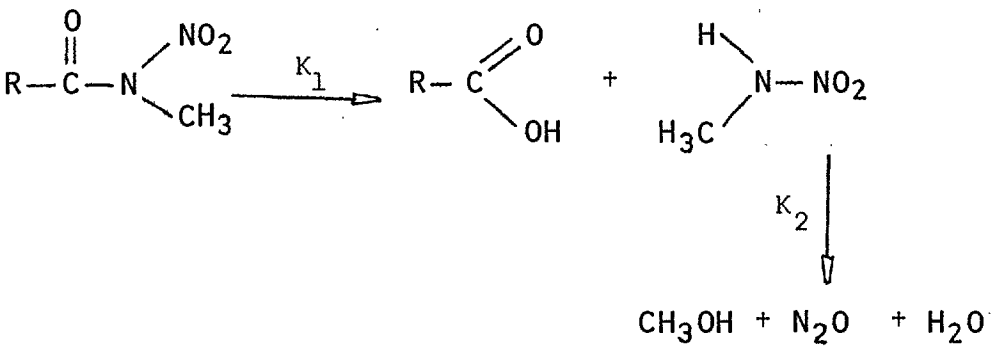
Table 4.4,2c. 2.5 ml 1M KH_2PO_4 + 1.9 ml 1M NaOH + 2.5 ml 5 M NaCl + 5 ml 10^{-3} M morpholine, diluted to 25 ml; T = 25°C; μ = 0.75 M; pH = 7.00; λ_{max} = 251 nm; Initial $[C_6H_5CON(NO_2)CH_3]$ ca. 10^{-4} M

t min	A_t	A_t-A_∞	% Reaction	$10^4.K_o s^{-1}$
7	0.670	0.420	-	-
9	0.650	0.400	4.7	4.1
11	0.630	0.380	9.5	4.2
13	0.615	0.365	13.1	3.3
15	0.595	0.345	17.8	4.7
25	0.520	0.270	35.7	4.2
33	0.470	0.220	47.6	4.2
45	0.410	0.160	61.9	4.4
59	0.365	0.115	72.6	3.9
79	0.320	0.070	83.3	4.2
89	0.305	0.055	86.9	4.0
∞	0.25	-	-	-
				Ave.= 4.1

Table 4.4,2d. 3.1 ml borate buffer ($Na_2B_4O_7$ 0.125 M, NaOH 0.12 M) + 9 ml 2 M $NaClO_4$ diluted to 25 ml; T = 25°C; μ = 0.75 M; pH = 9.42; λ_{max} = 2.44 nm; Initial $[C_6H_5CON(NO_2)CH_3]$ ca. 10^{-4} M

t min	A_t	A_t-A_∞	% Reaction	$10^4.K_o s^{-1}$
0.67	0.700	0.430	-	-
1.17	0.630	0.360	16.3	59.3
1.67	0.570	0.300	30.2	60.6
2.17	0.520	0.250	41.9	60.6
2.67	0.480	0.210	51.2	58.3
3.17	0.445	0.175	59.3	60.6
3.67	0.415	0.145	66.3	62.6
4.17	0.390	0.120	72.1	63.3
5.17	0.350	0.080	81.4	67.5
6.17	0.325	0.055	87.2	62.3
∞	0.27	-	-	-
			Ave. =	61.7

Hydrolysis of N-nitroamides under acidic conditions gives the corresponding carboxylic acid and methylnitramine as products. However, further hydrolysis of methylnitramine also occurs under the reaction conditions giving CH_3OH, N_2O and water (Scheme 4.4,2a).



Scheme 4.4,2a

As a consequence, when following the decomposition of the nitroamides by U.V., the read infinity is lower than it should be expected for the absorbances due to the carboxylic acid and methylnitramine. A theoretical infinity (A_∞^{th}) was calculated from equation 4.4,2a.

$$A_\infty^{th} = A_{Acid} + A_{Nit} = C_o^{Acid}\ \varepsilon^{Acid} + C_o^{Nit}\ \varepsilon^{Nit}$$

... eq. 4.4,2a

where C_o is the initial [substrate].

The values of ε used are listed in Table 4.42e and Table 4.4,2f

Table 4.4,2e. ε values for substrate and methylnitramine in water

	λ nm	ε^{sub}	ε^{nit}
$CH_3CON(NO_2)CH_3$	244	8446.	5000.
$C_6H_5CON(NO_2)CH_3$	251	10700.	2900.
$ClC_6H_4CON(NO_2)CH_3$	256	11600.	1800.

Table 4.4,2f. ε values for carboxylic acids at several acidities

	λ mn	$[H_2SO_4]$M	ε
CH_3COOH	244	1 to 9	10
C_6H_5COOH	251	1	200
	"	2	400
	"	4	800
	"	6	1000
	"	8	1200
	"	9	1600
ClC_6H_4COOH	256	1	3400
	"	2	4300
	"	3	4500
	"	4	4800
	"	5	5100
	"	6	6200

Hence $\underline{K}_o$ values were obtained from plots of $\ln(A_t-A_\infty^{th})$ against time. These plots were linear for at least two half-lives, for N-methyl-N-nitrobenzamide and N-methyl-N-nitro-p-chlorobenzamide.

In the case of N-methyl-N-nitroacetamide the value of A_∞^{th} is only slightly lower than the absorbance of the reaction solution A_t, so at high acidities, when the reactions are very fast, the results calculated by this method were considered unreliable. However, being a case of consecutive first order reaction (Scheme 4.4,2b) it is

possible to determine the concentrations of the different species involved at time t (eq. 4.4,2b) [92].

$$A \xrightarrow{K_1} B \xrightarrow{K_2} C$$

Scheme 4.4,2b

$$A = A_o e^{-K_1 t}$$

$$B = \frac{A_o K_1}{K_2 - K_1} (e^{-K_1 t} - e^{-K_2 t}) \qquad \text{... eq. 4.4,2b}$$

$$C = A_o - A - B$$

And thus the contributions of each species for the observed absorbance at time t (eq. 4.4,2c).

$$A_{obs} = {}_{obs}A_o = E_A A + E_B B + E_C C \qquad \text{... 4.4,2c}$$

Thus if we have a set of approximate values of K_1, K_2, E_A, E_B, and E_C it is possible to calculate values of A_{obs} as a function of time and compare them with the observed values. The K_1 values used were the ones calculated with A_∞^{th} mentioned before and K_2 values were measured independently using an authentic sample of methylnitramine and hydrolysing it in the same conditions as the N-methyl-N-nitroacetamide (Appendix 1).

A computer programme [92] was used to perform these calculations. The average deviation between the calculated values and the observed

values of A_{obs} was used as a criteria of accuracy, and the computer varied the approximate values of K_1, K_2, E_A, E_B and E_C so as to minimize the deviation.

Rate coefficients for these reactions were usually reproducible to better than 50% depending on the rate of reaction. Typical kinetic runs are given in Tables 4.4,2g; 4.4,2h; 4.4,2i; and 4.4,2j.

Table 4.4,2g 4ml 10M D_2SO_4 diluted to 10 ml; T = 25°C; λ_{max} 244 nm; Initial $[CH_3CON(NO_2)CH_3]$ ca. $2 \cdot 10^{-4}$ M

t min	A_t	$A_t - A_\infty$	% reaction	$10^4 \cdot K_o s^{-1}$
1	1.420	0.620	-	-
5	1.290	0.490	20.9	2.6
7	1.240	0.440	29.0	9.2
9	1.185	0.385	37.9	10.8
11	1.140	0.340	45.2	10.8
13	1.095	0.295	52.4	11.7
15	1.055	0.255	63.7	11.7
19	0.990	0.190	69.3	12.5
24	0.930	0.130	79.0	12.7
∞	0.80	-	-	-
				Aver. 11.1

Value obtained by the computer method: 9.1×10^{-4} s^{-1}

Table 4.4,2h 2ml 10M H_2SO_4 diluted to 10 ml; T = 44°C; λ_{max} = 244 nm; Initial $[CH_3CON(NO_2)CH_3]$ ca. $2 \cdot 10^{-4}$ M

t min	A_t	$A_t - A_\infty$	% reaction	$10^4 \cdot K_o$ s^{-1}
1.0	1.460	0.60	-	
1.5	1.430	0.57	5.0	16.7
2.0	1.400	0.54	10.0	16.7
2.5	1.360	0.50	16.7	26.6
3.0	1.330	0.47	21.7	20.0
3.5	1.300	0.44	26.7	23.3
4.5	1.240	0.38	36.7	25.0
6.0	1.170	0.31	48.3	22.2
7.0	1.130	0.27	55.0	23.3
8.0	1.090	0.23	61.7	26.6
9.0	1.060	0.20	66.7	23.3
∞	0.800	-	-	-

Value obtained by the computer method: $21.1 \times 10^{-4} s^{-1}$ Aver. 22.37

Table 4.4,2i. 1 ml 10M H_2SO_4 diluted to 10 ml; T = 25°C; λ_{max} = 251 nm

Initial $[C_6H_4CON(NO_2)CH_3]$ ca. $1 \cdot 10^{-4}$ M.

t min	A_t	$A_t - A_\infty$	% reaction	$10^4 \cdot K_o s^{-1}$
1	1.080	0.750	-	-
7	1.020	0.690	8.0	2.2
27	0.930	0.600	20.0	1.2
47	0.840	0.510	32.0	1.3
67	0.770	0.440	41.3	1.2
87	0.710	0.380	49.3	1.2
117	0.630	0.300	60.0	1.3
142	0.585	0.255	66.0	1.1
182	0.525	0.195	74.0	1.1
202	0.500	0.170	77.4	1.2
362	0.420	0.090	88.0	0.4
∞	0.330	-	-	-
				Aver.1.2

Table 4.4,2j. 2 ml 10M H_2SO_4 diluted to 10 ml; T = 15°C; $\lambda_{max.}$ = 251 nm

Initial $[C_6H_4CON(NO_2)CH_3]$ ca. $1 \cdot 10^{-4}$ M

t min	A_t	$A_t - A_\infty$	% reaction	$10^4 \cdot K_o s^{-1}$
1	1.17	0.81	-	-
61	1.07	0.71	12.3	0.36
121	0.95	0.59	27.2	0.53
181	0.85	0.49	39.5	0.50
221	0.80	0.44	45.7	0.46
281	0.72	0.36	55.5	0.55
321	0.685	0.325	59.9	0.42
361	0.65	0.29	64.2	0.50
401	0.63	0.27	66.7	0.29
481	0.59	0.23	71.6	0.33
521	0.57	0.21	74.1	0.37
∞	0.36	-	-	-
				Aver. 0.43

4.5 O^{18} Exchange experiments

4.5,1 O^{18} Exchange in phosphate buffer

Extraction of starting material

Phosphate buffer (20 ml, KH_2PO_4 0.5 M, NaOH 0.375 M, NaCl 2.5 M) was added to a solution of N-methyl-N-nitrobenzamide (0.09 g) in dioxane (10 ml) and made up to 100 ml. Samples were taken at 10 min (25 ml of solution), 20 min (25 ml of solution) and 30 min (50 ml of solution). These samples were extracted with ether (3 x 25 ml) and the ethereal solution was dried over anhydrous magnesium sulphate and evaporated. The resultant solids were recrystallised from petroleum ether (40-60°C) giving white needles (Table 4.5,1).

Table 4.5,1. Extraction of starting material

Time (min)	Amount extracted (mg)	m.p. °C
10	19	57 - 59
20	15	58.5-59
30	25	57.5-59

Extraction of product

Another experiment was performed under identical conditions, except that the solution was left overnight until the reaction was complete. The solution was then acidified (H_2SO_4M) until pH ∿ 1 and extracted with ether (3 x 50 ml). The ether was dried over magnesium sulphate and evaporated under reduced pressure. A solid was obtained (67 mg) which on recrystallisation from petroleum ether (80-100) gave white plates m.p. 115-122°C.

4.5,2 Oxygen exchange in sulphuric acid

H_2SO_4 1 M

N-Methyl-N-nitrobenzamide (0.09 g) was dissolved in dioxane (10 ml) and sulphuric acid (10 ml, 10M) added. The solution was made up to 100 ml and samples taken at 42 min (25 ml solution, 85 min (25 ml solution) and 127 min (50 ml solution). The samples were extracted with ether (3x25 ml) and the ethereal solutions dried over magnesium sulphate and evaporated under reduced pressure. The resultant solids were recrystallised from petroleum ether (40-60°C) giving white plates (Table 4.5,2a).

Table 4.5,2a. Extraction of product

Time (min)	m.p.(°C)
42	115-120
85	100-115
127	110-115

H_2SO_4 9M

Another experiment was performed under similar conditions increasing the amount of sulphuric acid used (90 ml, 10M). Samples were taken after 5 min and 10 min (50 ml solution each). The samples were extracted with ether (3 x 25 ml). The ethereal extracts were then washed with sodium bicarbonate (5%) until neutral, dried and evaporated as before. The resultant solids were recrystallised from petroleum ether (40-60°C) (Table 4.5,2b)

Table 4.5,2b. Extraction of starting material and product

Time (min)	m.p. (°C)
5	58-60
10	118-122

PART 2

OXIDATION OF PHENOLIC COMPOUNDS

BY SODIUM NITRITE

CHAPTER 1

INTRODUCTION

1.1 PHENOLIC COMPOUNDS AS NITRITE TRAPS

N-Nitroso compounds(N-nitrosamines and N-nitrosamides) are known to be carcinogenic to laboratory animals[93]. They can be formed by the reaction of acidified nitrite with amines and amides, in vitro and in vivo[94]. Amines, ureas and carbamates occur widely as food constituents, food additives, drugs and pesticides. Nitrite occurs in water and human saliva and it is used as a preservative for meat and fish. In addition, nitrates which are widely distributed in vegetables can undergo bacterial reduction to produce significant concentrations of nitrite. Although it remains to be established whether N-nitroso compounds have a direct relationship to human cancer, the possibility cannot be ignored and has been intensively studied in the last decade.

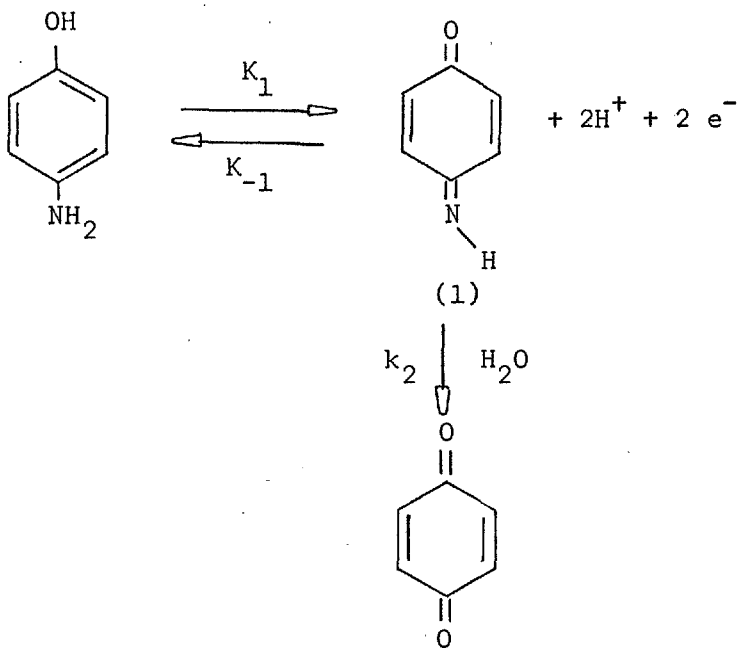
One direction of recent research has been to examine compounds which react faster with acidified nitrite than the amino compounds, hence avoiding the formation of N-nitroso compounds. Challis[95] noticed that phenols, which are common constituents of dietary products, react much faster than secondary amines under physiological conditions to form C-nitroso compounds. C-Nitrosation was observed in meats[96] and at a higher rate with phenolic compounds than with the amines present[97]. Other phenolic compounds were found to influence the amount of nitrosamine formation when present, e.g. catechols[98], gallic and tannic acid[99] and thymol, vanilin, hydroquinone and α- tocopherol [100].

Challis and Bartlett[101,102] studied the reaction of 4-methylcatechol with nitrous acid and found that the prevailing reaction is oxidation to the corresponding quinone with concurrent formation of nitric oxide. They also found that readily oxidizable phenols, such as 4-methylcatechol and chlorogenic acid (a constituent of fruits and coffee)

inhibit nitrosamine formation as reported earlier for ascorbic acid, both *in vitro* and *in vivo*[103] (c.f. Section 1.4).

1.2 OXIDATION OF p-AMINOPHENOLS

The mechanism of oxidation of p-aminophenol and its N-methyl and N,N-dimethyl derivatives has been studied extensively. Wieland[104] suggested that p-benzoquinonemonoimine (1) is an intermediate in the oxidation of p-aminophenol and Willstätter[105] has shown that p-benzoquinonemonoimine (1) rapidly hydrolyses to p-benzoquinone in acid solution. Conant and Pratt[106] suggested the mechanism in Scheme 1.2,1 for the oxidation of p-aminophenol with potassium ferricyamide. They postulated that the first step is rapid,



Scheme 1.2,1.

reversible, two electron oxidation and that the second step is the slow hydrolysis of (1). The rate of the overall reaction is independent of the dilution of the solution thus showing K_1 to be first order.

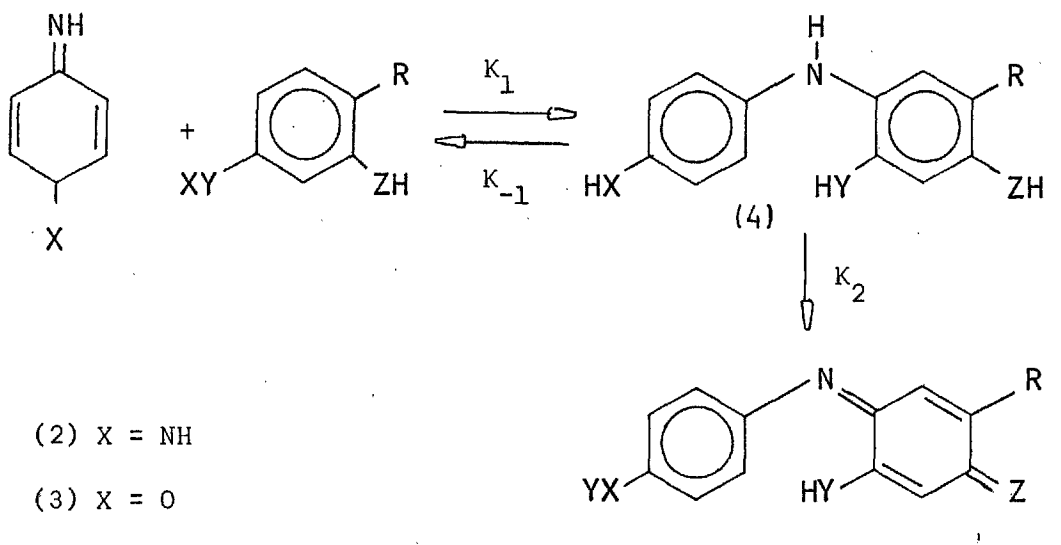
Fieser[107] studied the oxidation potentials of p-aminophenol-p-benzoquinonemonoimine and p-methylaminophenol-N-methyl-p-benzoquinone-monoimine (respectively E_o = 0.733 v and E_o = 0.693 v) and determined the basic dissociation constants of p-aminophenol and p-methylaminophenol (10.0 for both).

More recently, the anodic oxidation of p-aminophenol has been studied by polarography in both basic[108] and acidic[109] conditions. Results in both cases were found to agree with the mechanism in Scheme 1.2,1. Furthermore, the rate of hydrolysis of the p-benzoquinonemonoimine[110] was found to increase with pH. At pH > 5, 1,4-addition reactions of p-aminophenol to p-benzoquinone interfere in the reaction, resulting in disubstituted quinones. The electrochemical oxidation of p-dimethylaminophenol, (PDAP) however, proceeds <u>via</u> a different mechanism. Markus and Hawley[111] found that in acidic media the unprotonated PDAP suffers two electron oxidation to form the N,N-dimethyl-p-benzoquinonemonoimine which undergoes hydrolysis to give p-benzoquinone and dimethylamine. The oxidation of PDAP in the range 6-9 is a four electron process which yields 2-dimethylamino-p-benzoquinone as the product. In basic media, oxidation proceeds in two one-electron steps giving first the phenoxy radical and then the p-benzoquinonemonoimine.

Studies on the rate of autooxidation of p-aminophenols[112] established that N-methylation increases the rate of oxidation, but the rate of the dimethyl compound lies between the rate of the monomethyl and the parent compound.

p-Aminophenols and p-phenylenediamines have been used extensively

in colour photography as developing agents. Coupling reactions are used between the products of oxidation of both compounds, quinonemonoimines and quinonediimines respectively, and anions such as hydroquinone[113,114] and phenolates[115]. These types of reaction have also been used extensively in the dyeing of furs. Corbett studied the reactions of p-benzoquinone diimine (2) and monoimine (3) with m-diamines[116], phenols[117] and m-aminophenols[118] having at least one vacant position para to an electron donor. In each case, the reaction follows a two-step mechanism in which the initial reaction of the imine with the coupling reagent is rate controlling and is followed by rapid oxidation of the intermediate diphenylamine (4) by a second molecule of imine or some other oxidant (Scheme 1.2,2).



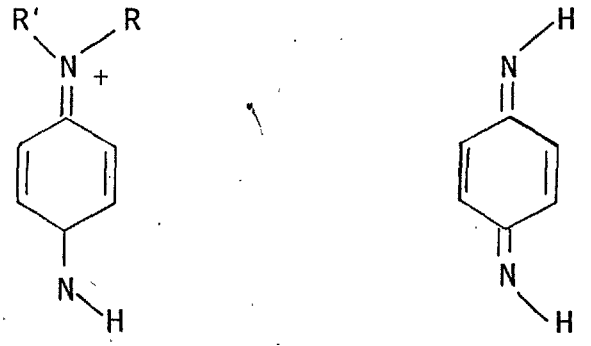
(2) X = NH

(3) X = O

Scheme 1.2,2

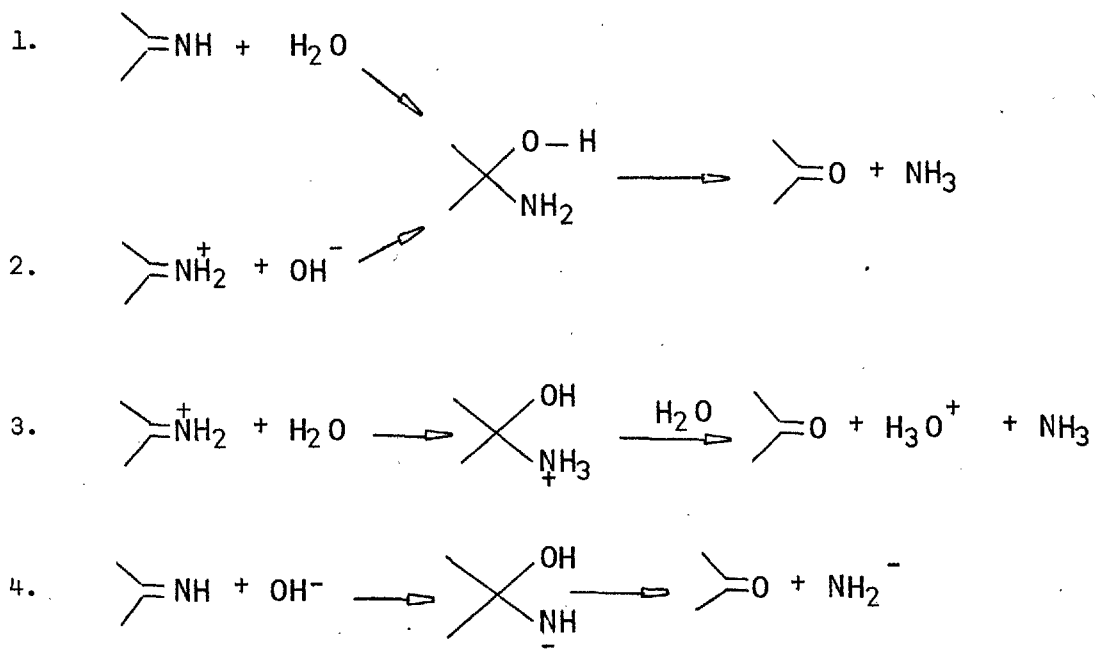
Tong[119] studied the effect of pH on the rate of hydrolysis of p-benzoquinoneimines over the pH range 7-12. For (5) he found the rate increased 10 fold for each pH unit and concluded that the reaction involved is OH^- catalysed hydrolysis of the cationic amine. For (6) he found that the rate of hydrolysis was independent of pH over the range 8.88-12.15

and concluded that since this imine existed in the central form at pH > 8, the hydrolysis in this region is uncatalysed. According to



(5) (6)

Corbett[120], the hydrolysis of imines can be considered to involve the mechanisms shown in Scheme 1.2,3 (c.f. hydrolysis of Schiff bases[121])



Scheme 1.2,3.

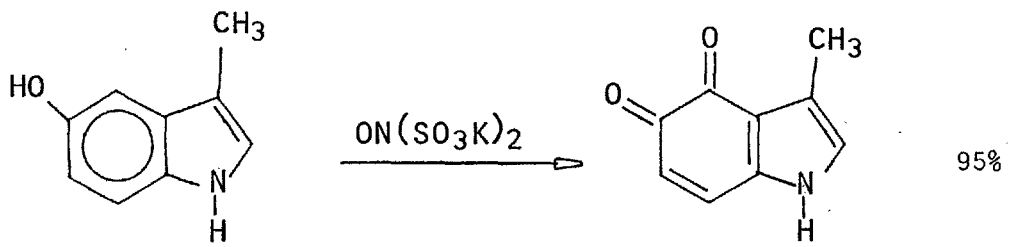
Over the pH range 6-12, the hydrolysis of p-benzoquinonediimine is considered to proceed via mechanisms 1,2 and 3[122]. The same happens to the p-benzo-

quinone over the pH range 2-10, although at higher pH there is evidence from a contribution of mechanism 4[119] (Scheme 1.2,3).

Nogami and co-workers[123] report that O-benzoquinonemonoimine and O-benzoquinonediimine were obtained by the oxidations of O-aminophenol and O-phenylenediamine, respectively with lead dioxide in organic solvent and potassium hexacyanoferrate (III) in aqueous buffer solution.

1.3 OXIDATION OF 5-HYDROXYINDOLES

The oxidation of hydroxyindoles has been studied by Teuber and co-workers[124]. They have investigated particularly the oxidation of hydroxyindoles with potassium nitrosodisulphonate and have obtained quinone derivatives. 5-Hydroxy-3-methylindole is oxidized in 95% yield to the quinone (7) (Scheme 1.3,1). 5-Hydroxy-2-phenylindole is oxidized in a similar manner. The reaction has also been successfully extended to a



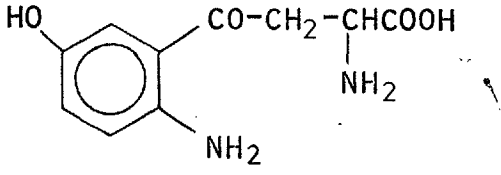
(7)

Scheme 1.3,1.

number of 2-methyl-3-carbethoxy-5-hydroxyindoles and again 4,5-quinones are the products[125].

The oxidation of 5-hydroxytryptophon with performic acid[126] gave

5-hydroxykynurenine (8), 6-hydroxy-2,4-quinoline-dicarboxylic acid (9) and 6-hydroxy-4-quinolinic acid (10).



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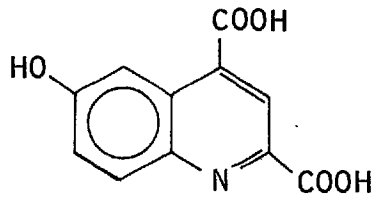

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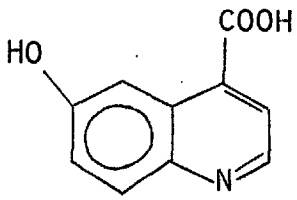


(10)

1.4 OXIDATIONS BY NITROUS ACID

Oxidation by nitrous acid is thought to involve an initial nitrosation followed by rapid transformation to give the oxidized products[122]. Empirical rate laws have been determined for many redox reactions of nitrite ion and nitrous acid with a substrate. R. Generally, the rate was found to be first order in R, or zero order,or between zero and one. These three types of rate law can be explained by Scheme 1.4,1 where either K_1 or K_2 is rate limiting[128].

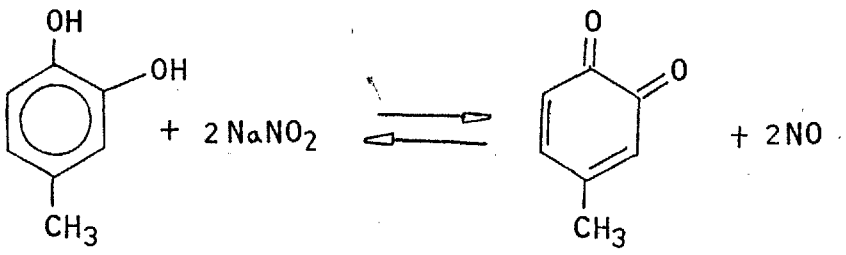
$$H_2ONO^+ + X \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} NOX + H_2O$$

$$NOX + R \xrightarrow{K_2} \text{products}$$

Scheme 1.4,1

X can be I, Br, Cl, OAc^-, NO_2^-, NO_3^-, etc. depending on the species present in solution. Bartlett[102] studied the oxidation of 4-methylcatechol with

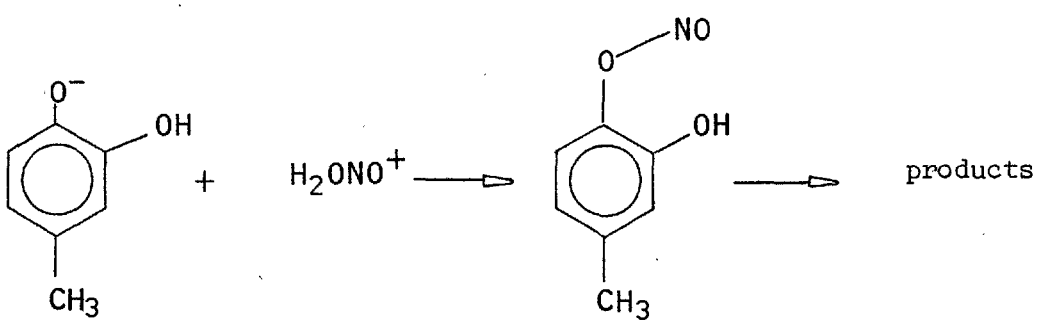
sodium nitrite (Scheme 1.4,2) and found that the reaction follows the rate equation 1.4,1.



Scheme 1.4,2

$$\text{rate} = K_1 [AcO^-][NaNO_2][H^+]^2 + K_2[\text{catechol}][NaNO_2][H^+] \quad \ldots\text{eq. } 1.4,1$$

This was interpreted as reaction occurring via two concurrent pathways, one involving rate determining formation of nitrosyl acetate and an other involving rate limiting formation of catechyl nitrite from attack of H_2ONO^+ on the catechol anion (Scheme 1.4,3).

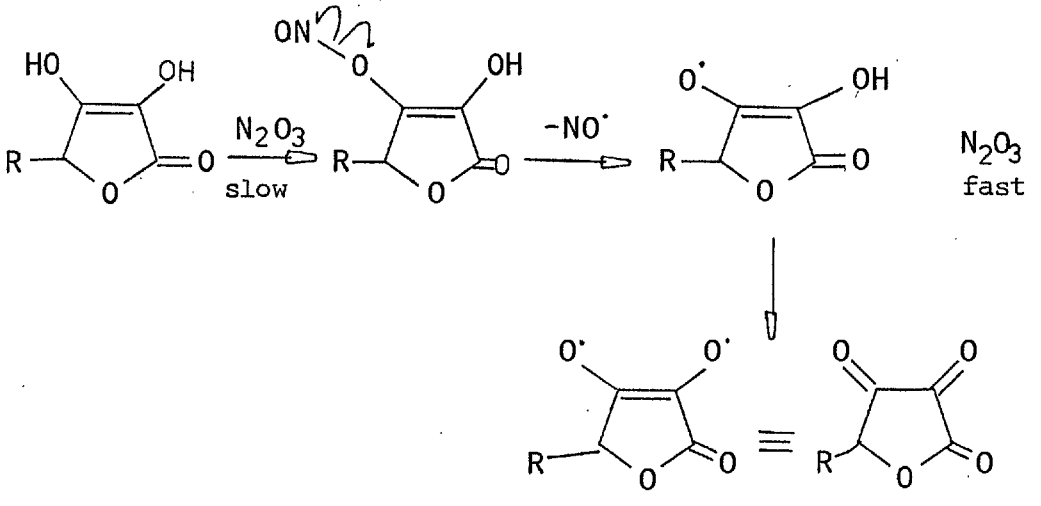


Scheme 1.4,3

Addition of inorganic salts was found to add a further term to the rate equation (eq. 1.4,2), probably indicating reaction via NOX in another concurrent pathway.

$$\text{rate} = K_x [X^-][NaNO_2][H^+]^2 \qquad \ldots \text{ eq. } 1.4,2$$

The kinetics of the oxidation of ascorbic acid by nitrous acid was studied by Dahn and his colleagues[129]. They identified two concurrent routes, one via nitrous acidium ion and one via dinitrogen trioxide or nitrosyl halide, the latter being by far the more predominant. A mechanism was postulated involving a radical analogous to a phenoxy radical (Scheme 1.4,4). The ascorbic acid radical has some resonance stabilization



R = $CH_2OHCHOH^-$

Scheme 1.4,4

and Lagercrantz[130] has shown that radicals produced during the autooxidation of 0.1M L-ascorbic acid at pH 6.6-9.6 are stable for several hours in solution.

CHAPTER 2

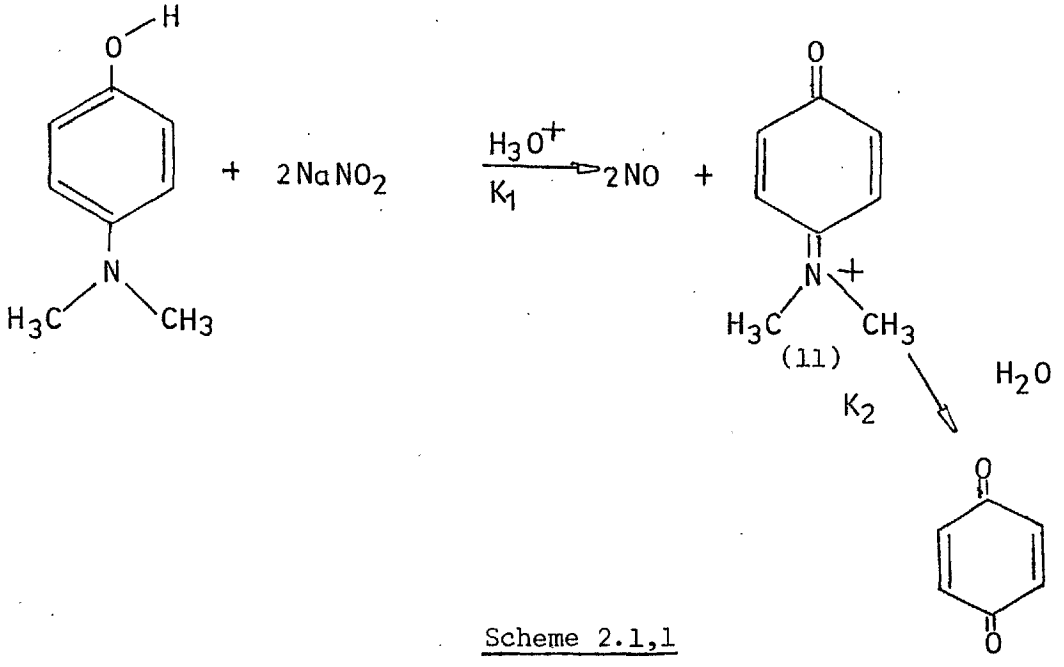
OXIDATION OF PHENOLIC COMPOUNDS BY SODIUM NITRITE

RESULTS AND DISCUSSION

2.1 RESULTS OF THE OXIDATION OF PHENOLIC COMPOUNDS BY SODIUM NITRITE

2.1,1 p-Dimethylaminophenol

The rate of oxidation of p-dimethylaminophenol by HNO_2 was determined by measuring, at timed intervals, the u.v. absorption of an intermediate at λ_{max} 280 nm. This intermediate, probably the N,N-dimethyl-p-benzoquinonemonoimine (11) hydrolysed to give p-benzoquinone (Scheme 2.1,1). The latter was isolated and identified by spectral analysis. Also, it was found that 2 moles of NO were evolved from the reaction mixture (Section 3.3,1). The reactipn path involves two consecutive



Scheme 2.1,1

reactions for which values of the pseudo first order rate coefficients K_1 (rate = K_1 [substrate]) and K_2 (rate = K_2 [substrate]) were calculated by the computer method discussed in Section 3.4,1b. In the following tables, t_{max} is the time at which the absorption of the intermediate (11) is a maximum.

The reaction was studied in aqueous solutions at 25°C with various [substrate] (Table 2.1,1a), various [HCl] (Table 2.1,1b), various $[Cl^-]$ (Table 2.1,1c) and various $[HNO_2]$ (Table 2.1,1d).

Table 2.1,1a. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C [HCl]-0.8 M; Initial ($NaNO_2$) -2 . 10^{-4} M

10^5.(substrate)M	$10^4 . K_1\ s^{-1}$	$10^4 . K_2\ s^{-1}$	t_{max} min
3.0	6.2	2.9	39
5.0	6.0	3.0	38.5
7.5	8.1	2.2	39

Table 2.1,1b. Oxidation of p-dimethylaminophenyl by sodium nitrite at 25°C. μ = 0.8 M (NaCl); Initial (substrate) - $7.5.10^{-5}$M; Initial ($NaNO_2$) - 2 . 10^{-4} M

[HCl]M	$10^4 . K_1\ s^{-1}$	$10^4 . K_2\ s^{-1}$	t_{max} min
0.01	6.2	2.5	44
0.04	7.2	2.1	39
0.1	8.0	2.2	37
0.4	7.3	2.7	37
0.8	8.1	2.2	39

Table 2.1,1c. Oxidation of p-dimethylaminophenyl by sodium nitrite at 25°C. Initial (substrate) - 7.5 . 10^{-5} M; Initial ($NaNO_2$) - 2 . 10^{-4} M

[HCl]M	$[HClO_4]$ M	$10^4 . K_1\ s^{-1}$	$10^4 . K_2 s^{-1}$	t_{max} min
0.0	0.8	5.3	2.2	50
0.2	0.6	5.5	2.3	45
0.6	0.2	6.2	2.3	42
0.8	0.0	8.1	2.2	39

Table 2.1,1d. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C. [HCl] - 0.5 M; Initial (substrate) - $7.5 \cdot 10^{-5}$ M

$10^4 \cdot [HNO_2]$ M	$10^4 \cdot K_1$ s^{-1}	$10^4 \cdot K_2 s^{-1}$	t_{max} min
2	4.9 (4.7)	3.9 (2.7)	38 (38)
4	10.8 (9.9)	3.9 (4.1)	21 (20)
7	17.0	3.9	12

Variation of initial [substrate] shows that the pseudo first order rate constant for the oxidation (K_1) is independent of [substrate], first order in $[HNO_2]$* (Figure 2.1,1a) and independent of acidity in the region 0.04 M to 0.8 M HCl. Varying Cl^- effects the K_1 values (Figure 2.1,1b) probably due to some reaction by NOCl. The pseudo first order rate constant for the hydrolysis of N,N-dimethyl-p-benzoquinone-monoimine (K_2), is approximately constant under all the conditions studied, suggesting that this reaction is an hydrolysis by the solvent H_2O.

The reaction was also studied in dichloroacetic acid buffers at several dichloroacetate concentrations (Table 2.1,1e and Figure 2.1,1c).

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* At the pH of these reactions, $NaNO_2$ exists as HNO_2.

Figure 2.1,1a. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C

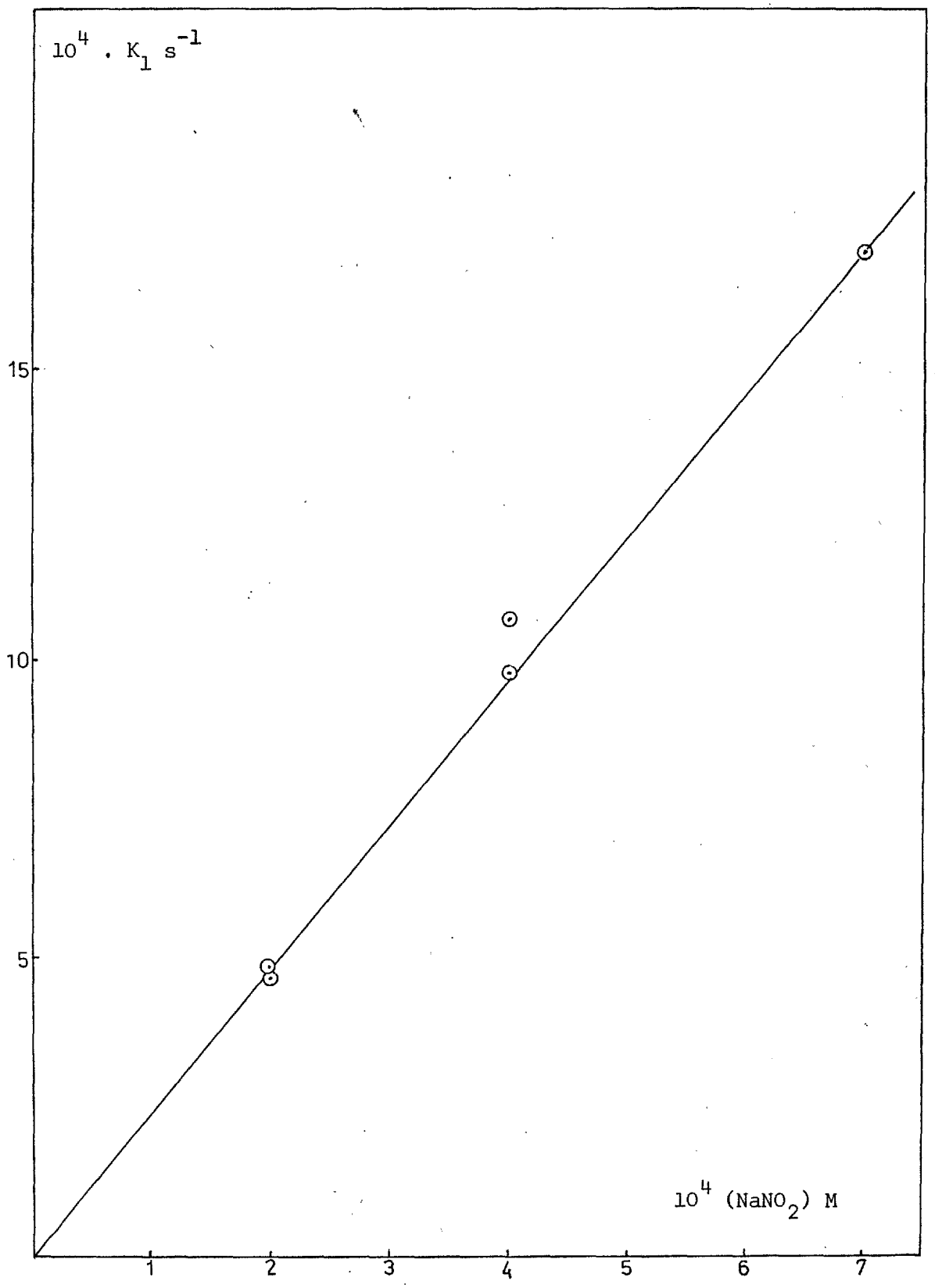


Figure 2.1,1b. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C

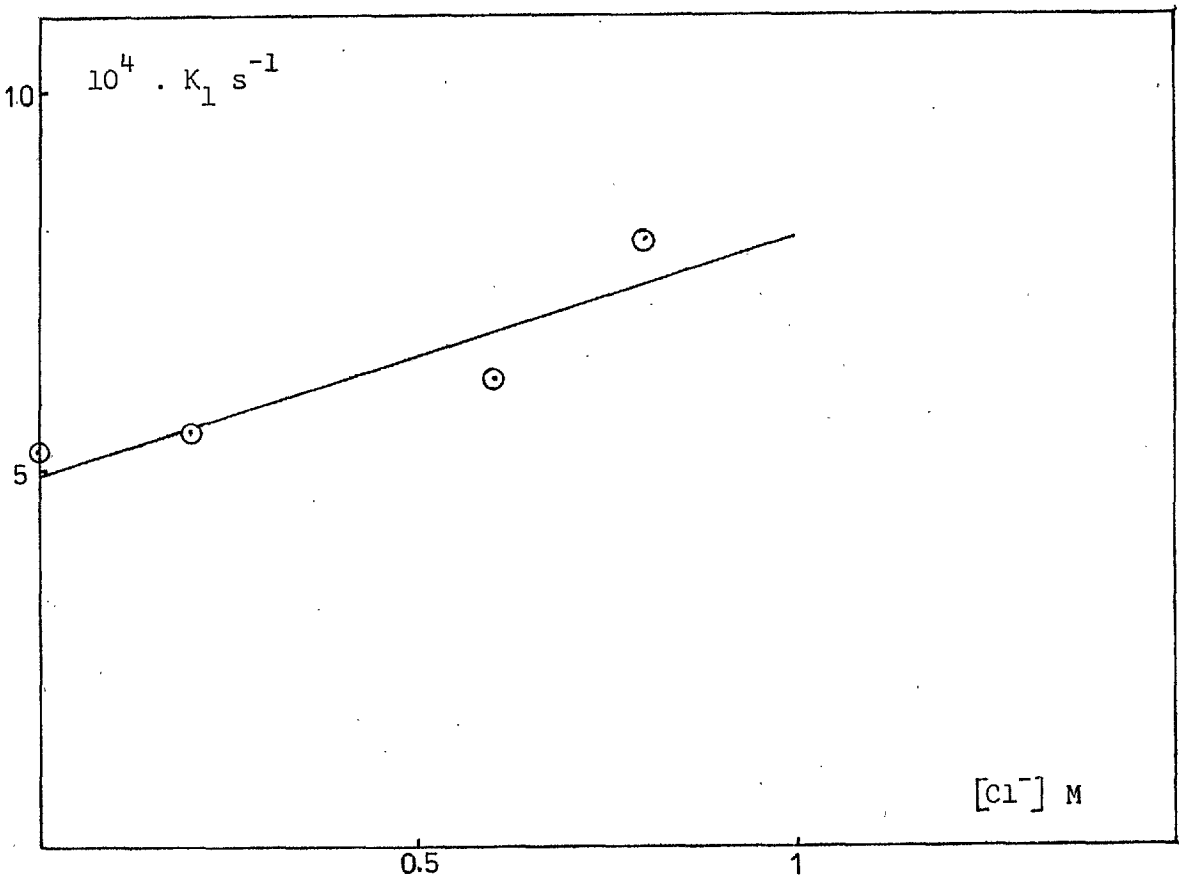


Table 2.1,1e. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C. μ = 0.4 M ($NaClO_4$); buffer ratio - 1; Initial (substrate) -7.5 . 10^{-5} M; Initial ($NaNO_2$) - 2 . 10^{-4} M.

$(Cl_2CHCOO^-)M$	pH	$[Cl_2CHCOO^-]M$	$10^4 . K_1 s^{-1}$	$10^4 . K_2 s^{-1}$	t_{max} min
0.05	1.60	0.057	3.9	3.2	53
0.10	1.48	0.100	5.5	2.4	46
0.15	1.44	0.143	6.2	2.9	38
0.20	1.38	0.176	6.7	3.4	35

As expected from the previous results, K_1 increases with $[Cl_2CHCOO^-]$ * and K_2 is constant.

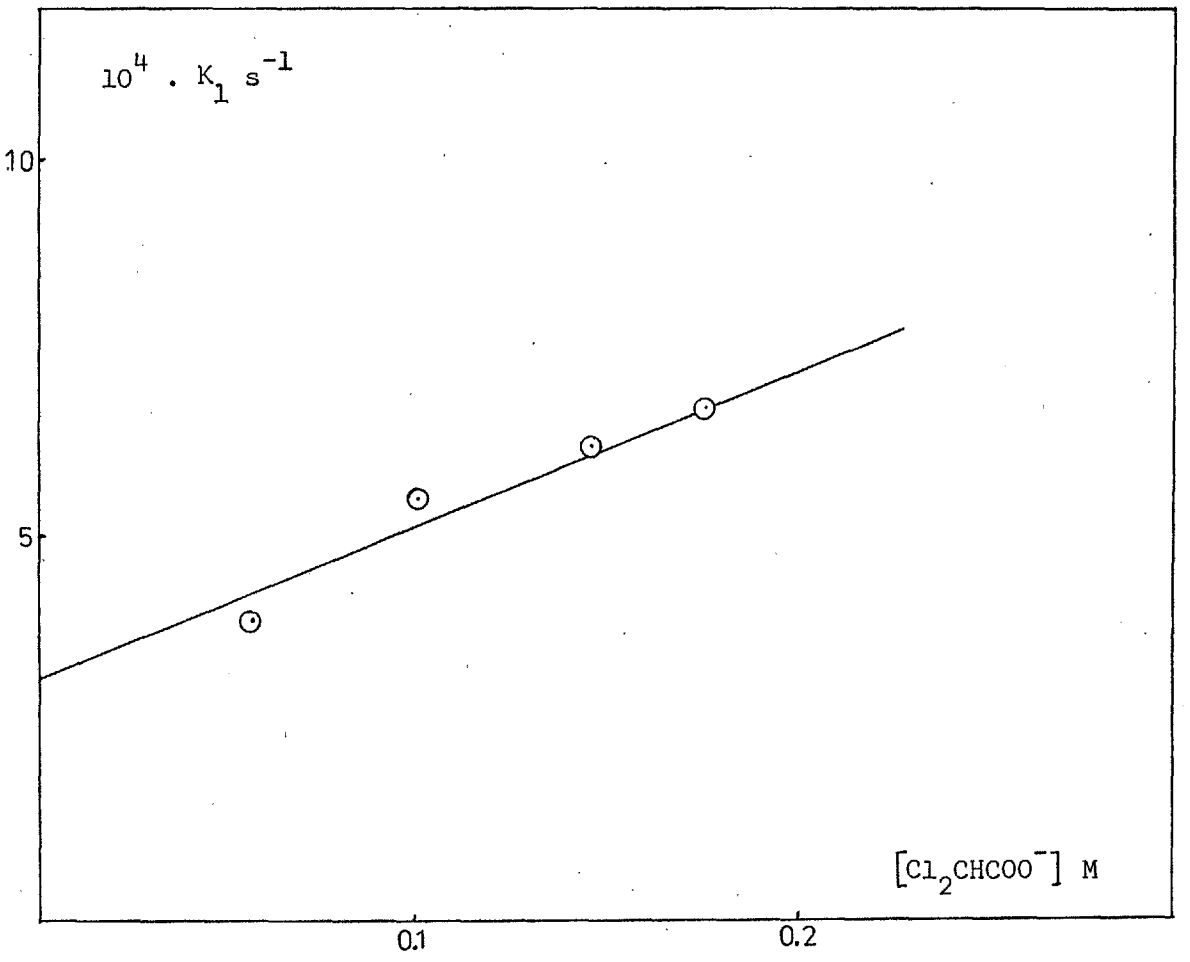
The results obtained for varying $NaNO_2$ are shown in Table 2.1,1f and plotted in Figure 2.1,1d. The experiments from which these results

Table 2.1,1f. Oxidation of p-dimethylaminophenyl by sodium nitrite at 25°C. μ = 0.4 M ($NaClO_4$); 0.1 M $Cl_2CHCOOH$ - Cl_2CHCOO^- (1:1); pH - 1.60; Initial (substrate) - 7.5 . 10^{-5} M

Run	$10^4(NaNO_2)M$	$10^4 . K_1 s^{-1}$	$10^4 . K_2 s^{-1}$	t_{max} min
A	2	3.9	3.2	53
B	3	6.6	2.9	36
C	4	9.1	3.6	27
D	5	14.1	3.3	20

* Although the pH varies, the previous results show that K_1 is independent of acidity, hence this variation must refer to the $[Cl_2CHCOO^-]$ term.

Figure 2.1,1c. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C



were calculated, are shown in Figure 2.1,1e. The oxidation step is clearly first order in $[HNO_2]$. The hydrolysis step is independent of $[HNO_2]$ at these concentrations, but not at higher $[HNO_2]$ as shown in Table 2.1,1g and Figure 2.1,1f. Here the oxidation step is so fast that

Table 2.1,1g. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C. [HCl] - 0.5 M; Initial (substrate)- $7.5 \cdot 10^{-5}$ M

$10^2.(NaNO_2)M$	$10^4. K_2 s^{-1}$
1	4.1
2	5.5
3	6.9
4	7.6

only the hydrolysis of the N,N-dimethyl-p-benzoquinonemonoimine is followed and K_2 values can be obtained from plots of $\ln(OD-OD_\infty)$ versus time. From the intercept of Figure 2.1,1f, a value of K_2 ca $2.8 \cdot 10^{-4} s^{-1}$ is calculated which compares favourably with values of K_2 as determined from the later stages of the reactions shown in Figure 2.1,1e (A = $2.3 \cdot 10^{-4} s^{-1}$; B = $2.8 \cdot 10^{-4} s^{-1}$; C = $2.8 \cdot 10^{-4} s^{-1}$; D = $2.5 \cdot 10^{-4} s^{-1}$) and with the values calculated by the computer programme (Table 2.1,1f).

All these results for the oxidation of p-dimethylaminophenol are rather scattered and an error of at least ± 25% is indicated. The main reason for this error lies in the sensitivity of the computer programme to small deviations in the estimated values of E_B, K_1 and K_2 given as input.

Figure 2.1,1d. Oxidation of p-dimethylaminophenyl by sodium nitrite at 25°C

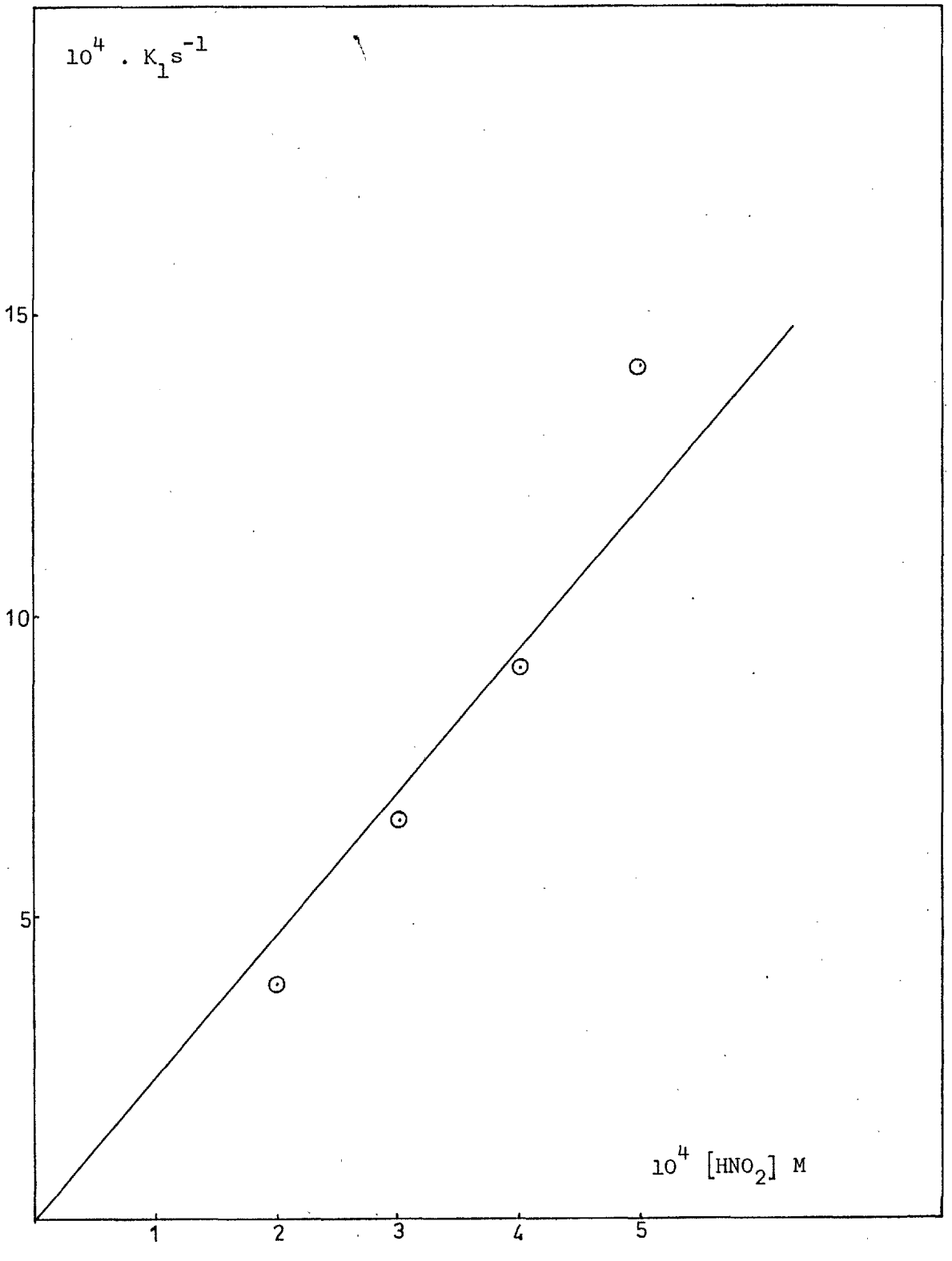


Figure 2.1,le. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C

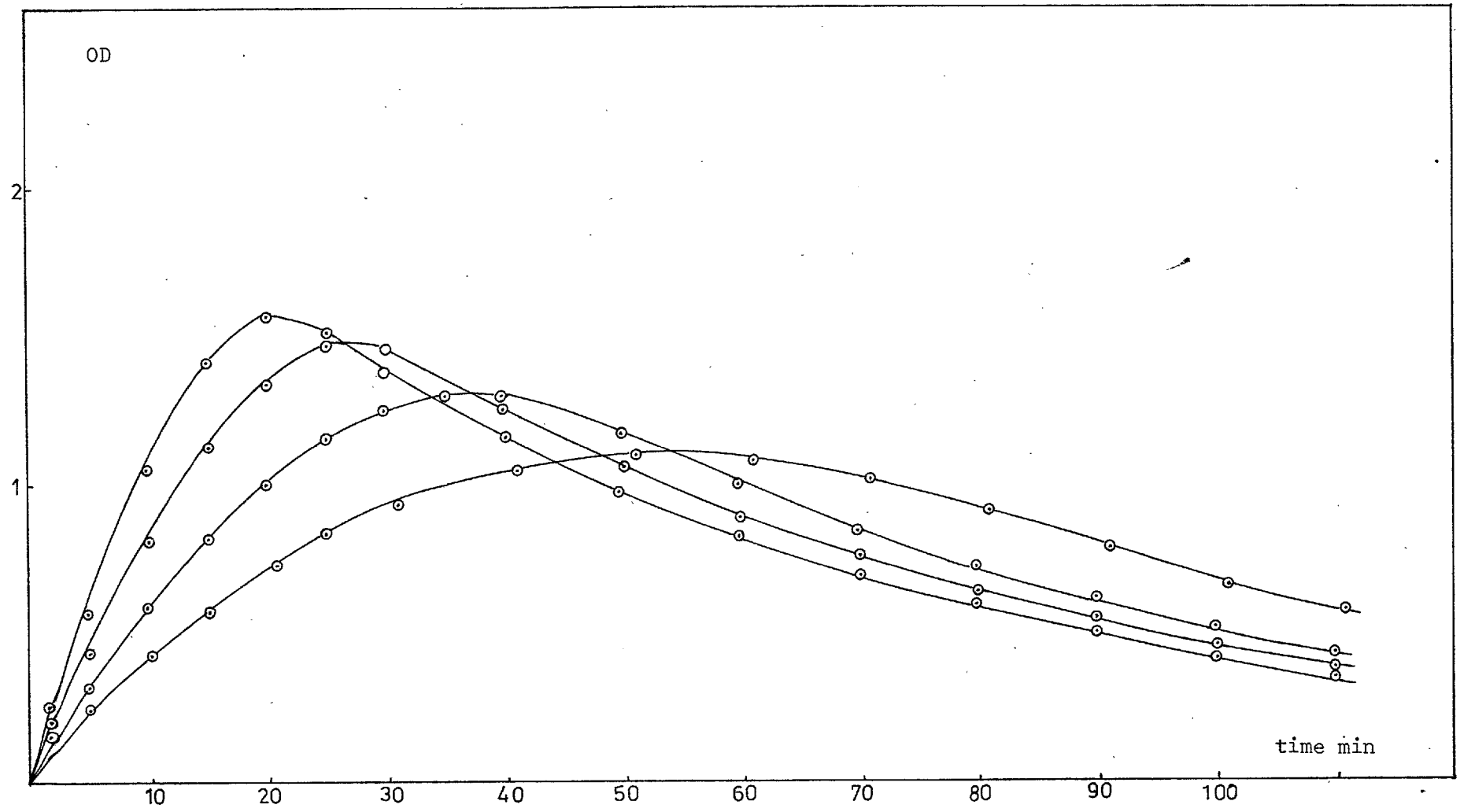
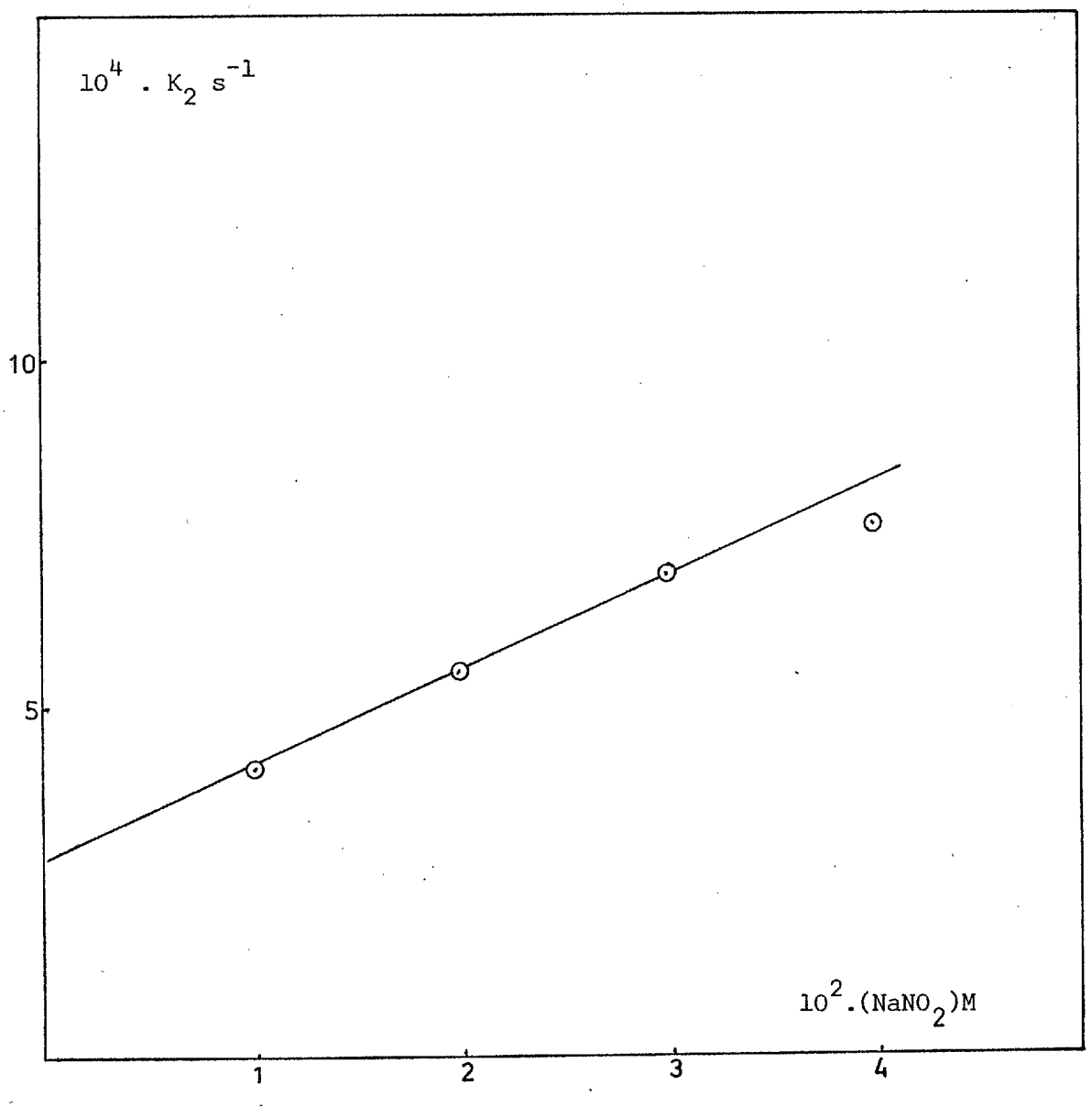


Figure 2.1,1f. Oxidation of p-dimethylaminophenol by sodium nitrite at 25°C



2.1,2 5-Hydroxyindoles

Rates of oxidation of 5-hydroxy-1,3-dimethylindole by sodium nitrite were measured, in acetate buffers, at 25°C, by following the evolution of nitric oxide in a gas burette. The reaction rate was found to be independent of [substrate], first order in $[NaNO_2]$ and [NaOAc] and to have a second order dependence on solvent acidity (eq. 2.1,2a).

$$\text{rate} = K\,[NaNO_2][H_3O^+]^2[NaOAc] \qquad \ldots \text{ eq. 2.1,2a}$$

The products of reaction appeared to be a complex mixture of polymers plus nitric oxide (Section 3.3). To avoid possible complications from the interference by products, the initial rate method was used to calculate rate coefficients (Section 3.4). Other 5-hydroxyindoles were studied and the initial rates of reaction compared with those for 5-hydroxy-1,3-dimethylindole.

$$IR = K_1\,[HNO_2] \qquad \ldots \text{ eq. 2.1,2b}$$

2.1,2a 5-Hydroxy-1,3-dimethylindole

This reaction was studied at several [substrate] and the results obtained are shown in Table 2.1,2a1.

Table 2.1,2a1. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C. (HOAc) 0.2 M; (NaOAc) 0.1 M; ethanol 20%; Average pH 4.70.

10^3.[substrate]M	$10^3(NaNO_2)$M	% reaction	10^2.IR ml s^{-1}	10^5.IR mol $l^{-1}s^{-1}$
2.0	9	114.	1.2	0.97
4.5	9	111.	1.3(1.4)	1.05(1.1)
6.5	9	107.	1.3	1.05
6.0	5	82.	0.33(0.34)	0.27(0.28)
9.0	5	81.	0.42(0.54)	0.34(0.44)
12.0	5	79.	0.45(0.44)	0.36(0.35)

Values of the initial rates (IR) in ml s^{-1} were converted to mol l^{-1} s^{-1}, correcting for temperature and reaction volue. Clearly, these initial rates are independent or in excess. Table 2.1,2a2 shows the results obtained when varying $[NaNO_2]$.

Table 2.1,2a2. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C. (HOAc) 0.2 M; (NaOAc) 0.1 M; ethanol 20%; Average pH 4.70. Initial [substrate] - $4.5 \cdot 10^{-3}$ M.

10^3 $(NaNO_2)$M	$10^4 \cdot [HNO_2]$M	% reaction	10^2.IR ml s^{-1}	10^5.IR mol $l^{-1}s^{-1}$
9.0	2.5	111	1.3(1.4)	1.05(1.1)
13.5	3.7	127	2.2	1.75
18.0	4.9	134	2.7	2.2
24.0	6.6	165	4.2	3.4

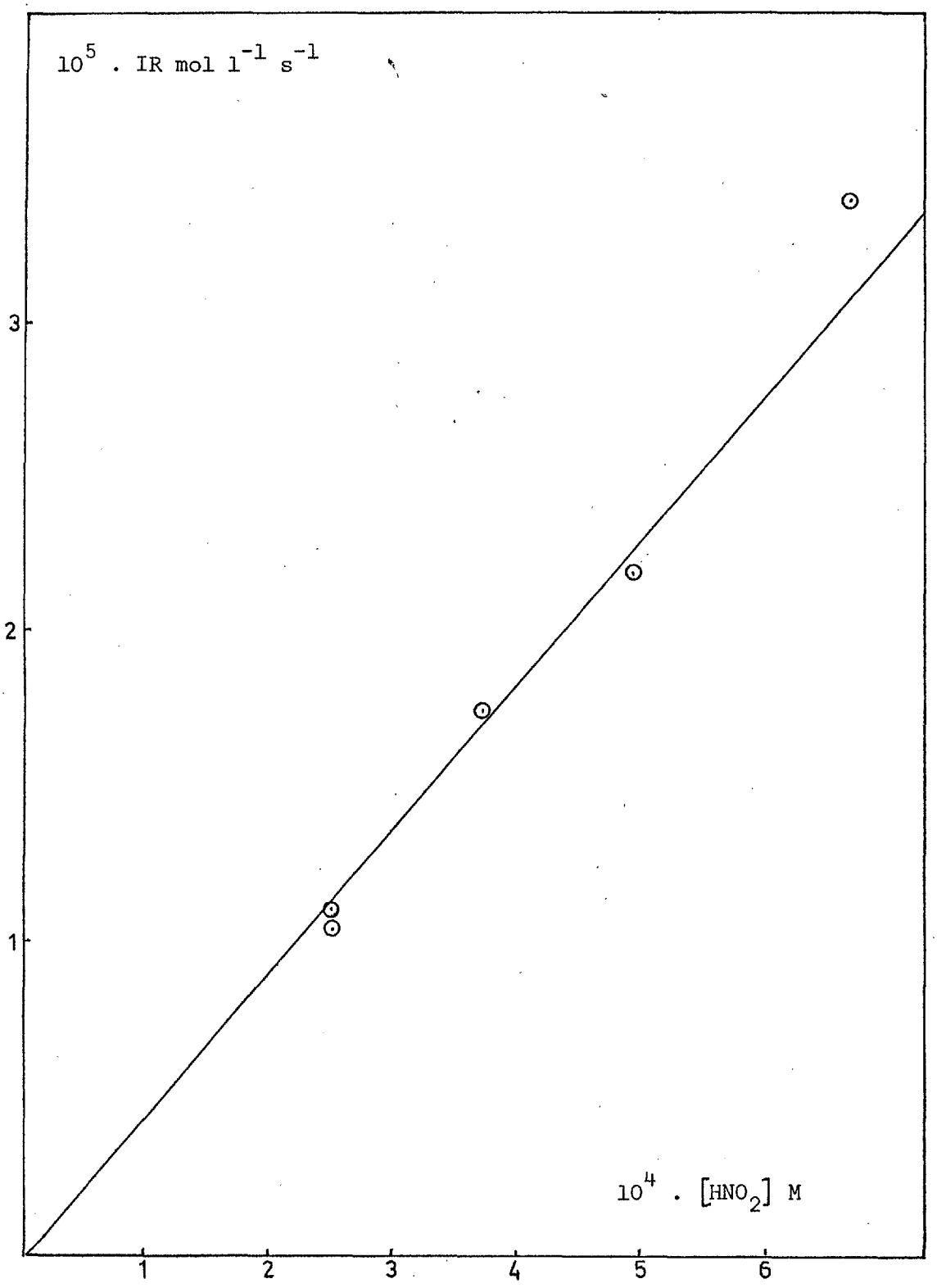
Figure 2.1,2a1 which is a plot of these results, shows a straight line passing through the origin. Thus the reaction is also first order in $[HNO_2]$.

The rate of reaction was found to have second order dependence upon $[H_3O^+]$. Table 2.1,2a3 shows the initial rates obtained at varying, albeit limited pH, and Figure 2.1,2a2 is a plot of these results.

Table 2.1,2a3. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C. $(NaNO_2)$ $9 \cdot 10^{-3}$ M; (NaOAc) 0.09 M; ethanol 20%. Initial [substrate] - $4.5 \cdot 10^{-3}$ M.

(HOAc)M	pH	% reaction	10^2 . IR ml s^{-1}	10^5 . IR mol l^{-1} s^{-1}
0.40	4.41	118	3.9	3.1
0.30	4.51(4.53)	110	2.6(2.7)	2.1(2.2)
0.24	4.62	108	2.0	1.6
0.18	4.72(4.71)	113	1.3(1.4)	1.05(1.1)
0.16	4.78	124	0.9	0.73

Figure 2.1,2a1. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C



The dependence of the reaction rate on $[OAc^-]$ was also studied. The results are summarised in Table 2.1,2a4.

Table 2.1,2a4. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C. Ethanol 20%; average pH 4.70

(a) $(NaNO_2)$ 9.10^{-3}M; [substrate] $4.5 . 10^{-3}$ M

(HOAc)M	(NaOAc)M	% reaction	10^2 . IR ml s^{-1}	10^5 . IR mol $l^{-1}s^{-1}$
0.18	0.09	113	1.3(1.4)	1.05(1.1)
0.3	0.15	112(110)	1.5(1.5)	1.2
0.4	0.20	102(105)	1.8(1.8)	1.4
0.5	0.25	105	2.7	2.2
0.6	0.30	104	3.1	2.5

(b) $(NaNO_2)$ $5 . 10^{-3}$M; [substrate] $4.5 . 10^{-3}$ M; μ = 0.2 M $(NaClO_4)$

(HOAc)M	(NaOAc)M	% reaction	10^2 . IR ml s^{-1}	10^5 . IR mol $l^{-1}s^{-1}$
0.08	0.04	89	0.25	0.20
0.12	0.06	90	0.36	0.29
0.16	0.08	91	0.42	0.34
0.2	0.1	78	0.55	0.44

These two sets of results are plotted in Figure 2.1,2a3. The initial rates for $[NaNO_2]$ = $9 . 10^{-3}$ M are very scattered. However, they are self consistent with those at $[NaNO_2]$ = $5 . 10^{-3}$ M. The reaction is first order in [NaOAc].

When excess $NaNO_2$ was used more than the calculated 100% of nitric oxide was evolved from the reaction solutions. This is probably due to spontaneous decomposition of $NaNO_2$ in the acetate buffer solutions, as explained in Table 2.1,2a5.

Figure 2.1,2a2. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C

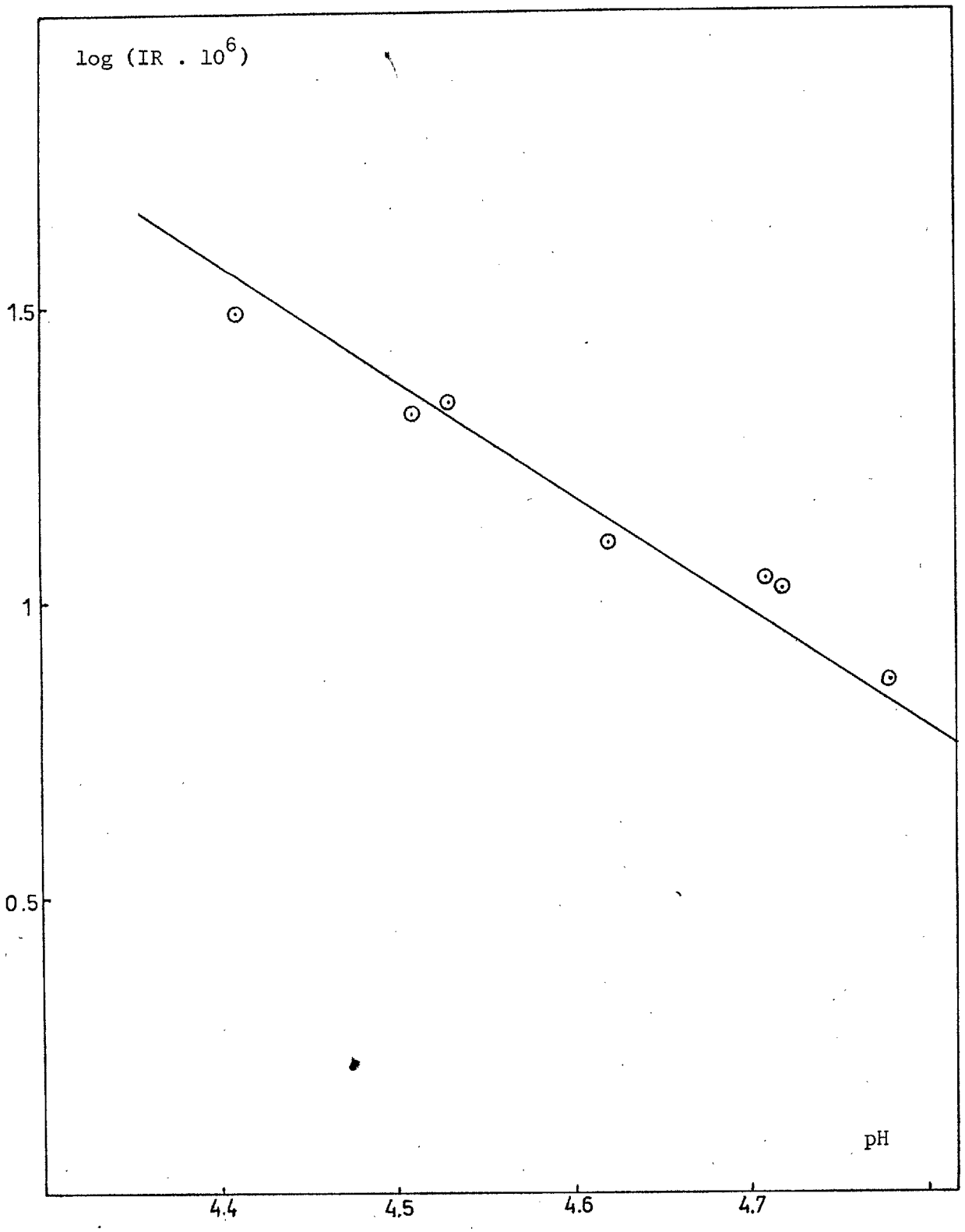


Table 2.1,2a5. Comparison between % NO evolved in the reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite and % NO evolved in blank solutions of acetate buffer

$10^3.(NaNO_2)M$	% NO evolved in reaction solution	% NO evolved in blank solution	Difference
9.0	115.	17	98
13.5	127.	28	99
18.0	133.	38	95

2.1,2b. Other 5-hydroxyindoles

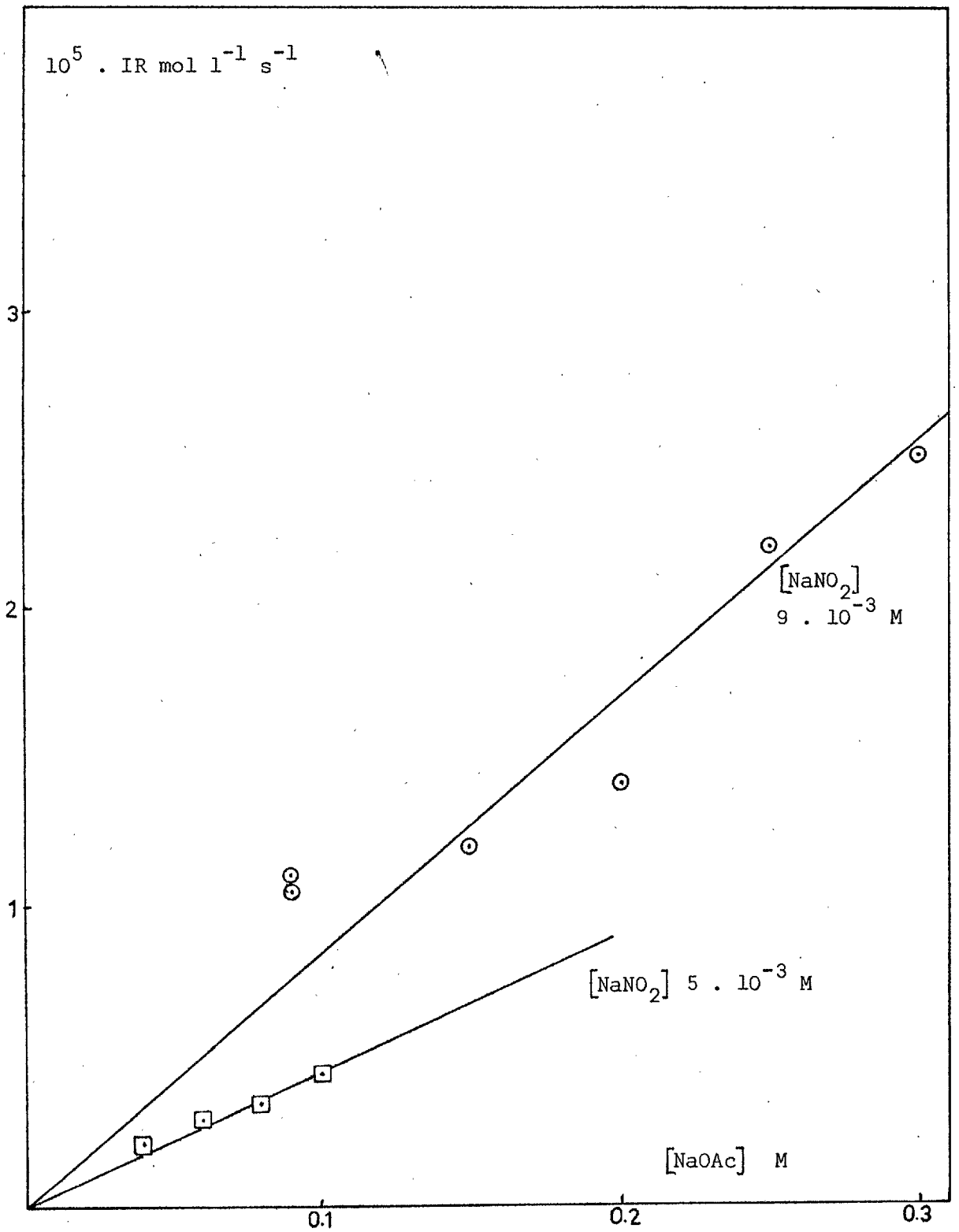
The rates of reaction of 5-hydroxytryptophan, 5-hydroxy-3-methylindole and ascorbic acid, with $NaNO_2$ were measured in acetate buffer at 25°C again by following the evolution of nitric oxide. The reaction of 5-hydroxyindole with $NaNO_2$ takes place without gas evolution so it was not possible to measure the rate of reaction by the gas burette method. The results obtained are summarized in Table 2.1,2b.

Table 2.1,2b. Reaction of 5-hydroxyindole with sodium nitrite at 25°C (HOAc) 0.2 M; (NaOAc). Initial $(NaNO_2)$ $5 \cdot 10^{-3}$ M. Initial [substrate] $6 \cdot 10^{-3}$ M

substrate	10^5 . IR mol l^{-1} s^{-1}	% NO	Reproducibility
5-hydroxy-1,3-dimethylindole	1.04	82(72)	good
5-hydroxytryptophan	1.25	57(64)	difficult
5-hydroxy-3-methylindole	1.47	63(65)	very difficult
5-hydroxyindole	-	No gas	-
ascorbic acid	2.50	80(86)	good

The reaction rates are of the same order of magnitude for all substrates studied suggesting that the reactions proceed by similar mechanisms.

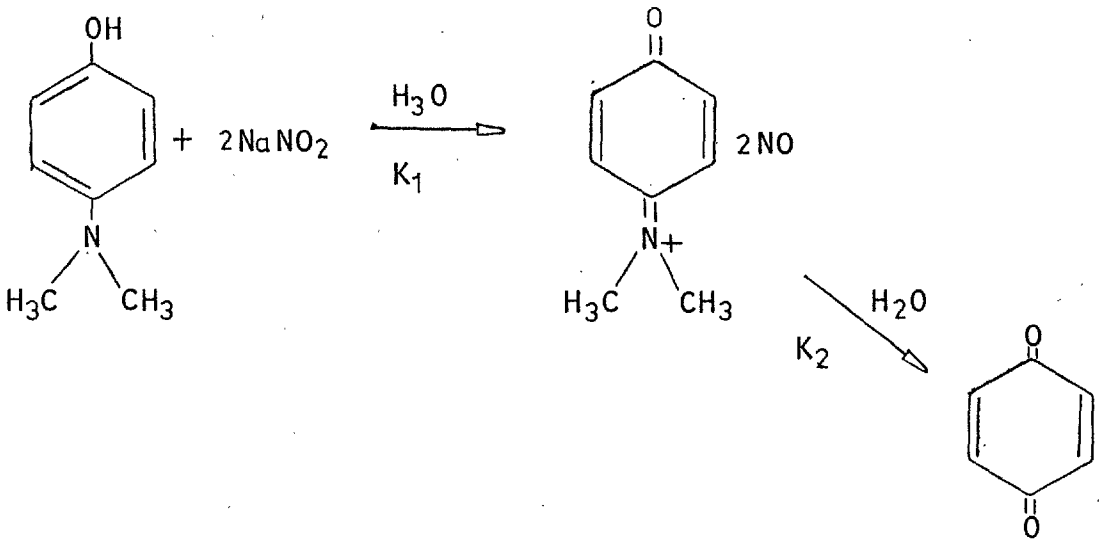
Figure 2.1,2a3. Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite at 25°C



2.2 DISCUSSION OF THE OXIDATION OF PHENOLIC COMPOUNDS BY SODIUM NITRITE

2.2,1 p-Dimethylaminophenol

The reaction of p-dimethylaminophenol with nitrous acid is a two-electron oxidation giving the N,N-dimethyl-p-benzoquinonemonoimine which hydrolysis to p-benzoquinone (Scheme 2.2,1a). A similar pathway applies to oxidation by potassium ferricyanide (Section 3.3,2).



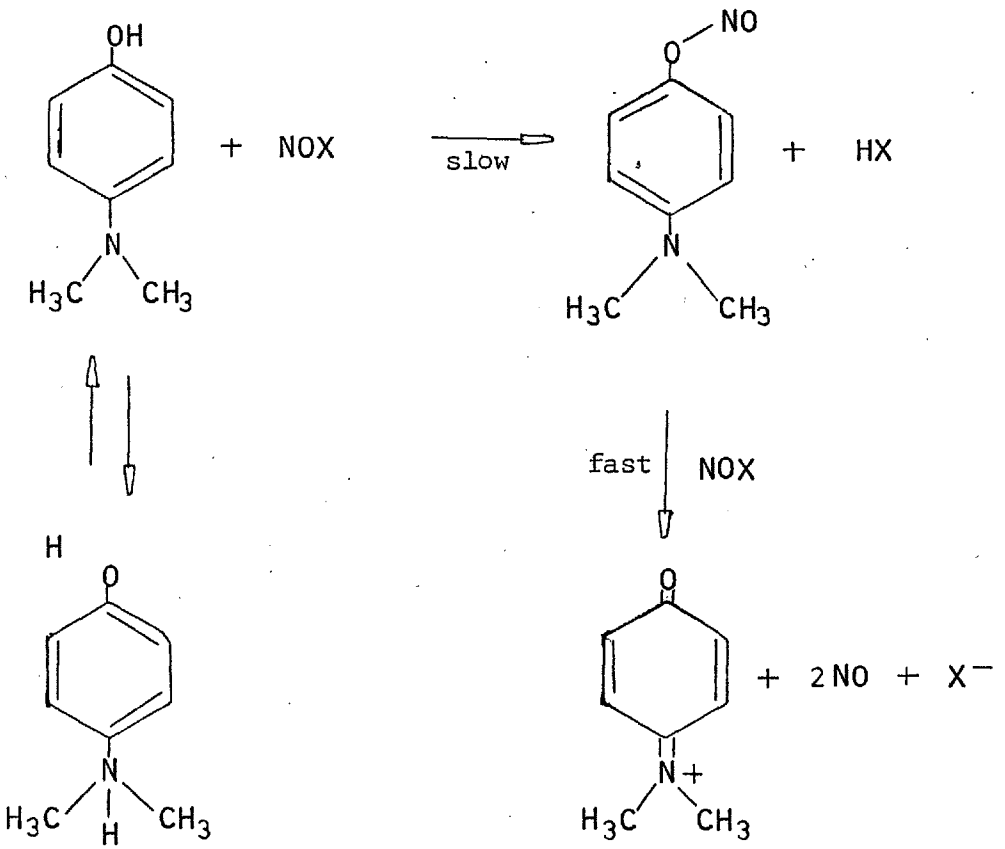
Scheme 2.2,1a

The results presented on Section 2.1,1, suggest that K_1 is first order in [substrate] and $[HNO_2]$ and independent of acidity under the conditions studied. K_1 Is also found to be dependent on both the $[Cl^-]$ and $[Cl_2CHCOO^-]$ suggesting that the rate determining step is attack of the nitrosating agent on the substrate. The nitrosating agent (NOX) may be the nitrosoacidium ion (H_2ONO^+), nitrosyl chloride or nitrosyl dichloro-acetate or a combination of these according to the conditions of the reaction (eq. 2.2,1a and eq. 2.2,1b). The concentration of these nitrosating agents is known to increase with increasing acidity. The fact that K_1 is acidity independent arises from extensive protonation

of the substrate under the reaction conditions.

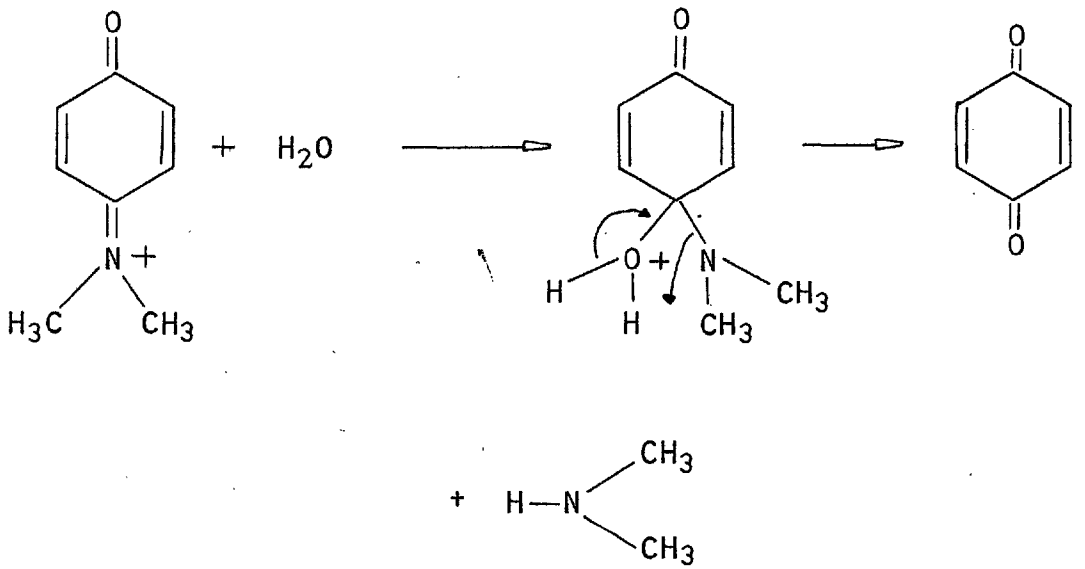
$$V = K_x [\text{substrate}][HNO_2] + K_y [\text{substrate}][HNO_2][Cl^-] \quad \ldots \text{ eq. 2.2,1a}$$

$$V = K_x [\text{substrate}][HNO_2] + K_z [\text{substrate}][HNO_2][Cl_2CHCOO^-] \text{ eq. 2.2,1b}$$



Scheme 2.2,1b

The rate of hydrolysis of N,N-dimethylamino-p-benzoquinone monoimine appears to be first order in [substrate] and independent of acidity under these conditions and independent of $[Cl^-]$ and $[Cl_2CHCOO^-]$. This is consistent with an uncatalysed hydrolysis by water (Scheme 2.2,1c).

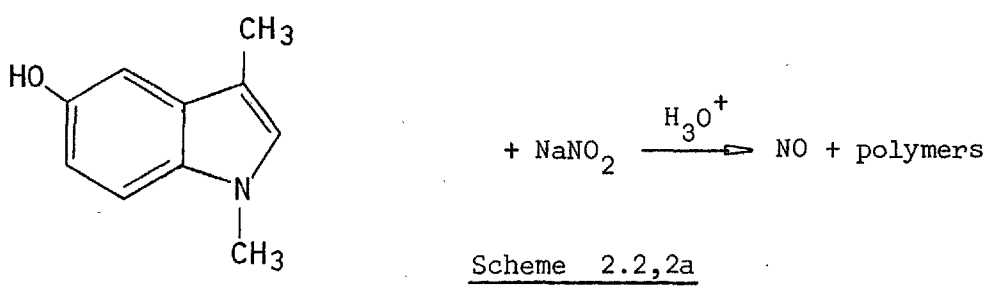


Scheme 2.2,1c

The fact that K_2 is slightly larger in dichloroacetate buffers may imply that the hydrolysis is catalysed by HO^- but the scatter of the results does not allow a definitive conclusion. The dependence on nitrite at high $[HNO_2]$ is not fully understood.

2.2,2 5-Hydroxyindoles

The oxidation of 5-hydroxy-1,3-dimethylindole by sodium nitrite in acetate buffers at 25°C was found to give nitric oxide plus a complex mixutre of products (Scheme 2.2,2a).

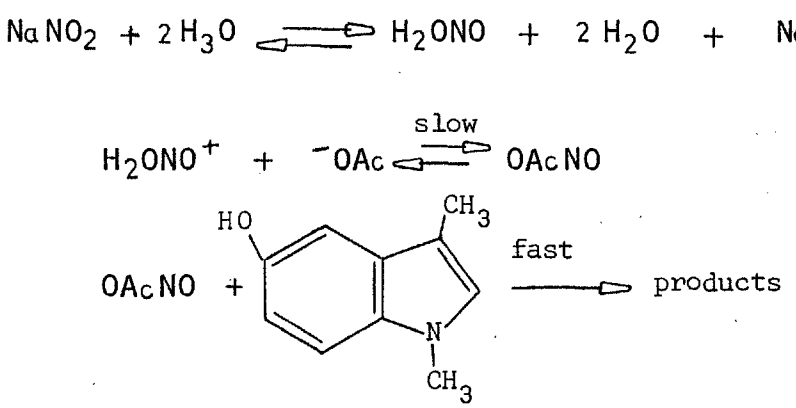


Scheme 2.2,2a

Further, the reaction was found to be independent of [substrate], first order in $[NaNO_2]$, second order in $[H_3O^+]$ and first order in $[OAc^-]$ (eq. 2.2,2a).

$$\text{rate} = K\,[NaNO_2][H_3O^+]^2[OAc^-] \qquad \ldots \text{ eq. 2.2,2a}$$

This equation can be interpreted as evidence for determining formation of nitrosyl acetate, followed by a rapid reaction with the substrate (Scheme 2.2,2b).

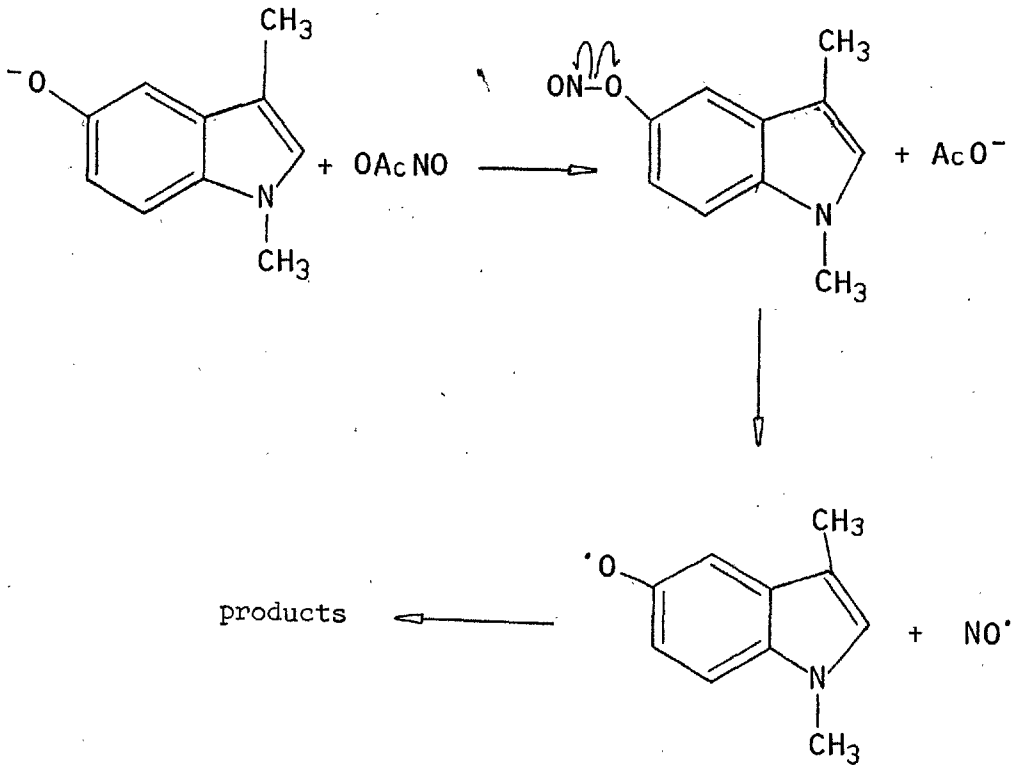


Scheme 2.2,2b

The oxidation step probably involves nitrosation either of the neutral phenol or of the phenoxy ion followed by the formation of phenoxy radicals which undergo polymerization reactions (eg. Scheme 2.2,2c).

The reactions of the other indoles studied, 5-hydroxy-3-methyl-indole and 5-hydroxytryptophan, gave lower NO yields, very poor reproducibility and, in some instances, required an induction period. Hence only a comparison of reaction rates was attempted, (Table 2.1,2b). It was found that all compounds reacted at a similar rate, suggesting that the reactions proceed by similar mechanisms. Compounds with the 3 position free, e.g. 5-hydroxyindole, do not show NO evolution,

probably due to preferential nitrosation at the 3 position[132].



Scheme 2.2,2c

CHAPTER 3

EXPERIMENTAL DETAILS

3.1 PURIFICATION OF REAGENTS AND SOLVENTS

Acetic acid, sodium acetate, sodium perchlorate, sodium chloride were AnalaR reagents and were dried where necessary. Sodium hydroxide and hydrochloric acid were BDH volumetric solutions. Sodium nitrite was an AnalaR reagent and was vacuum dried over P_2O_5. 5-Hydroxyindole (Aldrich) was purified by sublimation, m.p. 107-108°C (lit.[157]: 103-7°C). 5-Hydroxy-tryptophan (Aldrich) was used without further purification, m.p. 290°C with decomposition, (lit.[158]: m.p. 293-5° (decomp.)). AnalaR ethanol was used without further purification. Other solvents (dichloromethane, chloroform, diethyl ether) were either AnalaR or GPR grade.

3.2 SYNTHESIS OF SUBSTRATES

3.2,1 p-Dimethylaminophenol

3.2,1a N-Dimethylanisidine[133]

p-Anisidine (10 g, 0.08 moles) and dimethylsulphate (7.7 ml, 0.08 moles) were mixed in a round-bottomed flask. When the reaction was over, more dimethyl-sulphate (7.7 ml, 0.08 moles) was added. The mixture was heated under reflux for 1 hour, allowed to cool, neutralized with 1 M NaOH and extracted with diethyl ether (4 x 50 ml). The ether fractions were dried over anhydrous $CaSO_4$ and evaporated under reduced pressure. The resultant violet oil was added to acetic anhydride (4.1 g, 0.04 moles) and steam-distilled. The distillate gave a white precipitate on addition of 1M NaOH which was collected by filtration. Yield 2.4 g (20%), m.p. 47°C (lit.[134]: 48°C).

IR. $\nu_{max.}$ (nujol) 1510, 1250, 1180, 1045, 950, 820 cm^{-1}.

3.2,1b p-Dimethylaminophenol[135]

N-Dimethylanisidine (1.5 g, 0.009 moles), glacial acetic acid (9g) and hydrobromic acid (15 ml, 48% aqueous solution) were heated under reflux for ca. 4 h. The solution was evaporated under reduced pressure, the resultant residue dissolved in water and treated with activated charcoal. The solution was made alkaline with Na_2CO_3 (1.9 g) and extracted with ether (4 x 20 ml). The ethereal fractions were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The resultant solid was recrystallised from petroleum-ether (80-100°C): yield 0.6 g (44%), m.p. 75-76°C (lit.[136] 75-77°C).

UV. λ_{max} (CH_3COOH 0.2 M) 222 nm (log ε 3.9), 272 nm (log ε 3.1)

IR. ν_{max} (nujol) 3300-2400 (broad), 1600, 1510, 1250, 1190, 1170, 1150, 1100, 1050, 930, 830 cm^{-1}.

NMR. δ (DMSO) 2.67 (6H, s), 6.61 (4H, s), 8.6 (1H, s)

Mass.Spec. M/e 137 (M^+), 121, 94, 65, 39.

Analysis. Found: C, 69.83; H, 8.19; N, 10.18%. $C_6H_{11}NO$ requires C, 70.07; H, 8.03; N, 10.22%.

3.2,2 5-Hydroxy-3-methylindole

3-Carboxyl-5-hydroxyindole (Aldrich) was decomposed by heating under vacuum (140-160°C, 0.55-0.58 mmHg) a white solid which on recrystallization from petroleum ether (60-80°C) gave white plates, m.p. 110-120°C (lit.[137] 116°C). The compound rapidly oxidizes when exposed to the air and so was kept under nitrogen.

UV. λ_{max} (water) 277 nm (log ε 3.7) (lit.[138] λ_{max} (ethanol) 278 nm)

IR. ν_{max} (nujol) 3480, 3385, 1620, 1590, 1480, 1200, 1170, 1085, 1060.

NMR. δ (DMSO) 2.18 (3H, s), 6.85 (4H, m).

3.2,3 5-Hydroxy-1,3-dimethylindole

3.2,3a N-Acetylmethylphenetidine

To a solution of N-acetylphenetidine (10 g, 0.048 moles) in benzene (80 ml, Na dried) sodium hydride (3 g, 0.13 moles) was added with stirring. After precipitation of the phenetidine Na salt, methyl iodide (10 g, 0.07 moles) was added and the mixture heated under reflux for 2 hours. The solution was allowed to cool, filtered and the filtrate evaporated under reduced pressure. A yellow oil (10.8 g, 100% yield) was obtained which was used in the next step without further purification.

3.2,3b N-Methylphenetidine

N-Acetylmethylphenetidine (2 g, 0.009 moles) was heated under reflux in HCl (20 ml, 20%) for 4 hours. The resultant mixture was extracted with ether (3 x 20 ml), the ethereal solutions dried over anhydrous $CaSO_4$ and evaporated under vacuum to give an oil which was distilled at 4 mmHg. The fraction boiling at 102-106^{o}C was collected (1.08 g, 75% yield).

IR. ν_{max} (neat) 3400, 1620, 1510, 1390, 1240, 1150, 1120, 1000, 920, 820 cm^{-1}.

NMR. δ ($COCl_3$) 1.29 (3H, t), 2.79 (3H, s), 3.2 (s, 1H), 3.95 (2H, q), 6.65 (4H, m).

3.2,3c N-Propane-2-one-N-methyl-p-phenetidine

N-Methyl-p-phenetidine (4.5 g, 0.03 moles), triethylamine (3 g, 0.03 moles) and chloroacetone (2.75 g, 0.03 moles) were dissolved in benzene (10 ml) and heated at 60-70°C for 12 hours. The solution was filtered and evaporated under reduced pressure. A yellow oil was obtained (6 g) which was used in the next step without further purification.

3.2,3d 5-Ethoxy-1,3-dimethyindole

N-Propane-2-one-N-methyl-p-phenetidine (0.1 g) and N-methylphenetidine hydrobromide (0.1 g) were heated at 125°C for 30 min. HCl (2 ml, 50%) was then added and the solution was boiled, allowed to cool and extracted with ether (3 x 10 ml). The ether extract was dried over $CaCl_2$, evaporated under reduced pressure and the residue sublimed. The resultant white product was recrystallised from ethanol giving white plates, m.p. 83-87°C.

IR. ν_{max} (nujol) 1590, 1500, 1260, 1140 cm^{-1}

NMR. δ ($CDCl_3$) 1.4 (3H, t, J = 6Hz), 2.3 (3H, s), 3.6 (3H, s), 4.1 (2H, qu, J = 6Hz), 6.9 (4H, m).

Analysis. Found: C, 76.07; H, 7.77; N, 7.33%. $C_{12}H_{15}NO$ requires C, 76.19; H, 7.94; N, 7.41%.

3.2,3e 5-Hydroxy-1,3-dimethylindole

5-Ethoxy-1,3-dimethylindole (0.2 g, 1.10^{-3} moles), aluminium chloride (1 g, freshly sublimed) and chlorobenzene (15 g, dried over $CaSO_4$) were heated under reflux for 15 minutes. The reaction was stopped by adding cold hydrochloric acid (5 ml, 5 M) and the solution extracted with diethyl ether (5 x 10 ml). The ether extract was dried over anhydrous $CaSO_4$ and

evaporated under reduced pressure. The resultant solid was purified by sublimation (0.01 mmHg, 80°C) giving a white solid (0.13 g, 76% yield), m.p. 96-104°C. Recrystallization from petroleum ether (80-100°C) gave white needles, m.p. 104-106°C (lit.[139] 98-100°C)

UV. λ_{max} (20% aqueous methanol) 229 (log ε 4.3), 284 (3.75), 310 (3.66) nm (lit.[139] λ_{max}(95% ethanol) 229(4.32),283(3.77),310(3.66)nm).

IR. ν_{max} (nujol)3180 (br), 1620, 1580, 1490, 1210, 1190, 1060, 900, 840 cm^{-1} (lit.[139]: 3190 m (broad), 1624, 1582 cm^{-1})

NMR. δ ($CDCl_3$) 2.3 (3H, s), 3.75 (3H, s), 7.15 (4H, m).

Mass Spec. M/e 161 (M^+), 146, 131, 117, 103, 91, 77, 51.

Analysis. Found C, 74.37; H, 7.01; N, 8.65%. $C_{10}H_{11}NO$ requires C, 74.53; H, 6.83; N, 8.69%.

3.3 PRODUCT ANALYSIS EXPERIMENTS

3.3,1 Reaction of p-dimethylaminophenol with sodium nitrite

3.3,1a Products in solution

p-Dimethylaminophenol (0.137 g), hydrochloric acid (250 ml, 0.1 M) and sodium nitrite (0.069 g) were mixed at 25°C. The reaction was monitored by U.V. and when complete, the solution was extracted with diethyl-ether (4 x 50 ml). The ethereal fractions were dried over anhydrous $CaSO_4$ and evaporated under reduced pressure. A yellow solid was obtained which is light sensitive. Recrystallization from petroleum ether (60-80) gave bright yellow crystals, m.p. 80-89°C (lit.[90] m.p. 115-7°C). By comparing spectral characteristics, this compound was found to be p-benzoquinone.

UV. λ_{max} (water) 246 nm (log 4.35) (lit.[140] λ_{max} (water) 246 (4.33))

IR. ν_{max} (nujol) 3045, 1675, 1650, 1590, 1365, 1305, 1085, 1075, 942, cm^{-1} (lit.[91] ν_{max} (solid) 3063, 1679, 1658, 1593, 1369, 1310, 1084, 1074, 943).

NMR. δ ($CDCl_3$) 6.85 (4H, s) (lit.[141] δ ($CDCl_3$) 6.98).

3.3,1b Gaseous products

p-Dimethylaminophenol (0.0107 g) and HCl (50 ml, 0.1 M) were transferred to the jacketed (25°) reaction vessel attached to a gas burette (Section 3.4,2a). The reaction solution was degassed with oxygen free nitrogen for 1 hour and the pressure was then allowed to equilibrate to atmospheric. A solution of sodium nitrite (1 ml, 0.5M) was injected into the cell and the volume of gas evolved measured in the gas-burette (8.44 ml, 114% reaction corresponding to $(CH_3)_2NC_6H_4OH$ + $2NaNO_2$). A sample was transferred to an IR gas cell and analysed.

IR. ν_{max} (gas) 1900, 1740, 1620 cm^{-1} (lit.[142] ν_{max} (NO_2) 1920, 1750, 1620 cm^{-1}).

3.3,1c Search for dimethylnitrosamine

A reaction of p-dimethylaminophenol with sodium nitrite was performed as in 3.3,1a. After completion, it was neutralised (NaOH) and extracted with dichloromethane (2 x 25 ml). The dichloromethane solution was dried over anhydrous $CaSO_4$ and evaporated under reduced pressure to a small volume. This was examined by GLC (carbowax 20M on chromosorb W, 80-100 mesh column, 100°C) and compared with authentic dimethylnitrosamine. No dimethylnitrosamine was detected. When a sample of dimethylnitrosamine was added to the reaction solution (in the concentration expected, if it was formed) and the solution extracted in an identical manner, dimethylnitrosamine was detected.

3.3,2 Reaction of p-dimethylaminophenol with potassium ferricyanide

p-Dimethylaminophenol (1 ml, 10^{-3} M in acetate buffer) was added to potassium ferricyanide (1 ml, 10^{-2} M water) and the solution made up to 10 ml with acetate buffer 0.1 M (1:1). The subsequent reaction was followed in the thermostated (25°C) cell of a U.V. spectrophotometer at timed intervals. The U.V. spectra showed absorptions for an intermediate and product similar to those obtained for the reaction with sodium nitrite. (Figure 3.3,2a and 3.3,2b.) These correspond to the intermediate N,N-dimethyl-p-benzoquinonemonoimine (280 nm) and p-benzoquinone (246 nm) (cf. Section 2.1,1).

3.3,3 Reaction of 5-hydroxy-1,3-dimethylindole with sodium nitrite

3.3,3a Products in solution

5-Hydroxy-1,3-dimethylindole (0.08 g) was dissolved in ethanol (20 ml) and HCl (80 ml, 0.1 M) and a solution of sodium nitrite (1 ml, 0.5 M) added. A violet precipitate formed which was filtered off and vacuum dried. By T.L.C. it showed to contain a complex mixture of products ranging from dark brown to bright yellow. The precipitate was dissolved in dichloromethane and chromatographed in a silica gel column, using mixtures of benzene, dichloromethane, chloroform and ether as eluent. Two products were isolated and analysed by Mass Spectrometry:

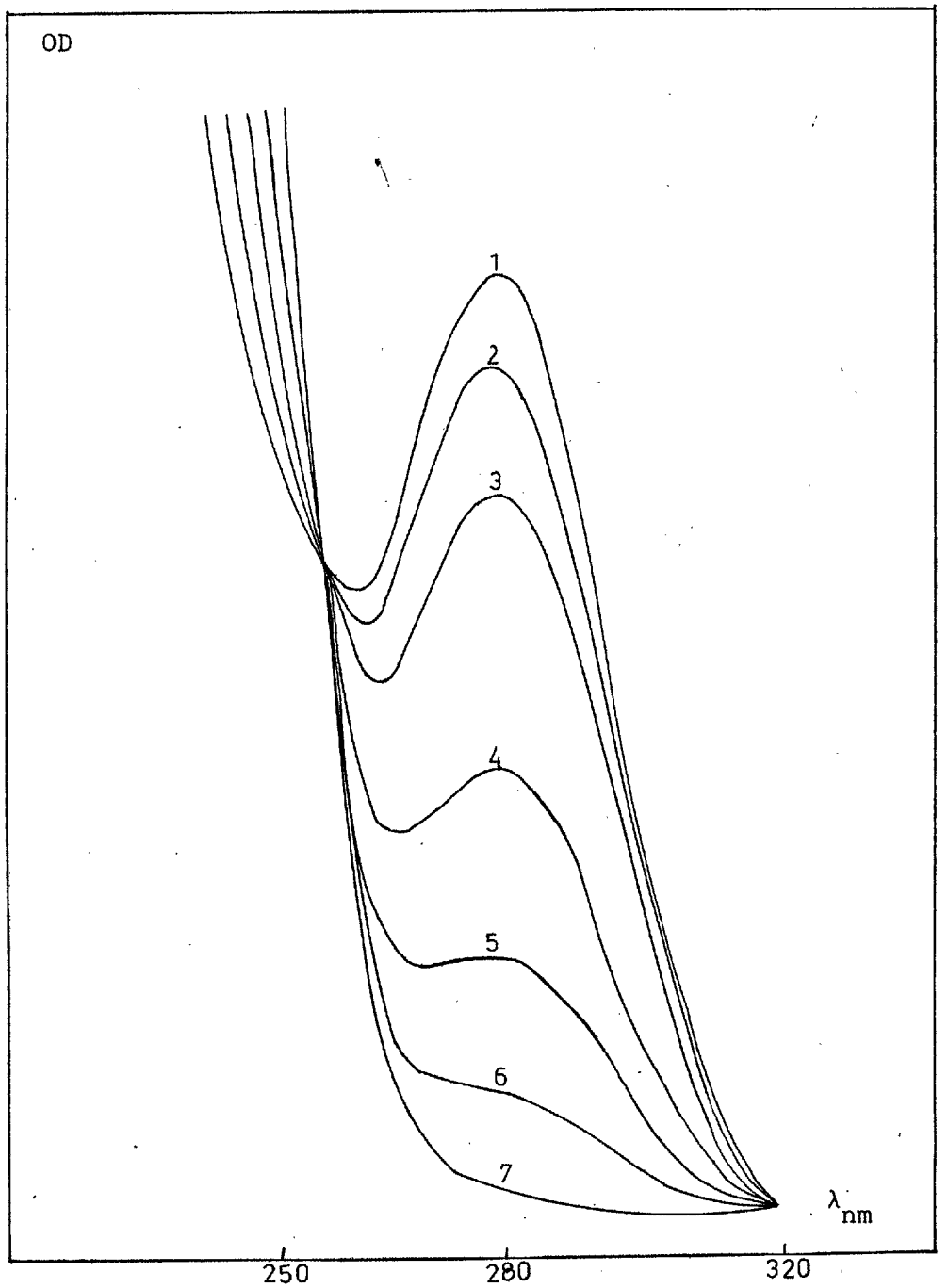
1. M/e 320 (M^+), 305, 189, 160, 132, 122, 57
2. M/e 438 (M^+), 410, 382, 362, 293, 279, 149, 70, 57.

These are consistent with the formation of polymeric products.

3.3,3b Gaseous products

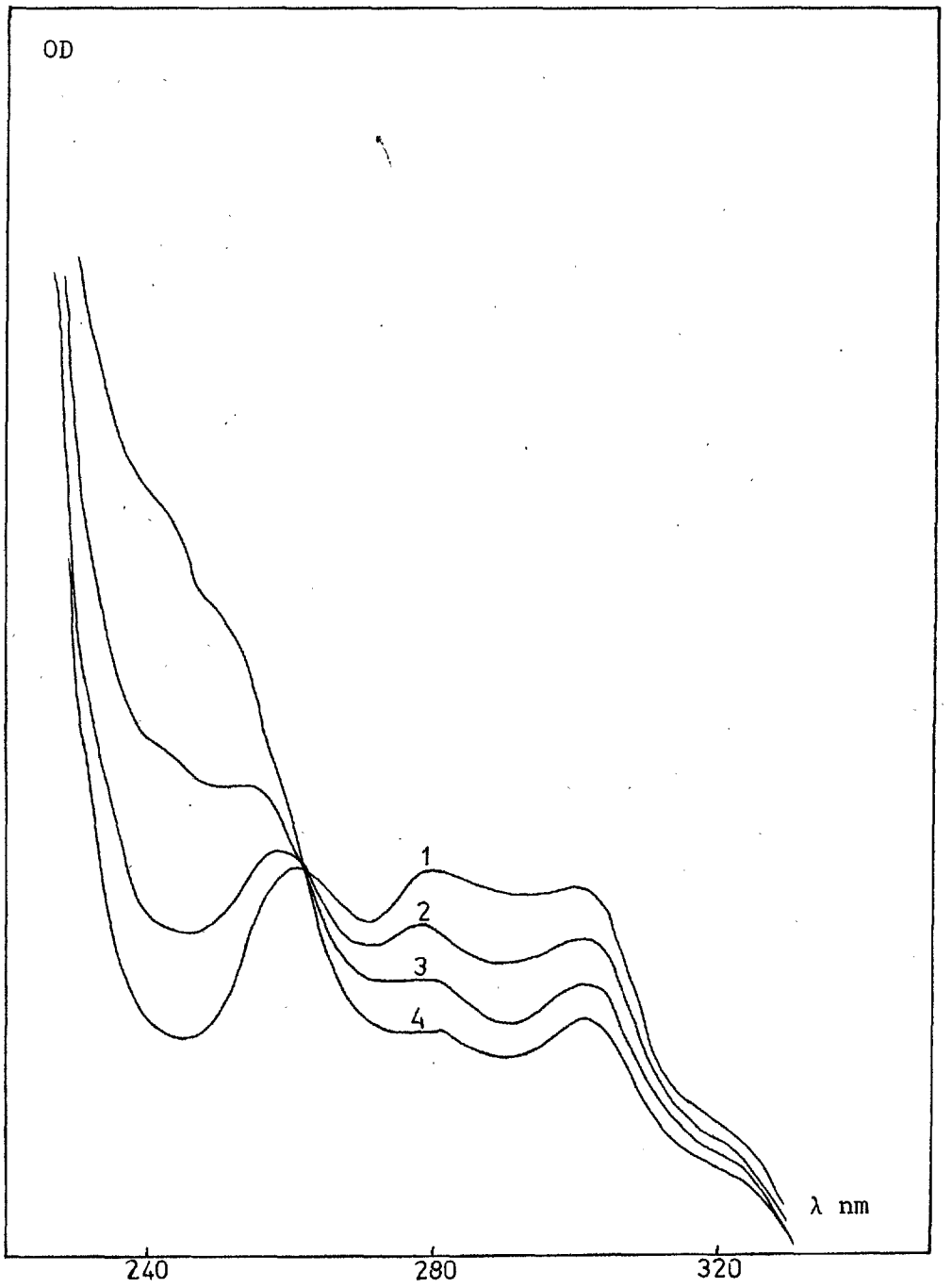
5-Hydroxy-1,3-dimethylindole (0.0355 g), ethanol (10 ml) acetic

Figure 3.3,2a. U.V. spectra of the reaction of p-dimethylaminophenol with sodium nitrite.



1. Reaction after 1 min.
2. " " 4 min.
3. " " 8 min.
4. " " 20 min.
5. " " 34 min.
6. " " 54 min.
7. " " 86 min.

Figure 3.3,2b. U.V. spectra of the reaction of p-dimethylaminophenol with potassium ferricyamide.



1. Reaction after 1 min.
2. " " 5 min.
3. " " 20 min.
4. " " 74 min.

acid (5 ml, 2M), sodium acetate (5 ml, 1M) sodium nitrite (1 ml, 0.5 M) and water up to 50.5 ml were mixed in a gas-burette as explained in 3.3,1b. The volume of gas evolved was measured (6.57 ml, 118% reaction corresponding to 5-hydroxy-1,3-dimethylindole + $NaNO_2$). A sample was transferred to an IR gas cell and analysed.

IR. ν_{max} (gas) 1810, 1740, 1620 cm^{-1} (lit.[142] ν_{max} (NO_2) 1920, 1750, 1620 cm^{-1}; ν_{max} (NO) 1840 cm^{-1}).

3.4 DETAILS OF KINETIC EXPERIMENTS

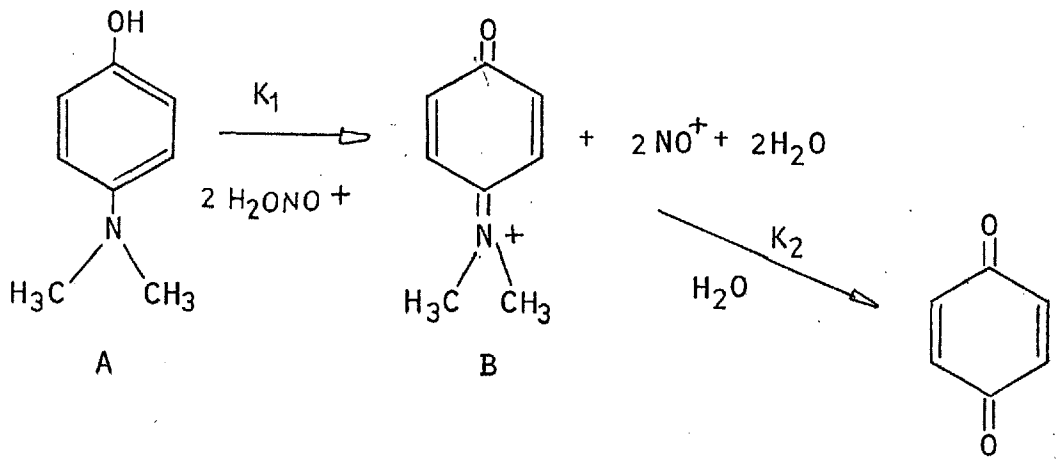
3.4,1 UV method

3.4,1a Details of the method

The rate of oxidation of p-dimethylaminophenol by HNO_2 was determined by measuring the UV absorption of an intermediate (λ_{max} 280 nm) using a Unicam SP 1800 spectrophotometer. The measurements were made against a reference cell containing all components of the reaction solution except the substrate and $NaNO_2$. In a typical experiment, the reaction solution was prepared in a 10 ml volumetric flask and placed in a thermostated bath at 25°C. After equilibration, the reaction was started by addition of 1 ml of an aqueous solution of $NaNO_2$. The reaction solution was then made up to the appropriate volume, shaken vigorously and an aliquot transferred to the U.V. cell. The absorption of this solution was then monitored at timed intervals, using either the spectral scan or fixed wavelength mode. At the conclusion of the experiment, either the pH of the reaction solution was measured, or the acidity of the solution was determined by titration against standard alkali (methyl orange). The temperature of the runs was checked regularly, within the UV cell, using a calibrated thermometer and was found to be within 25° ± 0.1°C.

3.41b. Computation of rate coefficients

The oxidation of p-dimethylaminophenol to p-benzoquinone proceeds according to Scheme 3.4,1b1. Both K_1 and K_2 are pseudo first order



Scheme 3.4,1b1

rate coefficients. The concentration of B at time t can be obtained from equation 3.4,1b1, where A_o is the initial concentration of A.

$$B = \frac{A_o K_1}{K_2 - K_1} \left(e^{-K_1 t} - e^{-K_2 t}\right) \qquad \ldots \text{ eq. } 3.4,1b1$$

The time at which the concentration of B attains a maximum value (t_{max}) can be obtained by setting the derivative of the curve equal to zero (eq. 3.4,1b2). But, since $A_o K_1/(K_2 - K_1)$ will not normally be zero,

$$0 = \frac{dB}{dt} = \frac{A_o K_1}{K_2 - K_1} \left(-K_1 e^{-K_1 t} + K_2 e^{-K_2 t}\right) \qquad \ldots \text{ eq. } 3.4,1b2$$

eq. 3.4,1b3 may be written.

$$0 = -K_1 e^{-K_1 t} + K_2 e^{-K_2 t} \qquad \ldots \text{ eq. } 3.4,1b3$$

This can be rearranged to eq. 3.4,1b4.

$$t_{max} = \frac{1}{K_2 - K_1} \ln \frac{K_2}{K_1} \qquad \text{... eq. 3.4,1b4}$$

K_2 was determined from the latter stages of the reaction by assuming that only the second step was being followed. Thus, knowing K_2 and t_{max}, K_1 values can be calculated from eq. 3.4,1b4. However, this calculation must be done by an inspection method and very inaccurate values are obtained. Hence a modification of the computer programme used in Part I, Section 4.4,2 was used to obtain K_1 and K_2. From approximate values of K_1 and K_2 and values of the absorbances of A, B and C (E_A, E_B, E_C), the programme calculated the observed total absorbance at time t, compared the calculated with the observed values and minimized the deviation between them by an iterative process. Values of E_A and E_C were calculated from solutions of known concentrations of A and C and were held constant. E_B was estimated from the absorbtion of a reaction solution in which K_1 was so fast that all A was transformed into B immediately.

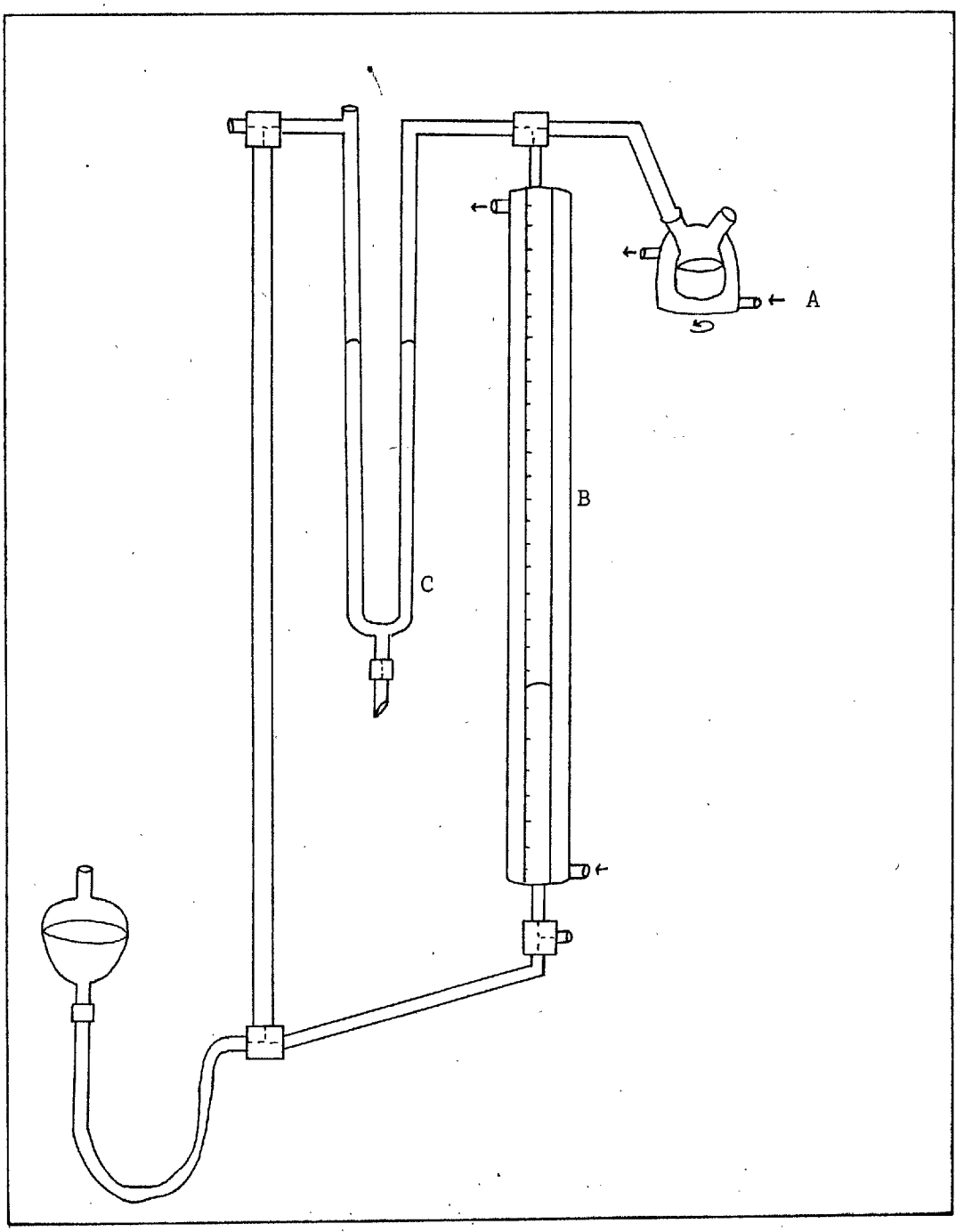
The values of K_1 and K_2 obtained are rather scattered (Section 2.1,1), although the average deviation of the calculated values of absorbance only varies from ca. 0.2 to ca. 0.04. It was noted that calculated values of K_1 and K_2 were very sensitive to small changes in the estimated values of E_B, K_1 and K_2 given as input.

3.4,2 Gas-burette method

3.4,2a Details of the method

The oxidation of 5-hydroxyindoles with sodium nitrite was followed by measuring the volume of nitric oxide evolved using a gas-burette (Figure 3.4,2a). The reaction vessel whose contents could be stirred magnetically, was connected to the gas-burette (capacity 10 ml) and both were thermostated at 25°C by circulating water. The temperature was checked throughout the course of the runs with a thermometer immersed in the water bath close to the gas-burette and was found to be constant to ± 0.1°C. The surface of mercury used in the manometer and in the gas-burette was protected from contact with nitric oxide by a layer of KOH (1M). In a typical experiment, the reaction solution was prepared in a 50 ml volumetric flask and transferred to the reaction vessel which was then connected to the gas-burette. The reaction solution was then degassed with oxygen-free nitrogen (after passage through a chain of 4 bottles of Fieser solution). From complete removal of oxygen from the reaction solution degassing was continued for at least 30 minutes, after which the system was allowed to equilibrate for ca. 10 minutes. The reaction was started by injecting a suitable amount (usually 0.5 ml) of aqueous $NaNO_2$ solution through a "Suba-Seal" stopper while the reaction solution was rapidly stirred. The volume of nitric oxide evolved at timed intervals was read in the gas-burette at atmospheric pressure. Independent checks established that the rate at which NO was evolved was independent of the rate of stirring. The reaction was followed to completion. The pH of the reaction solution was measured before the addition of $NaNO_2$.

Figure 3.4,2a. Gas-burette.



A Reaction vessel

B Gas-burette

C U-tube manometer

3.4,2b Computation of rate coefficients

The rate of reaction (v), as obtained from the slope of a plot of mls of NO versus time, is related to the concentration (c) of a reactant by eq. 3.4,2b1, where k is the rate constant and n the order

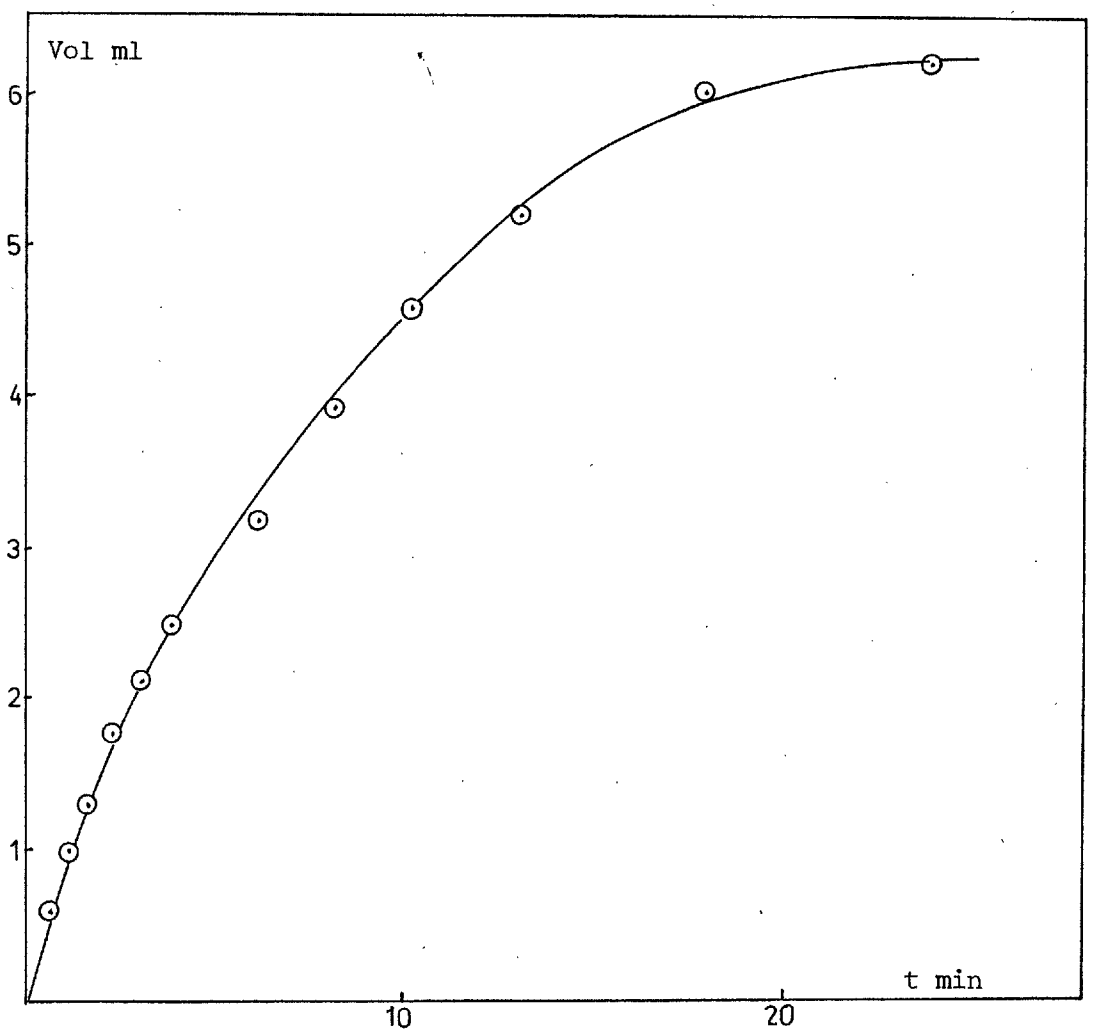
$$v = k\, c^{n} \qquad \text{... eq. 3.4,2b1}$$

of the reaction with respect to the substance which concentration is being varied. If v is measured at various values of the reactant concentration, a double-logarithmic plot of v against c gives a straight line, the slope of which is n.

Initial rates were calculated from plots of volume of nitric oxide evolved against time. They were corrected for temperature and expressed in $M\ s^{-1}$. Reproducibility was found to be about 10% for the slower runs and about 20% for the faster runs. Typical kinetic runs to show the reliability of this method are shown in Figures 3.4,2b1, 3.4,2b2 and 3.4,2b3.

Figure 3.4,2b1.

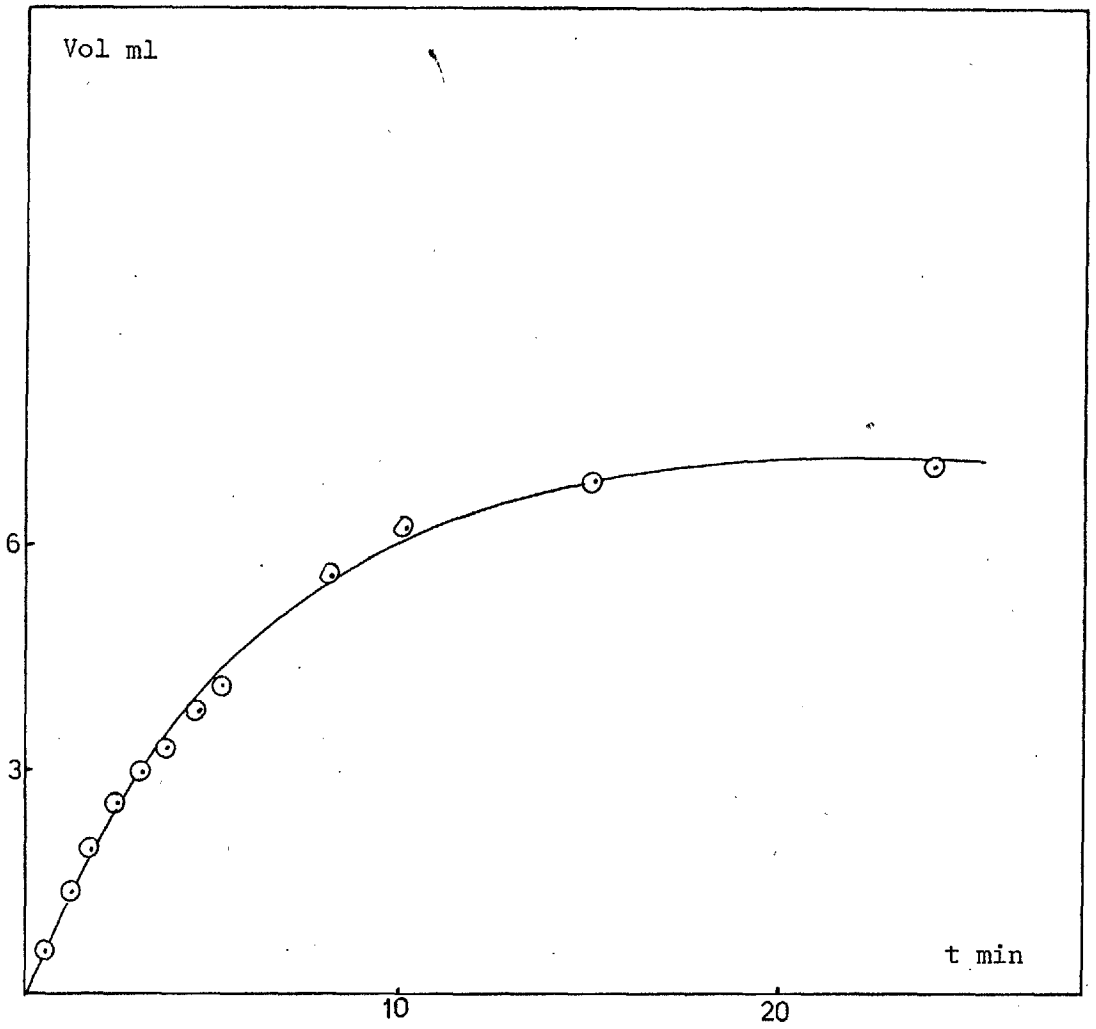
36.6 mg substrate + 10 ml C_2H_5OH + 10 ml 1M HOAc + 5 ml 1M NaOAc diluted to 50 ml + 0.5 ml 0.9M $NaNO_2$; pH = 4.72; temp. 25°C.



t(min)	vol(ml)	% reaction
0.67	0.58	10.4
1.25	0.97	17.5
1.75	1.27	26.7
2.42	1.75	31.5
3.25	2.11	38.0
4.0	2.47	44.5
6.25	3.16	56.9
8.25	3.93	70.8
10.3	4.59	82.7
13.2	5.17	93.1
18.0	6.01	108.3
24.2	6.18	111.4

Figure 3.4,2b2

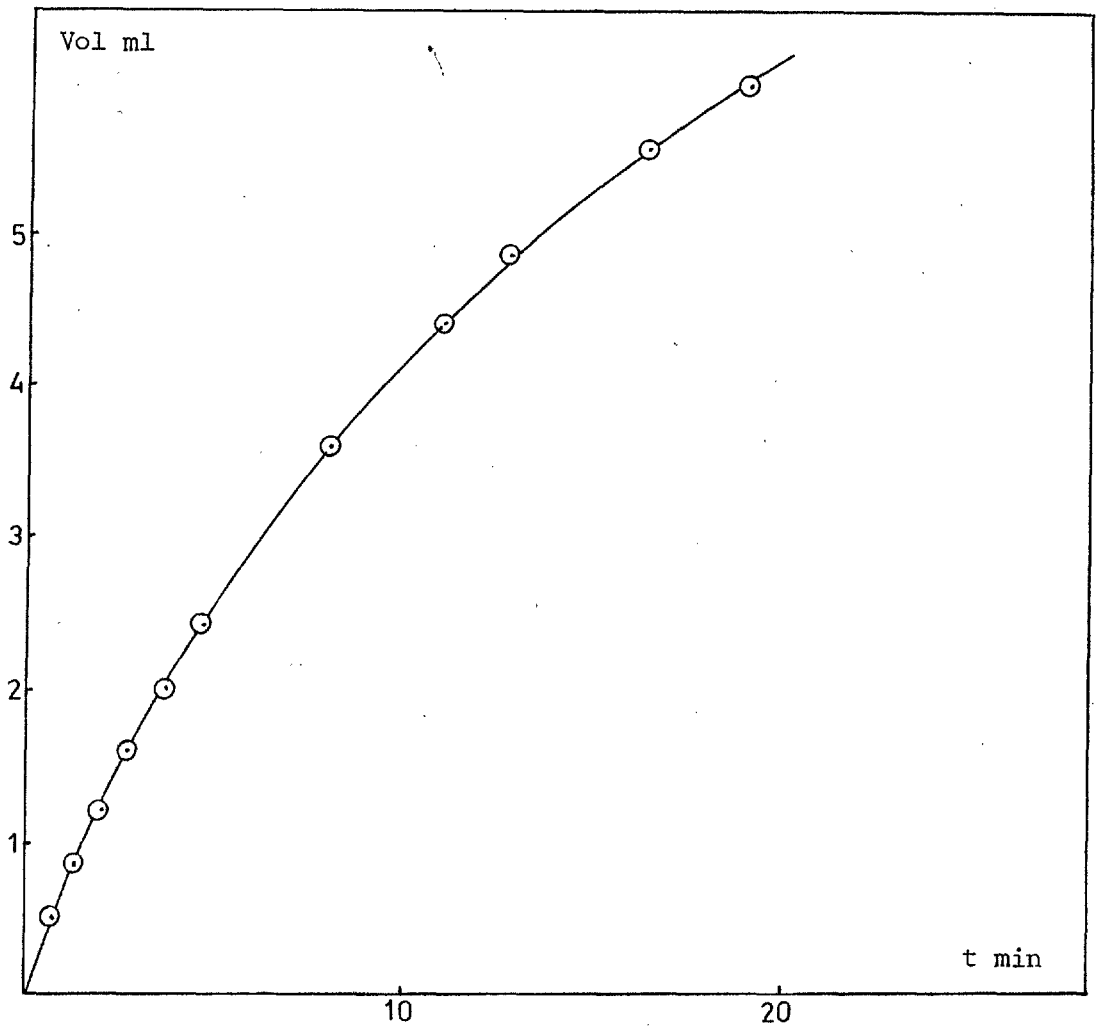
36.6 mg substrate + 10 ml C_2H_5OH + 10 ml 1M HOAc + 5 ml 1M NaOAc diluted to 50 ml + 0.5 ml 1.35 M $NaNO_2$; pH = 4.71; temp. 25°C.



t(min)	vol(ml)	% reaction
0.5	0.66	11.9
1.2	1.35	24.3
1.7	1.94	34.9
2.3	2.57	46.3
3.0	2.96	53.3
3.7	3.28	59.1
4.5	3.76	67.7
5.2	4.11	74.1
8	5.55	100.0
10	6.21	111.9
15	6.84	123.2
24	7.06	127.2

Figure 3.4,2b3

36.6 mg substrate + 10 ml C_2H_5OH + 8 ml 1M HOAc + 5 ml 1M NaOAc diluted to 50 ml + 0.9 M $NaNO_2$; pH = 4.78, temp. 25°C.



t(min)	vol(ml)	% reaction
0.67	0.50	9.0
1.25	0.83	14.9
2.0	1.21	21.8
2.75	1.62	29.2
3.75	2.02	36.4
4.75	2.43	43.8
8.0	3.61	65.0
11.0	4.44	80.0
12.75	4.89	88.1
16.3	5.59	100.7
19.0	6.02	108.5
29.0	6.92	124.7

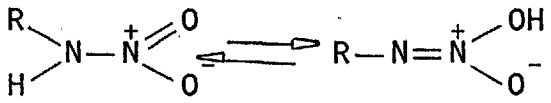
APPENDIX

ACID CATALYSED HYDROLYSIS OF PRIMARY NITRAMINES

1. INTRODUCTION

1.1 THE STRUCTURE OF PRIMARY NITRAMINES

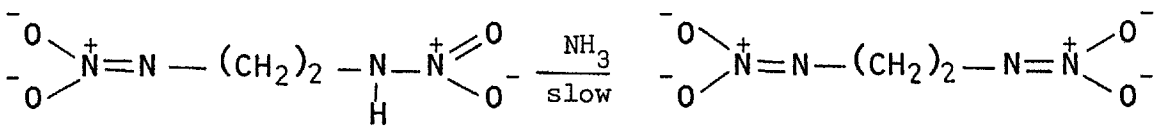
Primary nitramines are weak acids and on the basis of the temperature dependence of their dissociation constants, they have been considered to be pseudo acids[143] (Scheme 1.1,1). The existence of these



(1) (2)

Scheme 1.1,1

two mesomeric forms has been mentioned by Orton[144] in the case of 2,4-dibromo-6-nitrophenylnitramine. Bell and Pearson[145] studied the reaction of ethylenedinitramine with ammonia and found a measurable rate for the second dissociation (pK_1 = 5.35, pK_2 = 6.66) by using a flow method (Scheme 1.1,2). Since ethylenedinitramine was also found



Scheme 1.1,2

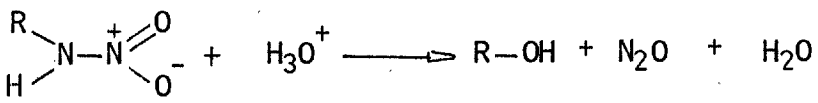
to be a low-activity acid catalyst, they classified it as a pseudo-acid. Nevertheless, no further report proving the real existence of the two forms of nitramine has appeared and recent reviewers[146,147] have considered it as a tautomeric equilibria.

On the basis of the infrared spectra, nitramide ($H_2N\text{-}NO_2$) and

methylnitramine[148] and arylnitramines[149] are thought to exist in form (1) (Scheme 1.1,1). Lamberton studied the NMR spectra of some primary nitramines and reported that the N-H proton gave a broad signal and that generally, coupling with the protons on the α-carbon did not occur.

1.2 ACID CATALYSED HYDROLYSIS OF PRIMARY NITRAMINES AND ITS O-ALKYLATED DERIVATIVES

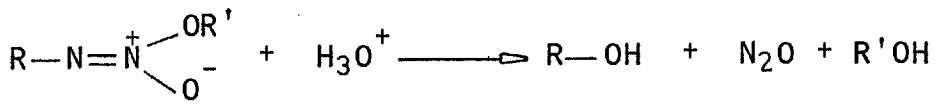
The decomposition of primary nitramines in mineral acid was studied by Lamberton[151,152] and co-workers and was found to give nitrous oxide and an alcohol as products (Scheme 1.2,1). The rate of reaction is first order in [nitramine] and $[H^+]$ and it is favoured by electron donation by the alkyl group R. The order of reactivity was found to be



Scheme 1.2,1

R = Me_3CCH_2 > EtMeCH > $EtMeCHCH_2$ > Pr^i > Et or Bu^t > Me > CH_2COOH.

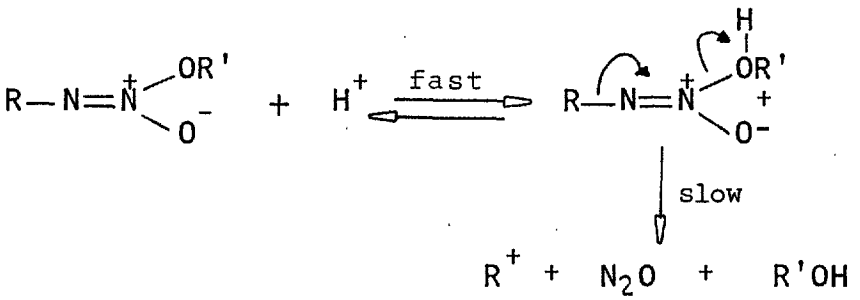
The reaction of isopropylnitramine was also studied in hydrogen sulphate buffers, phosphoric acid, phosphate, acetate and chloroacetate buffers and was found to be general acid catalysed. Similar results were found for the O-methylated derivatives of primary nitramines (Scheme 1.2,2) although they are much more reactive.[153,154] The reaction proceeds via



Scheme 1.2,2

scission of the R-N and N-OR' bond. Electron donation by R strongly favours decomposition but changes in R' act mildly in the opposite direction. General acid catalysis was also detected.

The mechanism of these reactions was formulated in an identical manner by Lamberton (Scheme 1.2,3). This mechanism, however, does not



R = alkyl, H

Scheme 1.2,3

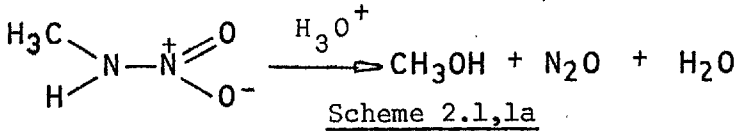
explain the observed general acid catalysis.

2. ACID CATALYSED HYDROLYSIS OF METHYLNITRAMINE AND O-METHYL-N-ISOPROPYLNITRAMINE

2.1 RESULTS

2.1,1 Hydrolysis of methylnitramine in H_2SO_4

Rates of hydrolysis of methylnitramine were measured in H_2SO_4 and D_2SO_4, following the reaction by the decrease in the U.V. absorption of the reactant (λ_{max} 232 nm). The reaction proceeds according to Scheme 2.1,1a, showing a first order dependence in [substrate](eq. 2.1,1a) and $\underline{K}_o$ was found to be acidity dependent.



Scheme 2.1,1a

$$\text{rate} = K\ [\text{substrate}]\ [H^+] \qquad \ldots \text{eq. 2.1,1a}$$

K_o values were obtained from plots of ln ($OD-OD_\infty$) *versus* time and were found to be constant for at least 3 half lifes.

Tables 2.1,1a and 2.1,1b show K_o values obtained for the hydrolysis of methylnitramine in H_2SO_4 and D_2SO_4 respectively (Figure 2.1,1a). The reaction is acid catalysed and is faster in H_2O than in D_2O.

Table 2.1,1a. Hydrolysis of methylnitramine in H_2SO_4 at 25°C.

Initial [substrate] *ca.* $2 \cdot 10^{-4}$ M

$[H_2SO_4]$ M	$10^6 \cdot K_o\ s^{-1}$
1.01	0.8
2.05	3.6
4.07	18.3
6.02	79.1
7.50	243.
9.10	723.

Table 2.1,1b. Hydrolysis of methylnitramine in D_2SO_4 at 25°C.

Initial [substrate] *ca.* $2 \cdot 10^{-4}$ M

$[D_2SO_4]$ M	$10^6 \cdot K_o\ s^{-1}$
1.9	1.5
3.6	6.6
5.6	26.
6.7	59.
8.	124.
9.	137.

The deuterium solvent isotope effect at several acidities is listed in Table 2.1,1c. The ratio $K_{H_2SO_4}/K_{D_2SO_4}$ is consistent with a slow proton transfer to the substrate in the rate determinin step.

Figure 2.1,1a. Hydrolysis of methylnitramine in H_2SO_4 and D_2SO_4 at 25°C.

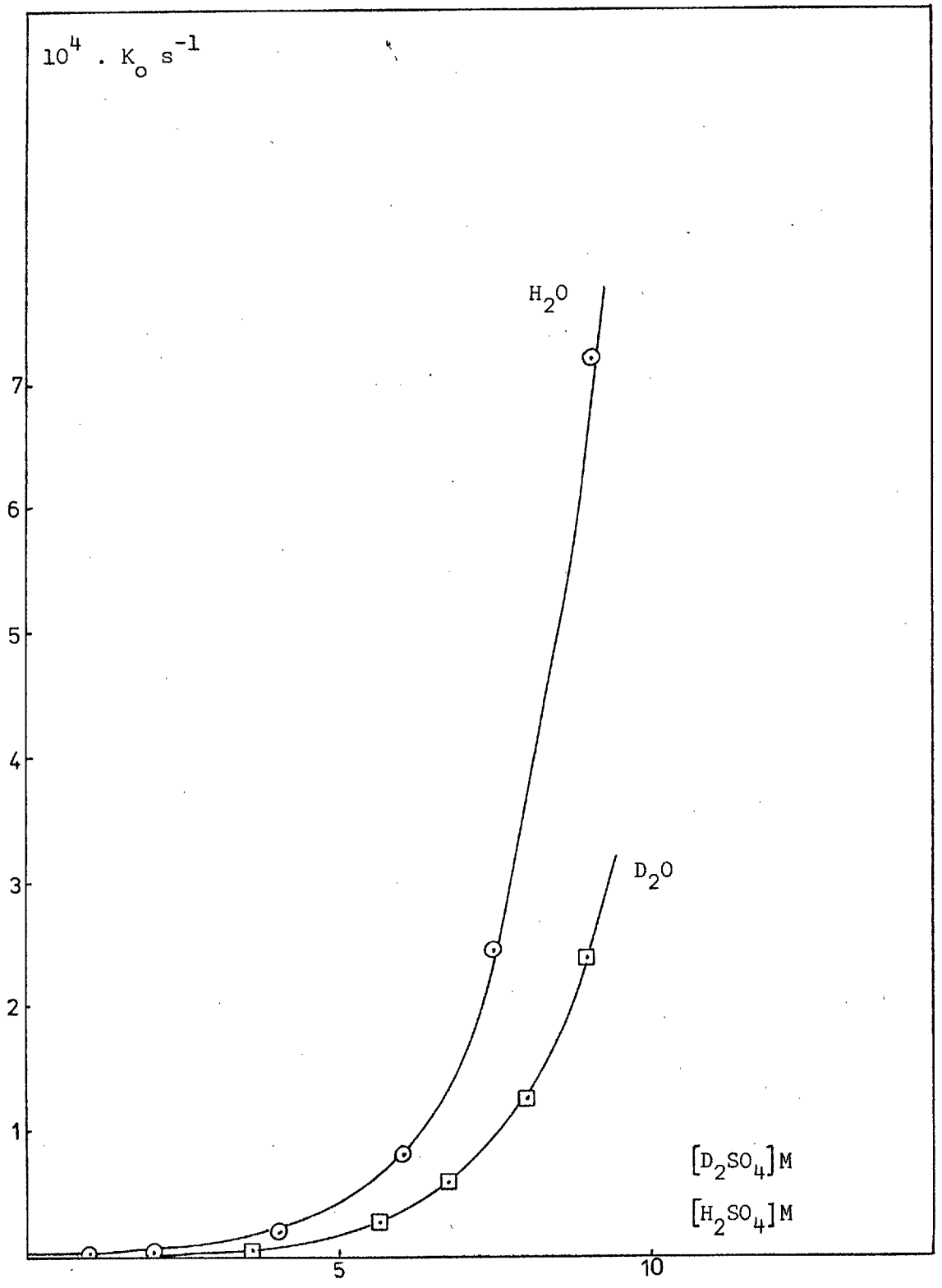


Table 2.1,1c. Deuterium solvent isotope effect for the hydrolysis of methylnitramine at 25°C

$[L_2SO_4]$ M	$K_{H_2SO_4}/K_{D_2SO_4}$
5	2.1
7	2.1
9	3.0

The effect of temperature upon the reaction rate was studied in 2M and 6M H_2SO_4. The results are listed in Table 2.1,1d.

Table 2.1,1d. Effect of temperature on the hydrolysis of methylnitramine in H_2SO_4

$[H_2SO_4]$ M	Temp °C	$10^4 . K_o$ s^{-1}
2	15	0.006
2	25	0.036
2	35	0.11
2	44	0.38
2	60	2.1
6	15	0.2
6	25	0.8
6	35	2.6

An Arrhenius plot for these data (Figure 2.1,1b) gives E_a (2M) = 103 k J mol^{-1} and E_a (6M) = 93 k J mol^{-1}. Values of the entropy of activation [80] at 298°K were $\Delta S^{\neq}$ (2M) = - 4 J mol^{-1} K^{-1} and $\Delta S^{\neq}$ (6M) = - 11 J mol^{-1} K^{-1}. The values of E_a and $\Delta S^{\neq}$ do not change significantly with acidity.

Values of the pseudo-first-order rate coefficients for the hydrolysis of methylnitramine were found to correlate with the H_A acidity function (Figure 2.1,1c).

Figure 2.1,1b. Arrhenius plot for methylnitramine in 2M and 6M H_2SO_4

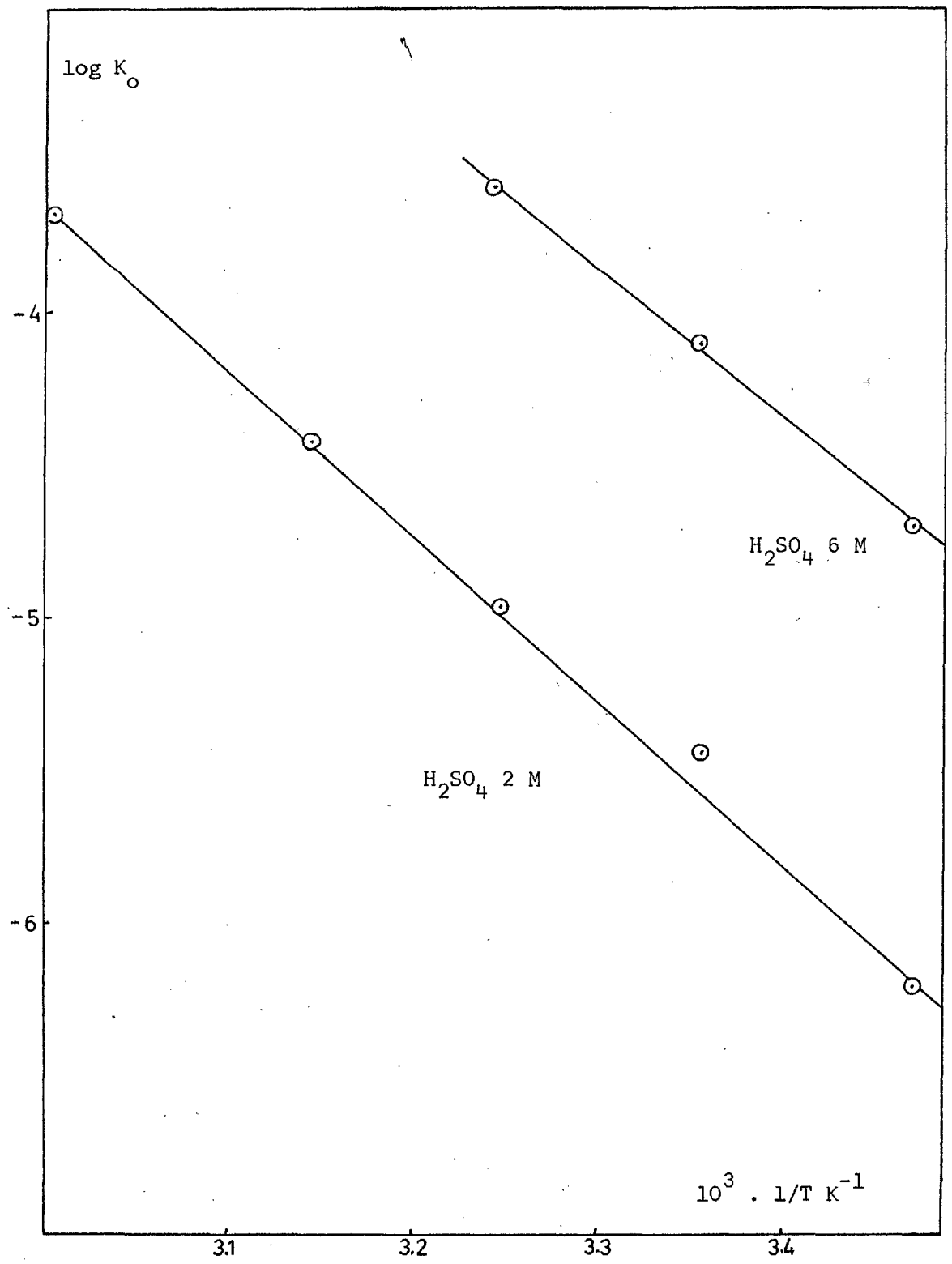
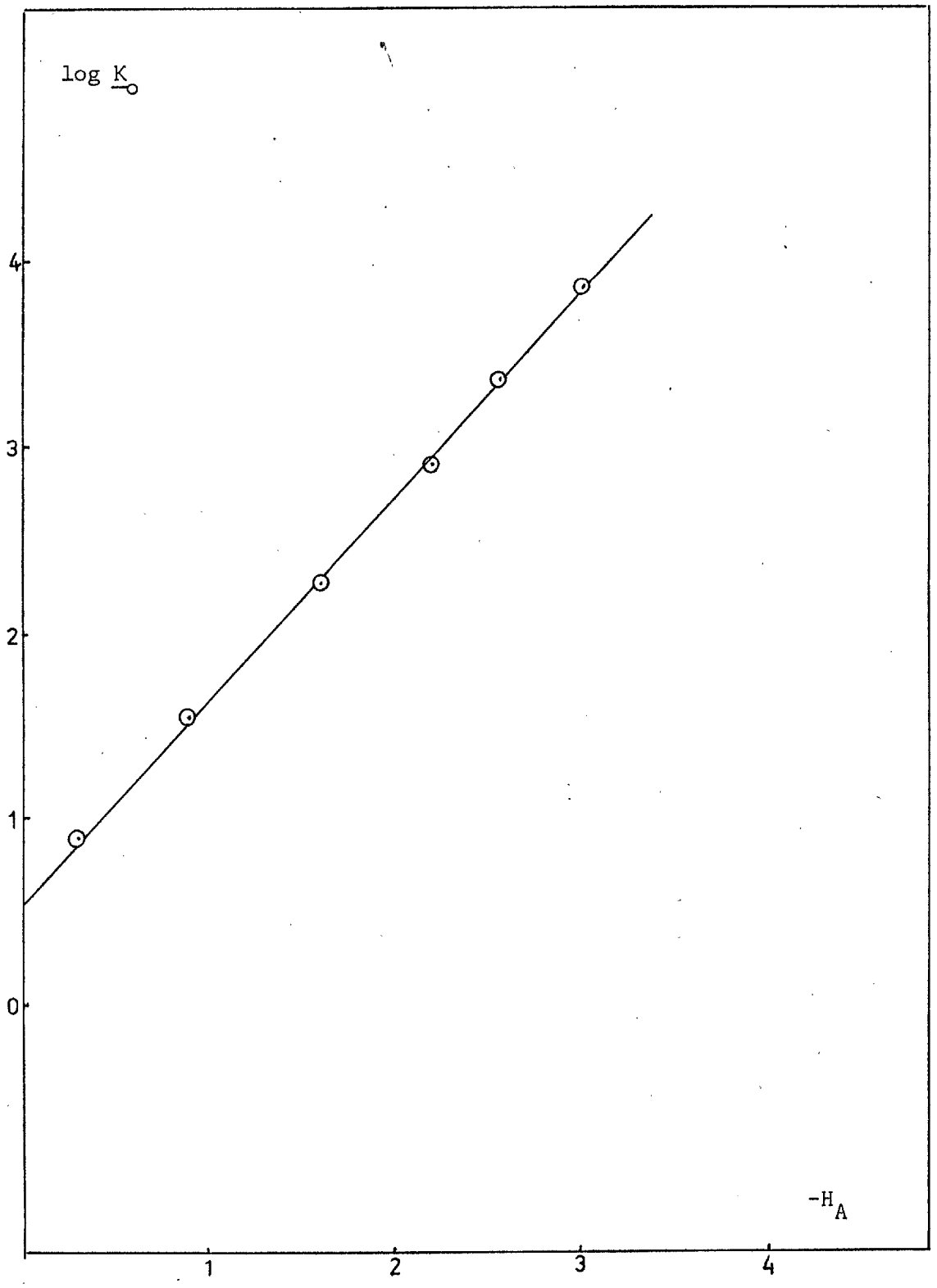
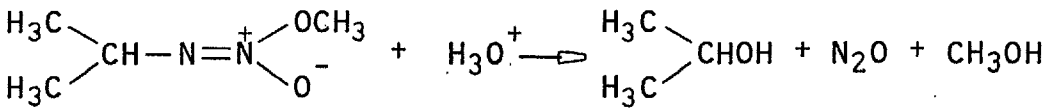


Figure 2.1,1c. Correlation of hydrolysis rates of methylnitramine with H_A acidity function



2.1,2 Hydrolysis of O-methyl-N-isopopylnitramine in HCl

Rates of hydrolysis of O-methyl-N-isopropylnitramine were measured in HCl and DCl, following the reaction by the decrease in absorption of the substrate (λ_{max} 214 nm). The reaction proceeds according to scheme 2.1,2a and equation 2.1,2a. Pseudo-first-order rate constants



Scheme 2.1,2a

$$\text{rate} = K\,[\text{substrate}]\,[H^+] \qquad \text{... eq. 2.1,2a}$$

were obtained from plots of ln (OD_t-OD_∞) *versus* time and were found to be constant for at least 3 half-lifes. Reproducibility was within ± 5%. Tables 2.1,2a and 2.1,2b show K_o values for the hydrolysis of O-methyl-N-isopropylnitramine in HCl and DCl respectively (Figure 2.1,2).

Table 2.1,2a. Hydrolysis of O-methyl-N-isopropylnitramine in HCl at 25°C. μ = 0.5 M (NaCl); Initial [substrate] *ca.* $1.2 \cdot 10^{-4}$ M.

[HCl] M	$10^3 \cdot K_o$ s^{-1}
0.01	0.10 (0.11)
0.05	0.52
0.075	0.78
0.101	1.07 (1.04)

Figure 2.1,2. Hydrolysis of O-methyl-N-isopropylnitramine in HCl and DCl at 25°C

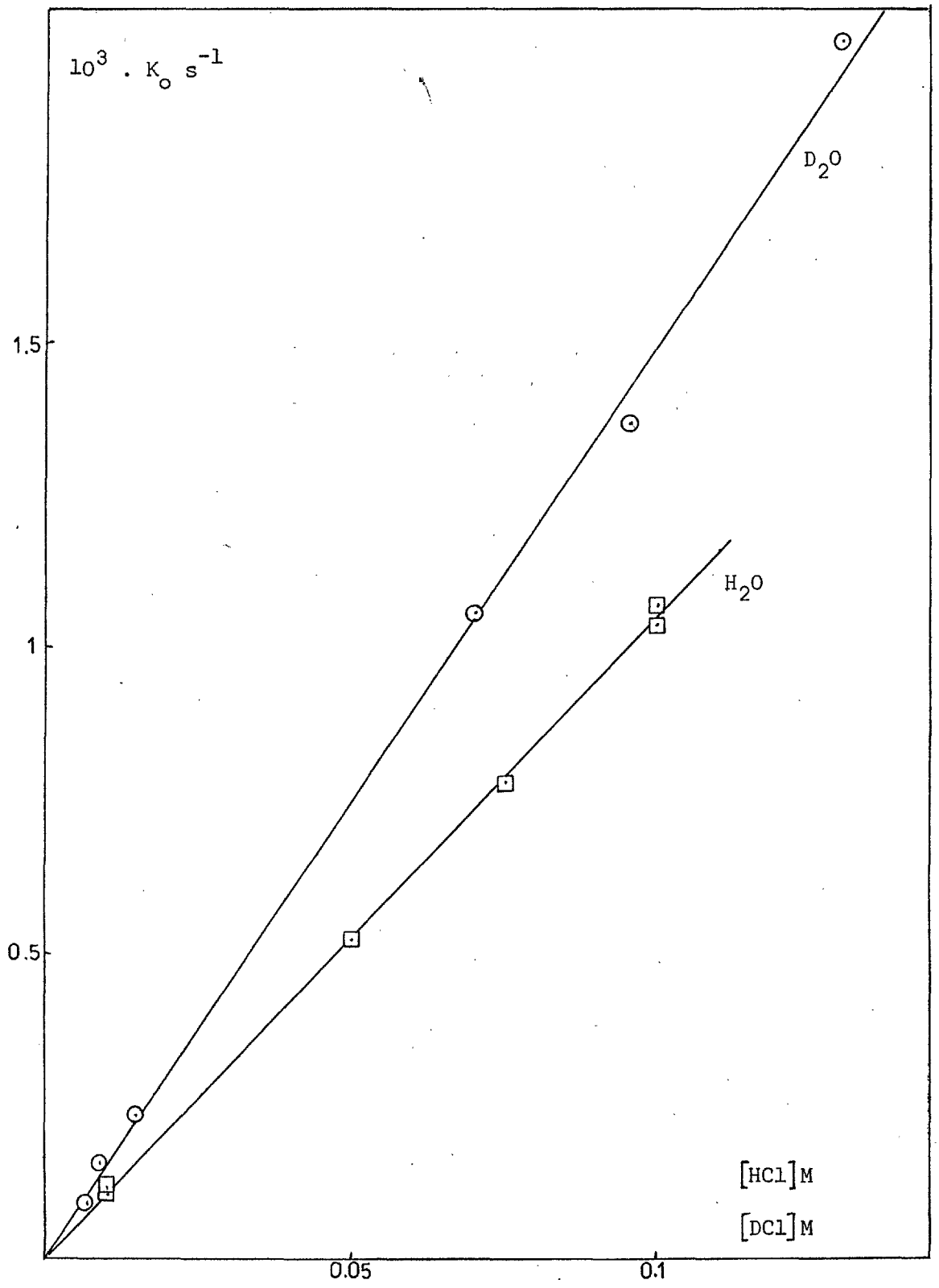


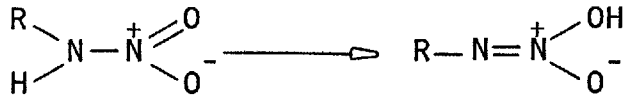
Table 2.1,2b. Hydrolysis of O-methyl-N-isopropylnitramine in DCl at 25°C. μ = 0.5 M (NaCl); Initial substrate ca. $1.1 \cdot 10^{-4}$ M

[DCl] M	$10^3 \cdot K_o s^{-1}$
0.007	0.09
0.009	0.15
0.015	0.23
0.07	1.06
0.095	1.37
0.130	2.06

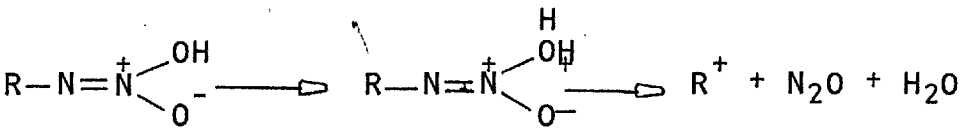
The reaction is acid catalysed and is faster in D_2O than in H_2O. A deuterium solvent isotope effect K_{HCl}/K_{DCl} ca. 0.7 was calculated from Figure 2.1,2 and is consistent with a fast pre-equilibrium protonation of the substrate.

2.2 DISCUSSION OF RESULTS

The solvent deuterium isotope effects found for the hydrolysis of methylnitramine ($K_{H_2SO_4}/K_{D_2SO_4}$ ca. 3) and for the hydrolysis of O-methyl-N-isopropylnitramine (K_{HCl}/K_{DCl} ca. 0.7) indicate that we are dealing with two different processes and that it is not possible to say that both reactions proceed via the same mechanism as Lamberton argues[154]. A slow proton transfer must take place in the rate determining step of the hydrolysis of methylnitramine but it is difficult to draw conclusions without more studies of the catalysis by acids and bases. However, if primary nitramines exist as tautomers, the isomerization step cannot be slow (Scheme 2.2,1). Another possibility would be slow protonation of the -OH group and although this would explain the general acid catalysis detected by Lamberton, this path is unlikely, taking into account present knowledge (Scheme 2.2,2).



Scheme 2.2,1



Scheme 2.2,2

The deuterium solvent isotope effect found for the hydrolysis of O-methyl-N-isopropylnitramine is consistent with a fast pre-equilibrium protonation of the substrate. Nevertheless, the mechanism in Scheme 1.2,3 is not consistent with general acid catalysis. Again, it is not possible to make conclusions without further studies of catalysis by acids and bases.

3. EXPERIMENTAL DETAILS

For purification of reagents and solvents, synthesis of methylnitramine and kinetic method refer to Chapter 4, Part 1.

3.1 SYNTHESIS OF O-METHYL-N-ISOPROPYLNITRAMINE

3.1,1 Synthesis of isopropylnitramine

To a dry-ice cooled mixture of diethylether and hexane (100 ml, 2:1), isopropylamine (5 g) was added with stirring, followed by n-butyllithium (30 ml, 3.8 M). Ethyl nitrate (4.23 g) was then added gradually.

The mixture was stirred for 1 hour and more n-butyllithium (15 ml, 3.8 M) and ethylnitrate (2.1 g) were added. After a further 30 minutes stirring, the mixture was allowed to attain room temperature and acidified until pH ca 1 (HCl). The hexane layer was separated and the aqueous layer extracted with ether (4 x 100 ml). The ether extracts were dried ($MgSO_4$) and evaporated. The resultant oil was distilled at 0.5 mm Hg and the fraction boiling at 50-51°C (3g) was retained. Yield 34%.

RI. n_D^{25} 1.4559

UV. λ_{max} (H_2O) 233 nm

IR. ν_{max} (liquid film) 3280, 2980, 1580, 1300 cm^{-1}.

NMR. δ ($CDCl_3$) 1.15 (6H, d), 4.0 (1H, m).

3.1,2 Synthesis of O-methyl-N-isopropylnitramine [153]

The isopropylnitramine silver salt (9.6 g) was suspended in diethyl ether (50 ml, sodium dried) and methyl iodide (10 g) added. The mixture was heated under reflux for 90 minutes and stirred at room temperature for 24 hours. The precipitate was filtered and washed with ether. The filtrate was combined with the ether washings and the ether removed by distillation. The resultant oil was distilled at 3.75 mm Hg and the fraction boiling at 56-59°C was retained (1.2 g). Yield 22%.

RI. n_D^{25} 1.4290

UV. λ_{max} (water) 214 nm

NMR. δ 1.2 (6H, d), 4.0 (4H, m, s)

3.2 TYPICAL KINETIC RUNS

Tables 3.2,1 and 3.2,2 are examples of kinetic runs which prove the reliability of the method.

Table 3.2,1. 4 ml 10 M H_2SO_4 diluted to 10 ml; T = 25°C
λ_{max} 232 nm; Initial $[CH_3NHNO_2]$ ca. $2 \cdot 10^{-4}$ M

t min	A_t	% reaction	$10^5 \cdot K_o$ s^{-1}
1	1.39	-	-
62	1.33	4.3	1.2
92	1.28	7.9	1.4
242	1.08	22.3	1.9
482	0.84	29.6	1.7
662	0.69	50.3	1.8
842	0.57	59.0	1.8
962	0.50	64.0	1.8
1102	0.39	71.9	2.9
1222	0.35	74.8	1.5
∞	0	-	-

Table 3.2,2. 1 ml 1 M DCl + 4 ml 1M NaCl diluted to 10 ml; T = 25°C;
λ_{max} 214 nm; Initial $[(CH_3)_2CHNNO(OCH_3)]$ ca.
1.1 . 10^{-4} M.

t min	A_t	$A_t - A_\infty$	% reaction	$10^3 \cdot K_o$ s^{-1}
1	0.587	0.573	-	-
3	0.466	0.452	21.1	2.0
5	0.368	0.354	38.2	2.0
7	0.289	0.275	52.0	2.2
9	0.232	0.218	61.9	1.9
11	0.186	0.172	69.9	2.0
15	0.121	0.107	81.3	1.9
17	0.100	0.086	84.9	1.8
19	0.084	0.070	87.8	1.8
21	0.068	0.054	90.6	2.1
23	0.061	0.047	91.8	1.2
∞	0.014	-	-	-

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