MECHANISTIC STUDIES OF CRESOL AND KETONE NITROSATION

by

IAN FRANKLIN

A THESIS SUBMITTED TO THE UNIVERSITY OF LONDON

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN THE FACULTY OF SCIENCE

Frankland Laboratory
Department of Chemistry
Imperial College of Science and Technology
London SW7

June 1979

Acknowledgements

I thank, sincerely, Dr. Brian C. Callis for his assistance and guidance during the course of this work.

I am grateful to Prof. Sir Derek H. Barton for the opportunity to work at Imperial College and to the Ministry of Agriculture, Fisheries and Food for financial support.

I also thank my colleagues in the Frankland Laboratory for their friendship, advice and assistance. Finally, thanks to the technical staff, particularly Jack Peppercorn, Dorothy and Lillian of the Frankland Laboratory.

ERRATA

Acknowledgements: Dr. Brian C. Challis

p. 11, line 19: Challis

p. 17, line 11: relationship

p. 28, line 20: observation

p. 31, line 13:) after (NO_2^-)

p. 34, line 14: ...limit for β of unity...

p. 35, equations (2.10) and (2.11): lines omitted from fractions on right hand side

p.38, line 12: ..(4-methylphenol)

p.77, line 2 of text: ...results since each...

p.87, line 6: spectra

p.88, line 18: ethylenediamine

p.89, line 19: deionised

p.90, line 16: employed

p.99, Section 7.1.2b; p.103, Section 7.1.3b and p.106, Section 7.2.1a: units of λ are nm

p.111, line 20: was not included...

ADDENDA

p.28, Section 2.2.3, lines 4 and 11: amend Figure 3 to Figure 2a, then insert p.28a (Figure 2a)

Insert p.42a after p.42 to obtain Table 15

ABSTRACT

Physiological, kinetic and mechanistic aspects of the nitrosation of phenols, in relation to the N-nitrosation of secondary amines, are reviewed. The kinetics of the nitrosation of 4-methylphenol for pH 1 to 6 are reported in detail and a mechanism is suggested to explain the novel finding that acetate ions catalyse the reaction at high pH. Rates of nitrosation of DL-tyrosine and N-acetyl-L-tyrosine are reported. The substrates studied showed a similar reactivity to that of phenol towards nitrous acid. It is suggested that the substrates may compete successfully with secondary amines for the nitrite in the diet.

The enolisation and electrophilic substitution of ketones is reviewed. Particular attention is given to the nitrosation of ketones. Results are reported showing that rates of nitrosation of alicyclic ketones are greater than rates of enolisation. This is explained by considering the formation of an O-nitroso intermediate prior to enolisation. The results support an earlier report but the required mechanism is contrary to that generally applied to the electrophilic substitution reactions of ketones.

CONTENTS

CHAPTER 1

LITERATURE REVIEW OF THE NITROSATION OF AMINES AND PHENOLS

1.1: Physiological Aspects of the Nitrosation of Amines and Phenols:

The induction of cancer in laboratory animals by N-nitrosamines, first reported by Magee and Barnes[1,2] in 1952, has initiated much research into the formation and occurrence of these compounds. Their facile production from either secondary or tertiary amines[3] and various nitrosating agents, all readily available in the environment, virtually ensures that all humans are exposed to N-nitrosamines. A recent review[4] presents a wide-ranging survey of the availability of carcinogens, including N-nitrosamines, and correlates this with the incidence of particular types of cancer. Further N-nitrosamine chemistry, with particular attention to their carcinogenic capability, has been reviewed extensively and recently summarised[5,6]. This survey concentrates on the most recent work in the literature with reference to earlier findings where appropriate.

Although N-nitrosamines occur naturally[5] at low levels (< 5 ppb), major concern has arisen from the realisation that N-nitrosamine precursors are ubiquitous in the environment. Secondary and tertiary amines are present in foodstuffs, pesticides and drugs, for example. Potential nitrosating agents, e.g. nitrite ion, are employed as food preservatives and others (nitrogen oxides) are formed during most combustion processes. The addition of nitrite and nitrate ions to meat products, principally those containing pork, to inhibit the growth of 'Clostridium Botulinum' (the micro-organism responsible for botulism, a fatal disease in humans) and to promote the "desirable" red colouration of bacon, provides a common source of potential nitrosating species. Domestic water supplies rich in nitrate from natural and

agricultural sources are thought to be significantly related to the incidence of specific types of cancer. For example, some correlation exists between the incidence of gastric cancer (58 % of the local population) in the Narino distict of Colombia and the relatively high level of nitrate in the water supply (up to 33 $\mu g/cm^3$ nitrate nitrogen)[7]. Similar epidemiological evidence from the United Kingdom has been reported[8] and later contended[9]. The nitrate is believed to be reduced to nitrite by bacterial action[6,8] but the precise mechanism is unknown. The manner in which nitrite exerts its bacteriostatic effect on 'Clostridium Botulinum' is also unclear although evidence suggests that the nitrite reacts with meat constituents to form compounds similar to Roussin salt which has the formula $NH_4[Fe_4S_3(NO)_6NO_2].H_2O$ [10]. Addition of this type of salt to meat mixes gave a red pigment; this was found to be bacteriostatic towards micrococci and 'Enterobacteriacea'. However, the bacteriostatic effect was reduced in the presence of meat protein. Other workers proposed the formation of antimicrobial agents from the nitrite[11] as a possible explanation of the Perigo effect[12] whereby bacterial growth in meat samples is still inhibited after all free nitrite has been consumed. Iron (II) - amino acid - nitrosyl complexes have been synthesized using many amino acids and $Fe(cystine)(NO)_2$, the only complex tested to date, inhibited growth of 'Staphylococcus Saprophyticus' [11].
It has been shown that nitrite is unnecessary for the control of 'Clostridium Botulinum' since sodium benzoate (5 g/kg) performs the same function[13]. Nevertheless, the use of nitrite as a preservative continues.

Production of N-nitrosodimethylamine (NDMA) in the atmosphere[14] by reaction of gaseous dimethylamine and nitrous acid

has been reported to occur at night in heavily polluted urban areas. The NDMA is photolysed by ultra-violet radiation and is removed from the atmosphere by the following noon. Any NDMA detected after this time must be from direct emission. Nitrosating agents of a more exotic nature are isoamyl and isobutyl nitrites[15] inhaled as "aphrodisiacs" by some inhabitants of the USA.

It was generally thought that nitrosation of amines in aqueous solution occurred only under acidic conditions[16] (pH < 5) in order to convert nitrite to nitrous acid, the supposed nitrosating agent. Very slow nitrosation of diethylamine at pH 6.4 and 24°C was reported[17] (ca. 1 % N-nitrosodiethylamine after 17 h). However, it has been reported[18] recently that rapid nitrosation of amines by gaseous N_2O_3 and N_2O_4 occurs from pH 7 to pH 14. The degree of nitrosation is virtually independent of the nucleophilic reactivity of the amine. It is significant that nitrosation also occurs when the reaction medium is plasma or whole blood. Since nitrogen oxides are common atmospheric pollutants arising from combustion processes, including cigarette smoke[19,20], this mode of nitrosamine formation may pose a greater danger than the formerly considered aqueous, acidic rections.

The volatile nitrosamines N-nitrosopiperidine and N-nitrosopyrrolidine (NNP) have been detected in salt premixes used to cure meat products[21]. It is not surprising, therefore, that the latter is found in cooked bacon although the nitrosation of amines also occurs during the cooking process. The most comprehensive survey of nitrosamine formation in foodstuffs[22] considered over 500 samples of foodstuffs including complete meals. The foods were extracted and volatile N-nitrosamines were detected by GC-MS techniques. The most frequently occurring were NDMA (1 - 5 μg/kg) and NNP (1 - 20 μg/kg, occasionally 200 μg/kg) in

cooked bacon samples. NDMA was also found in some other meat products, in which other nitrosamines were rarely detected, and in fish (1 - 10 μg/kg) and cheese (1 -5 μg/kg). N-nitrosamines other than NDMA were never found in fish and cheese in amounts greater than the detection limit of 1 μg/kg.

Earlier workers performed in vitro experiments[23] with pork, egg, bread, milk and cheese under simulated gastric conditions employing a nitrite concentration similar to that present in foods and including thiocyanate ion since this is found in saliva. Only cheese produced detectable amounts of volatile N-nitrosamines, a larger amount being formed at pH 3 (up to 7 μg/kg) than at pH 1. At pH 3 and a nitrite level of 100 ppm all foods produced non-volatile, dichloromethane soluble, N-nitroso compounds and also water soluble, N-nitroso compounds. Thiocyanate ion increased the amounts formed. Human subjects were found to have a fasting gastric pH of 1 which rose to pH 3 - 4 after a meal. Nitrite was rapidly lost in the stomach but no N-nitroso compounds were detected in the stomach contents following a meal.

Extracts of Japanese raw fish treated with nitrite showed mutagenic activity against 'Salmonella typhymurium' in the laboratory[24]. No activity was shown by similarly treated beef and hot dogs; this was taken to be possible evidence for the hypothesis that high incidence of gastric cancer in Japanese and certain other populations relative to low incidence in the USA and other western countries may be related to dietary factors. The finding that the 'Salmonella typhymurium' mutants most sensitive to mutagenesis were also those most sensitive to base-pair substitution suggests that structures such as alkylnitrosamides may be the mutagens present and that compounds like polycyclic or heterocyclic species are

unlikely to be the mutagens[24]. However, it has been noted[25] that the result of this type of test for mutagenicity may not be indicative of carcinogenicity. The test[25] was used to ascertain the mutagenicity of nitrogenous pesticides following *in vitro* nitrosation with sodium nitrite. The 37 pesticides tested contained either amide, carbamate or urea moieties. If the nitrosated species reacted positively to the mutagen test then the parent compound and nitrite were fed to mice to assess the *in vivo* formation and mutagenicity of the nitrosated compounds. With the exception of ethylenethiourea, already known to be mutagenic, none of the pesticides appeared to show *in vivo* mutagenicity. The factor[25] noted above is applicable, however, and lack of mutagenicity in this test does not exclude the possibility of carcinogenicity in man.

In vivo formation of *N*-nitrosamines from amine precursors and nitrite in laboratory animals[16] and in humans with hypoacidity[26] administered with large amounts of diphenylamine and nitrite is not very informative when related to the real-life situation due to the unrealistic dosage administered. However, it has been reported[27] that NDMA and *N*-nitrosodiethylamine have been detected in blood samples taken after human ingestion of a midday meal consisting of a bacon, spinach and tomato sandwich and beer. Spinach provided a large amount of nitrate (potentially nitrite) although at a realistic dietary level; the beer presumably provided desirably acidic gastric conditions. An unaccountable background level of *N*-nitrosamine in the blood samples was found but was removed if the human volunteer ate a low nitrate/nitrite, high ascorbate diet for the previous 24 hours.

The effect of ascorbic acid (vitamin C) and ascorbate ion upon N-nitrosamine formation is polemic. Sodium ascorbate inhibited NNP formation[28] in fried bacon and prevented mutagenesis by raw fish treated with nitrite[24] but in a model bacon fat system consisting of an aqueous buffer phase and a non-polar phase (pure corn oil or benzene) amine nitrosation was 5 - 25 times greater in the presence of ascorbate[29]. Pork cured with $Na^{15}NO_2$ with and without ascorbate[30] appeared to retain similar amounts of $^{15}NO_2^-$ during the curing, packaging and storage processes. The distribution of the nitrite varied, however, the lean fraction of ascorbate cured meat containing less than half the amount of nitrite present in the lean fraction of that cured without ascorbate. Nitrite loss *in vivo* was little affected by inclusion of ascorbic acid in the meal[23]. Positive catalysis of amine nitrosation by other polyhydroxy compounds, e.g. gallic acid[31] and chlorogenic acid[32], was probably a result of alkaline nitrosation[18] during work-up procedures.

The proposed inhibition of N-nitrosamine formation by phenols in the diet[33] due to the greater reactivity of phenols towards nitrosating agents derived from nitrite led to reports[34] that phenols and thiophenols present in foodstuffs did react must faster than amines. These results are discussed later, together with those of the author and other recent workers in this field. Nitrosated phenols have been obtained by various methods. Treatment of bovine serum albumin with nitrite under simulated gastric conditions[35] led to the isolation of 3-nitrotyrosine and 3,4-dihydroxyphenylalanine (dopa) both compounds being thought to derive from the initial product, 3-nitrosotyrosine. Analysis of extracts from spray smoked and traditionally smoked bacon[36] showed the presence of 2-nitro-5-methylphenol in both

types, whether raw or fried, and of other 2-nitro and 2-nitroso-phenols in the cooked meat. Nitrosation of smoke condensates[37] produced 30 nitrosated phenols, all detected as their nitro counterparts. Major products were 2-nitro-5-methylphenol, 2-nitro-4-methylphenol and 2-nitro-4-ethylphenol; only three of the products were nitrosated at a position other than the 2-position. 4-nitroso-2-methylphenol enhances formation of NNP at pH 5 and 37°C [38]. The efficiency of phenols as nitrite traps, thus preventing nitrosation of amines, appears to depend, therefore, upon the stability of the nitrosophenol to oxidation. If it does not rapidly oxidise to the corresponding nitrophenol it may form in sufficient quantity to catalyse the reaction of residual nitrite with the amines.

Nitrosating agents may also react with other metabolic components, e.g. haemes and amino acids. Nitrite, acting as a one electron donor, can oxidise haemoglobin to methaemoglobin[39], i.e. iron (II) to iron (III), causing methaemoglobinaemia in humans[15]. Reaction of nitrous acid with some porphyrins and haemes[40] gave nitrosylhaemes which were able to N-nitrosate secondary amines. This type of nitrosation, transnitrosation, was also demonstrated for N-nitrosamines[41] and proposed as a possibly significant biological mechanism. A mechanism of this kind, involving transnitrosation, may be the reason for the Perigo effect[12], discussed previously. Nitrosation of amino acids, as such and in proteins, is considered in Section 1.2.

N-nitrosamine formation promoted by carbonyl compounds[17] was demonstrated for formaldehyde and chloral at pH 6.4 and for hexahalogenated acetones, benzaldehyde,

nitrobenzaldehydes, 4-dimethylaminobenzaldehyde and pyridoxal at pH 4 with diethylamine as substrate and pH 6.5 with morpholine as substrate[62]. However, other workers[42] have reported no N-nitrosamine formation on addition of nitrite to mixtures of aliphatic aldehydes and primary amines.

1.2: Mechanistic Aspects of the Nitrosation of Phenols:

General aspects of electrophilic aromatic substitution have been reviewed at great length[43,44,45]. Factors influencing the rate determining step and the nature of intermediates were amplified by other authors[46,47]. Results most pertinent to the elucidation of mechanisms for these reactions, such as the effect of acidity and deuteriated substrate studies, have been summarised[48]. A very brief account of the mechanistic and kinetic argument applicable to these reactions is presented here.

Two processes are possible for the substitution of a hydrogen atom by an electrophile. A two step process, termed S_E2, is generalised in equation (1.1). The alternative one step process, S_E3, is generalised in equation (1.2).

$$\left.\begin{array}{l} ArH + E^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ArHE^+ \\ ArHE^+ + B \xrightarrow{k_2} ArE + HB^+ \end{array}\right\} \qquad (1.1)$$

$$ArH + E^+ \xrightarrow{k_3} (E....Ar..H....B)^{\cdots\cdots+\cdots} \longrightarrow EAr + HB^+ \qquad (1.2)$$

ArH denotes the aromatic substrate, E^+ the electrophile, B a basic entity and $ArHE^+$ an intermediate in the S_E2 reaction pathway. HB^+ is the conjugate acid of the base. The electrophile may be formed by a pre-equilibrium process. Investigation of the primary hydrogen isotope effect for these reactions shows that the S_E2 pathway is correct. No examples of the S_E3 process have been found[46].

The overall rate for the S_E2 mechanism, assuming a low steady state concentration of the intermediate, is given by equation (1.3):

$$\text{Rate} = \frac{k_1.k_2\,[ArH]\,[E^+]\,[B]}{k_{-1} + k_2\,[B]} \qquad (1.3)$$

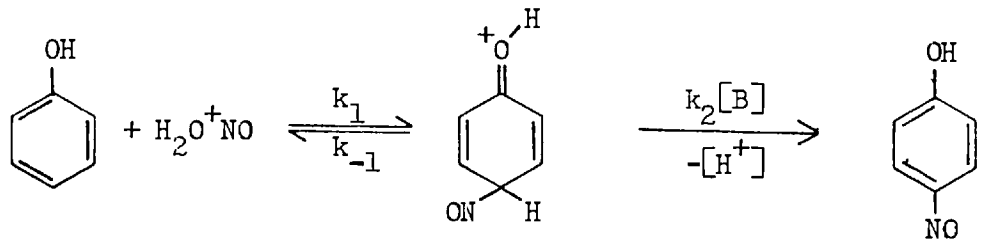
If the intermediate returns to substrate more readily than it proceeds to product ($k_{-1} \gg k_2$) the rate is given by the expression $(k_1.k_2 / k_{-1}).[ArH]\,[E^+]\,[B]$. Retention of k_2 in the expression implies that a primary hydrogen isotope effect will occur ($k_H > k_D$) if deuteriated substrate is used. There will also be a linear dependence of rate upon [B] if $k_2[B] \ll k_{-1}$. However, if $k_2[B] \gg k_{-1}$ then the rate is given by $k_1.[ArH]\,[E^+]$, showing kinetic dependence upon the first step only (k_1) and absence of both a primary hydrogen isotope effect and base catalysis.

In the case of aromatic nitrosation the above analysis was found to apply to phenol and 2-naphthol[49]. The reaction was studied from pH 1 to 5 in aqueous perchloric acid or carboxylate buffers with an excess of substrate over nitrite. Under these conditions the nitrosating species was either the nitrous acidium ion (H_2O^+NO) or nitrosyl salts (NOX) formed in pre-equilibrium reactions, equation (1.4).

$$HNO_2 + H_3O^+ \rightleftharpoons H_2O^+NO + H_2O$$

$$H_2O^+NO + X^- \rightleftharpoons NOX + H_2O \qquad (1.4)$$

Since the pre-equilibrium reaction involves acid, the mechanism for the overall process is termed A- S_E2. The mechanism for phenol is:



For phenol, the changeover from $k_{-1} > k_2$ to $k_{-1} < k_2$ occurred at ca. pH 4.5 and for 2-naphthol at ca. pH 2. This changeover is reflected in a graph of log k_{H_2O} (the rate constant derived when water is the only base) against pH. In the case of naphthol, this shows a pH independent region for pH < 2 and a pH dependent region for pH > 3.5 with a slope of unity, indicating a first-order dependence of k_{H_2O} upon $[H_3O^+]$.

Nitrosation of substituted benzenes[50] and 4-substituted phenols[51] in 10 - 12 M acid and 10^{-2} - 9 M acid, respectively, showed an A- S_E2 mechanism operated with proton expulsion for the dienone intermediate being rate determining. This factor prevented explicit identification of the nitrosating species involved for acidities up to ca. 5 M $HClO_4$ (or H_2SO_4) but at higher acidities the reagent was diagnosed to be the nitrosonium ion (NO^+) [52]. All substrates yielded nitro products; substitution occurred solely at the 2-position for the phenols[51] but predominantly at the 4-position

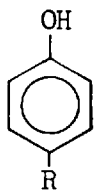
for the substituted benzenes[50]. The products were believed to form by rapid oxidation of an initial nitroso product. This was supported by reports that direct nitration of 4-methylphenol[46] and benzene[53] proceeds without a primary hydrogen isotope effect, i.e. step k_1 is rate determining, whereas the nitrosation studies showed such an effect to be present. Nitrosation of benzene in concentrated perchloric acid[50] also gave a rate several orders of magnitude less than that reported for direct nitration under similar conditions[54].

Nitrosation of resorcinol (1,3-dihydroxybenzene) and its mono- and dimethyl ethers[55] gave results similar to those for 2-naphthol[49] in the cases of resorcinol itself and the monomethyl ether. Insufficient data was reported for the dimethyl ether to observe similarity. However, these workers did not investigate fully the effect of added base and deuteriated substrate upon the reaction. Although the change in rate determining step at pH 3 was attributed to the fact that the "decomposition rate of the intermediate into products assumes higher values than the rate of the reverse reaction" (sic) the significance of the finding was unappreciated. Callis was able to claim, therefore, "the first 'fast' proton transfer for an aromatic nitrosation" in his contemporaneous publication[49].

The suggestion that phenol and nitrite may react as protonated phenol and nitrous acid in 20 - 60 % perchloric acid[56] is dubious in view of subsequent results[49].

Prior to the report of nitrosation of 4-methylphenol (Figure 1, Ia) in aqueous perchloric acid[51] the action of nitrite upon this substrate was included in investigations of the nitrosation of tyrosine (Ib) [57,58] and pepsin[58] in aqueous actate buffers at ca. pH 4. The aim of this work was to determine the amino acid in

pepsin (a protein) which was responsible for peptic activity by, in effect, removing the active group by nitrosation. Other substrates employed were tyrosol (Ic), α-bromopropionyltyrosine (Id), p-hydroxyphenylacetic acid (Ie) and tryptophan (II). Various



(I)

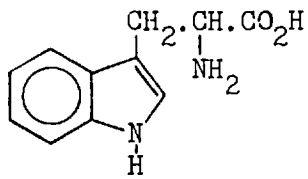
a, R = CH_3

b, R = $CH_2.CH(NH_2).CO_2H$

c, R = $CH_2.CH(OH).CO_2H$

d, R = $CH_2.CH(CO_2H).NH.CO.CHBr.CH_3$

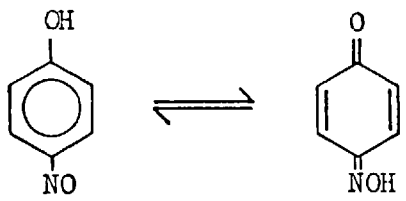
e, R = $CH_2.CO_2H$



(II)

products were reported for tyrosine and 4-methylphenol according to the relative concentrations of the reagents employed[57]. Kinetic measurements[58] were found to be most easily obtainable and reliable if copper(II) ions were present in the reaction mixtures. The ions complexed with the 2-nitrosophenol formed in the rate determining reaction and this complex was estimated colourimetrically. Rate measurements for pepsin were obtained *via* peptic activity. The results showed that tyrosine, α-bromopropionyltyrosine and pepsin gave identical rate constants. p-Hydroxyphenylacetic acid gave an apparently similar rate but was shown statistically to be reacting at a discretely different rate. Tyrosol, 4-methylphenol

and tryptophan all gave higher rate constants than tyrosine (ca. 50 %, 100 % and 200 %, respectively). The reactive entity in pepsin thus appeared to be the tyrosine residues. It is interesting to note that the peptide bond (...CO.NH...) in the side-chain of -bromo-propionyltyrosine did not affect the rate of reaction of the basic tyrosine molecule. This work is pertinent to that reported here, as are investigations of the reaction of N-acetyltyrosine and sodium nitrite[59] and ^{15}N-labelled sodium nitrite[60]. N-acetyltyrosine reacted with nitrite at pH 4 and 4°C to give a yellow chromophore with an absorption maximum at 401 nm, shifting to 409 nm when basified to pH 11.5 [59]. The product was thought to be the corresponding 2-nitrosophenol but this is at variance with literature data[61] for the model compound 2-nitroso-4-methylphenol. The reaction with ^{15}N-labelled nitrite[60] in aqueous solution at ca. pH 3 was observed by ^{15}N nuclear magnetic resonance (nmr) spectroscopy but no new signal appeared in the spectrum. This was due, possibly, to signal broadening by radical species (from the phenolic group) or to tautomerism of the expected 2-nitrosophenol species. 4-nitrosophenol also fails to show a signal when in chloroform and is known to readily tautomerise to 1,4-benzoquinone-monoxime, equation (1.5). Production of nitrosotyrosine residues



(1.5)

from reaction of bovine serum albumin[35] and nitrite has been discussed above. Interaction of tyrosine and other phenolic materials with nitrite[34], mentioned previously, led to results

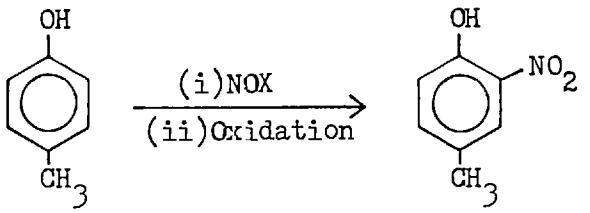
which were reported unsatisfactorily and will be discussed later in the context of the present investigation.

CHAPTER 2

RESULTS AND DISCUSSION FOR THE NITROSATION OF 4-METHYLPHENOL

2.1: Direct Spectrophotometric Method; Excess Sodium Nitrite:

Reaction of 4-methylphenol with an excess of sodium nitrite gave 4-methyl-2-nitrophenol as the only detectable product (> 85 % of quantitative yield), presumably by nitrosation at the 2-position followed by oxidation. Nitrous acid is known to act as an oxidant[50]. Equation (2.1) represents the overall reaction.



(2.1)

Rate of loss of substrate, determined via formation of product, exhibited a good pseudo-first order dependence upon the substrate concentration. Confirmation of this dependence by variation of the initial substrate concentration was not attempted. The rate of loss of nitrite was assumed to be first order, as in previous studies[49], and was not explicitly verified.

The overall rate expression for the nitrosation was thus considered to be given by equation (2.2):

$$\text{Rate} = \bar{k}_2[\text{4-methylphenol}](NaNO_2) \qquad (2.2)$$

Rates are reported here in terms of $\bar{k}_1$, the pseudo-first order rate constant, and k_2 (not $\bar{k}_2$), the second order constant relating to the actual concentration of nitrous acid present at a given pH, as in equation (2.3):

$$\text{Rate} = k_2[\text{4-methylphenol}][HONO] \qquad (2.3)$$

2.1.1: Acidity Dependence of k_2:

Variation of k_2 over the pH range 4 to 5.5 was determined at a fixed acetate concentration and therefore at a constant ionic strength (μ). The results are presented in Table 1 with a graphical representation in Figure 1. The rate constant k_2 appears to be independent of acidity at pH < ca. 4.7 but at higher pH shows an acidity dependence, the plot being linear with a slope of -0.55. This may be taken to indicate a half order dependence upon $[H_3O^+]$ but this is difficult to reconcile with a rational mechanism for the nitrosation reaction. Figure 1 shows a log k_2 / pH rlationship of a generally similar form to that previously obtained for phenol and 2-naphthol[49] but in these cases the pH dependent portion indicated a first order dependence upon $[H_3O^+]$ in accord with the expected mechanism. Further discussion will be given later in the context of results obtained by another experimental method.

2.1.2: Acetate Ion Catalysis:

The variation of $\bar{k}_1$ and k_2 with $[AcO^-]$ for pH 4 to 4.75 is presented in Table 2. The rate constants show only slight dependence upon $[AcO^-]$ at pH 4 and 4.5 and no dependence at pH 4.75. This implies that acetate ion is not catalysing the rate determining step of the reaction. Once again, this result is at variance with the results obtained for nitrosation of 2-naphthol where pronounced acetate catalysis was observed[49].

Table 1: Variation of k_2 with pH for the nitrosation of 4-methylphenol in aqueous acetate buffers at 38°

[4-methylphenol] = 10^{-4}M; ($NaNO_2$) = 10^{-2}M; [AcO^-] = 0.15 M

μ = ca. 0.15 M

Run	pH[a]	[AcOH] /M	10^5[HONO][b] /M	$10^6\overline{k}_1$ /s^{-1}	$10k_2$ /l $mol^{-1}s^{-1}$
139	4.06	0.62	65.97	258	3.91
146	4.21	0.50	47.62	230	4.83
158	4.21	0.50	47.62	241	5.06
140	4.28	0.39	40.82	162	3.97
147	4.40	0.31	31.28	136	4.35
178	4.45	0.25	27.97	121	4.33
141	4.47	0.25	26.74	103	3.85
145	4.60	0.20	19.96	81.8	4.10
159	4.63	0.20	18.66	70.8	3.79
152	4.79	0.13	12.98	48.0	3.70
142	4.95	0.080	9.017	22.2	2.46
153	5.08	0.064	6.700	17.2	2.57
154	5.19	0.051	5.208	16.8	3.23
155	5.28	0.042	4.238	8.47	2.00
164	5.47	0.023	2.740	4.31	1.57

a. Mean value of measured initial and final values.

b. Calculated from ($NaNO_2$) using pK_{HONO} 2.909 at 35°, μ = 0.15 [63].

Figure 1: Variation of log k_2 with pH for the nitrosation of 4-methylphenol.

Conditions as in Table 1

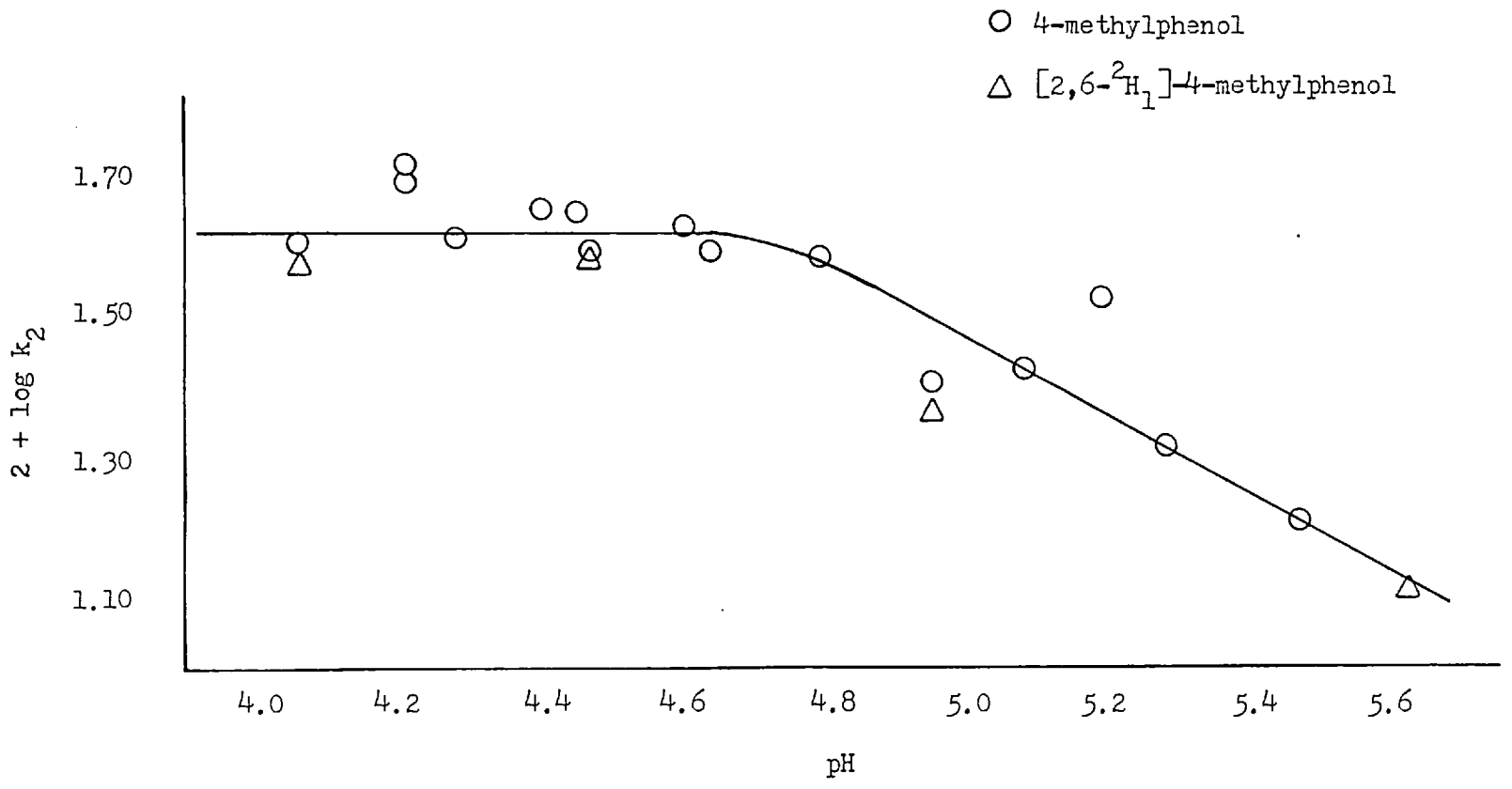


Table 2: Acetate dependence of k_2 for the nitrosation of 4-methylphenol in aqueous acetate buffers at 38°

[4-methylphenol] = 10^{-4}M; ($NaNO_2$) = 10^{-2}M; μ = ca.[AcO^-]

Run	pH	[AcO^-] /M	10^4[HONO][b] /M	$10^4 \bar{k}_1$ /s^{-1}	$10k_2$ /l $mol^{-1}s^{-1}$
190	4.03	0.15	7.04	2.80	3.97
191	4.04	0.30	6.68	2.73	4.09
192	4.06	0.45	6.50	3.24	4.98
193[a]	3.99	0.15	7.55	3.43	4.54
178	4.45	0.15	2.80	1.21	4.32
179	4.46	0.23	2.66	1.19	4.47
180	4.46	0.30	2.65	1.25	4.72
181	4.46	0.45	2.69	1.30	4.83
186	4.74	0.15	1.45	0.527	3.63
187	4.74	0.23	1.42	0.586	4.13
188	4.75	0.30	1.38	0.542	3.93
189	4.77	0.45	1.34	0.502	3.75

a. [$NaClO_4$] = 0.30 M added.

b. Calculated taking $\mu = \{[AcO^-] + [ClO_4^-]\}$ and the resulting pK_{HONO} at 35°, see reference 63, i.e.,

μ	$pK^{35°}_{HONO}$
0.15	2.909
0.23	2.897
0.30	2.895
0.45	2.902

2.1.3: Hydrogen Isotope Effect:

The reaction was performed with deuteriated substrate from pH 4 to 5.6. Results are given in Table 3 and are shown on Figure 1. The k_2^H/k_2^D value of unity over the whole acidity range provides evidence that the rate determining step, as detected by this experimental method, does not involve removal of a proton from the substrate. This conflicts with the case of 2-naphthol[49].

Table 3: Kinetic hydrogen isotope effect for nitrosation of [2,6-$^2H_1$]-4-methylphenol at 38°. k_2 in 1 $mol^{-1}s^{-1}$ throughout.

[substrate] = 10^{-4}; ($NaNO_2$) = 10^{-2}M; [AcO^-] = 0.15 M

Run	pH	$10k_2^H$ [a]	$10k_2^D$ [b]	k_2^H / k_2^D
194	4.07	3.91	3.67	1.07
195	4.48	3.85	3.86	1.00
196	4.97	2.46	2.19	1.12
197	5.64	1.32[c]	1.34	0.99

a. From data of Table 1; value of k_2^H for run of most similar pH taken.

b. Measured k_2 value corrected to 100 % deuteriation using,

$$k_2^D = k_2 n / \{1 - k_2(1 - n)\} \qquad (2.4)$$

where k_2 = experimental value for substrate of ($100n$) % deuteriation.

c. Extrapolated value from Figure 1.

2.1.4: Summary:

The disparities between the above results and those for the nitrosation of 2-naphthol suggested that the experimental method was not measuring the rate of the nitrosation reaction but, possibly, that of the subsequent oxidation of the nitroso- compound to the nitro- compound. Therefore, the reaction was re-investigated *via* a different procedure, *viz.* measurement of residual nitrite concentration during the reaction. This was the method by which the results for 2-naphthol were obtained.

2.2: Residual Nitrite Method; Excess 4-methylphenol:

Nitrosation of 4-methylphenol in dilute perchloric acid and aqueous acetate buffers of pH 1 to 6 occurred at the 2-position, *i.e.* *ortho* to the hydroxyl group, giving 2-nitroso-4-methylphenol and its oxidation product, 2-nitro-4-methylphenol.

The rate of loss of nitrite in the presence of excess substrate was shown to follow equation (2.5), where $\bar{k}_1$

$$\text{Rate} = -\frac{d(NaNO_2)}{dt} = \bar{k}_1(NaNO_2) \qquad (2.5)$$

has a first order dependence upon [4-methylphenol] as shown by the data of Table 4.

The overall rate equation was given by equation (2.6):

$$\text{Rate} = -\frac{d(NaNO_2)}{dt} = k_2[\text{4-methylphenol}]\,[HONO] \qquad (2.6)$$

where,

$$k_2 = \bar{k}_1(NaNO_2) \,/\, [\text{4-methylphenol}]\,[HONO]$$

The first order term in [HONO] was verified by the data of Table 5.

Table 4: Kinetic order in substrate for the nitrosation of 4-methylphenol in dilute perchloric acid and acetate buffers at 25°; first order dependence of $\bar{k}_1$ upon [substrate]

$(NaNO_2) = 10^{-4}$M; μ = 0.4 M

Run	pH	10^2[Sub] /M	10^5[HONO] /M	[AcO$^-$] /M	$10^5\,\bar{k}_1$ /s^{-1}	$10^2 k_2$ /l mol^{-1} s^{-1}
381	2.90	3.71	5.17	-	9.86	5.14
382	2.87	1.85	5.35	-	5.08	5.13
384	2.92	7.41	2.53	-	20.0	5.34
385	2.90	7.41	9.65	-	18.9	5.27
247	3.80	1.90	1.19	0.36	2.80	1.24
251	3.83	3.80	1.12	0.32	5.68	1.34
			10^7[HONO]			
275	4.96	7.56	9.25	0.30	9.46	1.35
383	4.97	15.1	9.04	0.30	17.2	1.28

Table 5: Kinetic order in nitrous acid for the nitrosation of 4-methylphenol in dilute perchloric acid and acetate buffers at 25°.

Run	pH	10^2[Sub] /M	10^5[HONO] /M	[AcO$^-$] /M	$10^5\,\bar{k}_1$ /s^{-1}	$10^2 k_2$ /l. mol^{-1}s^{-1}
384	2.92	7.41	2.53	-	20.0	5.34
381	2.90	3.71	5.17	-	9.86	5.14
293	3.06	7.56	4.26	-	21.9	6.79
385	2.90	7.41	9.65	-	18.9	5.27
			10^7[HONO]			
390	5.47	7.42	2.88	0.30	1.60	7.48
392	5.47	7.42	28.8	0.30	1.49	6.96

2.2.1: Acidity Dependence of k_{H_2O}:

From data derived from runs at constant pH and varying [AcO$^-$], to be presented later, values of k_2 in the absence of acetate ion, i.e. when water is the only base present, were determined. These values are referred to by k_{H_2O}. The data of Table 6 shows that k_{H_2O} was independent of acidity for pH < ca. 4.5 but acidity dependent for pH > 4.5. The data is represented by Figure 2. The slope of the pH dependent section of Figure 2 (calculated by a linear least squares procedure) is -0.92 for data for pH ≥ 5.17 and -1.09 for data for pH ≥ 5.50. The slope may be taken to be unity signifying a first order dependence upon [H_3O^+] in accord with the 2-naphthol results[49].

Table 6: Variation of k_{H_2O} with acidity for the nitrosation of 4-methylphenol at 25°.

[4-methylphenol] = 7.56×10^{-2}M; ($NaNO_2$) = 10^{-4}M

Runs from which k_{H_2O} extrapolated	pH	$10^3 k_{H_2O}$ /l $mol^{-1} s^{-1}$	4 + log k_{H_2O}
226 - 229 [b,c]	1.30	6.60	1.82
291 - 292 [c]	2.00	5.63	1.75
385 [a,c]	2.90	5.31	1.73
293 - 294 [c]	3.06	6.97	1.84
250 - 262	3.80	7.13	1.85
	4.32	5.95	1.77
263 - 266	4.55	5.95	1.77
267 - 270	4.76	5.23	1.72
275 - 278	4.90	3.29	1.52
279 - 282	5.17	3.10	1.49
271 - 274	5.50	1.60	1.20
283 - 286	5.65	1.41	1.15
287 - 290	5.85	0.683	0.83

a. [4-methylphenol] = 7.41×10^{-2}M

b. [4-methylphenol] = 10^{-2}M

c. Runs performed in dilute perchloric acid

2.2.2: Hydrogen Isotope Effect:

Reactions were performed using deuteriated substrate, i.e. [2,6-$^2H_1$]-4-methylphenol, over the range of pH 1 to 5.5. Table 7 gives the $k^H_{H_2O} / k^D_{H_2O}$ values obtained. The data presented has been smoothed via plots of log k_{H_2O} vs. pH for both normal and deuteriated substrate.

Figure 2: Variation of log k_{H_2O} with pH for the nitrosation of 4-methylphenol at 25°

Conditions as in Table 6

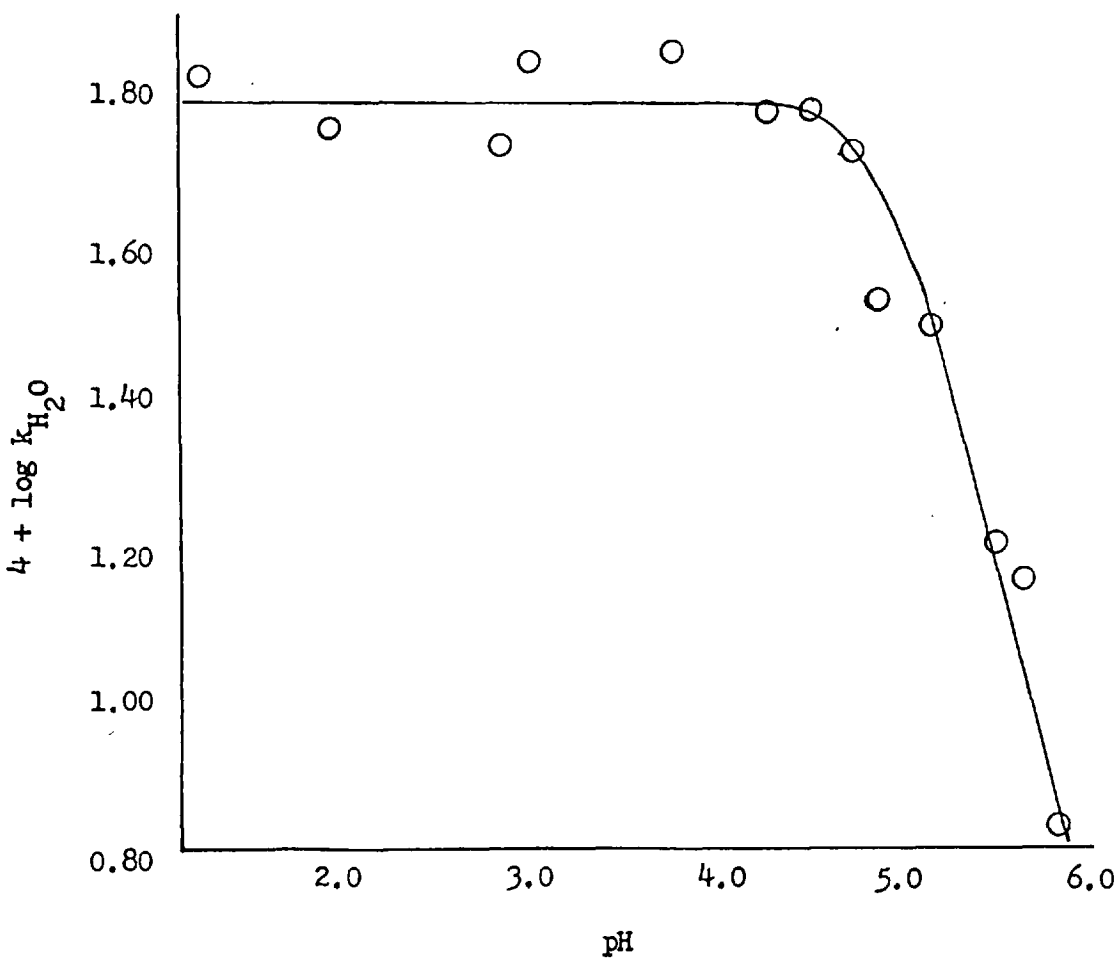


Table 7: Kinetic hydrogen isotope effect for the nitrosation of 4-methylphenol at 25°. Conditions as in Table 6.

Run	pH	$10^3 k^H_{H_2O}$ [b] /l mol^{-1}s^{-1}	$10^3 k^D_{H_2O}$ [b,c] /l mol^{-1}s^{-1}	k^H / k^D
315[a]	1.07			
307[a]	1.14	6.91	1.64	4.2
316[a]	1.97			
308[a]	2.03	6.91	1.64	4.2
317[a]	3.02	6.91	1.64	4.2
318, 322, 323	4.74	5.01	1.45	3.5
309, 313, 314	4.77	4.84	1.45	3.3
310, 311, 312	5.47	1.60	1.21	1.3

a. In dilute $HClO_4$, other runs in acetate buffers.

b. Data smoothed *via* log k_{H_2O} *vs.* pH plots.

c. Measured k_{H_2O} value corrected to 100 % deuteriation using equation (2.4).

The ratio is seen to decrease from 4.2 at acidities where the rate constant is pH independent to 1.3 at pH 5.5, *i.e.* where the rate constant is pH dependent. It is evident that the reaction is subject to a primary hydrogen isotope effect. The variation of the k^H/k^D ratio with pH and its magnitude in the regions of acid independence and dependence

parallel the results obtained for 2-naphthol. The data of Table 7 indicate that the primary isotope effect diminishes with increasing pH. The conclusion is drawn that as the pH increases the breaking of the C-H bond in the 2-position is progressively less involved in the rate determining step.

2.2.3: Acetate Ion Catalysis:

The nitrosation of 4-methylphenol was catalysed by acetate ion over the pH range studied, i.e. pH 4.3 to 5.8. Variation of k_2 with $[AcO^-]$ at several pH values is shown in Figure 3. The plots show that the rate constant has a first order dependence upon $[AcO^-]$ and that there is an intercept value of the rate constant. This intercept value is k_{H_2O} as described in Section 2.2.1. The rate constant k_2 is now described by equation (2.7):

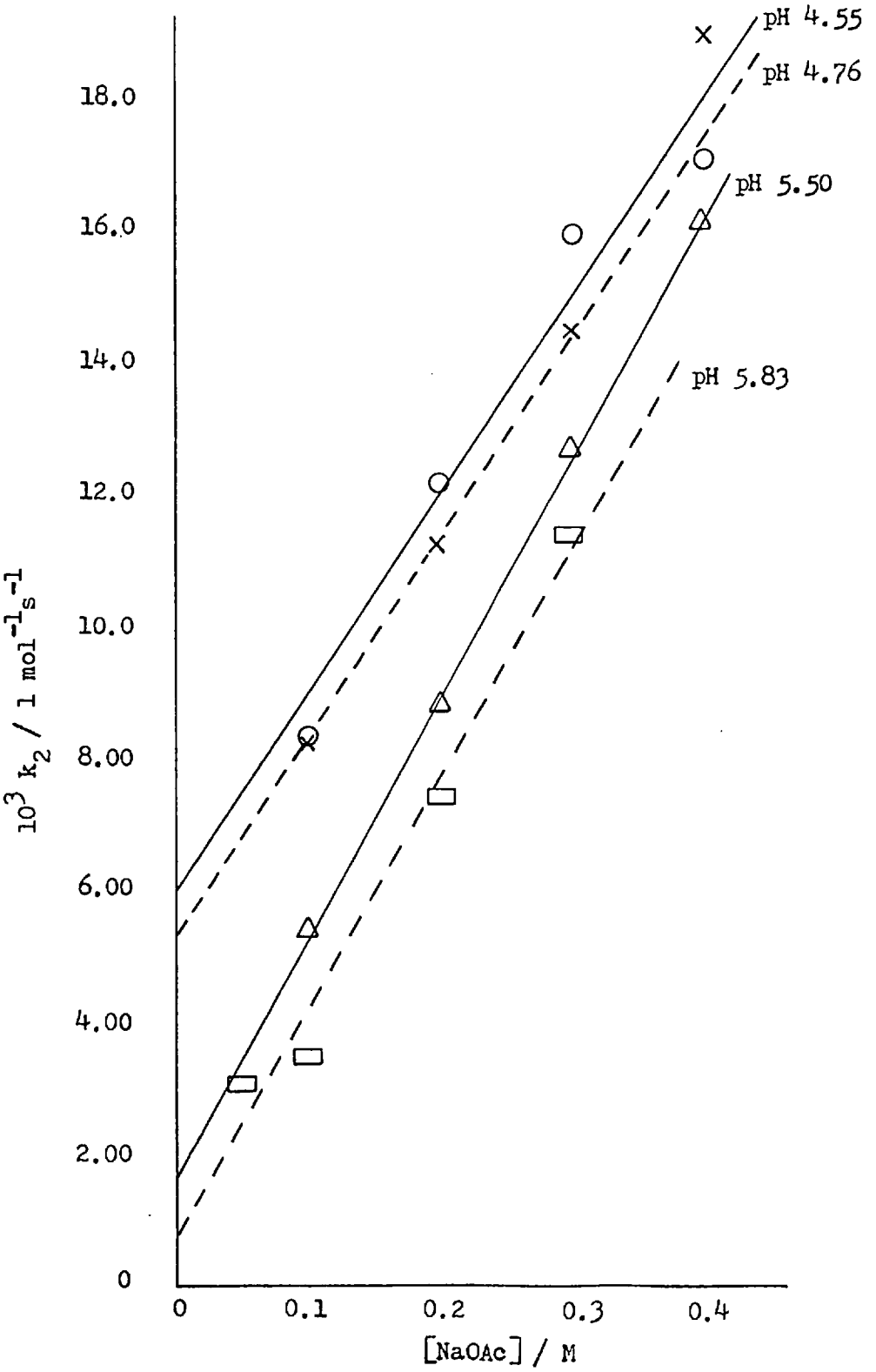
$$k_2 = k_{H_2O} + k_A[AcO^-] \qquad (2.7)$$

The catalytic rate constant, k_A, is given by the slopes of the lines on Figure 3. Table 8 presents the values of k_A at various acidities. It is seen that k_A remains essentially constant whereas k_{H_2O} decreases considerably over the pH range studied. This is a novel obsevation since previous work with similar substrates showed that k_A decreased with increasing pH [49].

When $[2,6\text{-}^2H_1]$-4-methylphenol was used as substrate at pH 5.5 k_A was reduced to 9.5×10^{-3} $l^2mol^{-2}s^{-1}$. This gave $k_A^H/k_A^D = 3.4$ at pH 5.5 suggesting that, for the reaction involving acetate ion, the breaking of the C-H bond

Figure 2a: Variation of k_2 with acetate ion concentration for the nitrosation of 4-methylphenol at 25°.

[4-methylphenol] = 7.56×10^{-2}M; ($NaNO_2$) = 10^{-4}M



in the 2-position was involved in the rate determining step at this pH, unlike the reaction in the absence of acetate ions (Section 2.2.2).

Table 8: Catalytic rate constants [equation (2.7)] for the nitrosation of 4-methylphenol at 25°.

pH	$10^3 k_{H_2O}$ /l mol^{-1}s^{-1}	$10^2 k_A$ /l^{2}mol^{-2}s^{-1}
4.32	5.95	2.98
4.55	5.95	2.99
4.76	5.23	3.08
4.90	3.29	3.43
5.17	3.10	3.26
5.50	1.60	3.65
5.65	1.41	2.56
5.85	0.683	3.48
		mean = 3.18

2.2.4: Effect of Chloride and Perchlorate Ions:

The data of Table 9 show that the addition of chloride and perchlorate ions to reaction mixtures did not affect the magnitude of k_{H_2O}. The nitrosation thus appears to be subject to base catalysis, e.g. H_2O and AcO^-, but not nucleophilic catalysis, e.g. Cl^-, and to be independent of ionic strength.

Table 9: Effect of Chloride and Perchlorate Ions for the nitrosation of 4-methylphenol in dilute perchloric acid and aqueous acetate buffers at 25°.

Run	pH	10^5[HONO] /M	[AcO^-] /M	[ClO_4^-] /M	[Cl^-] /M	$10^3 k_{H_2O}$ /l $mol^{-1} s^{-1}$
386 [a]	1.13	9.84	-	0.30	-	4.46
387 [a]	0.93	9.90	-	-	0.30	4.31
293 [b]	3.06	4.26	-	0.40	-	6.79
296 [b]	3 10	4.03	-	-	0.40	6.68
301 [b]	5.50	2.68	0.40	-	-	12.7
302 [b]	5.44	3.45	0.40	0.40	-	10.8
303 [b]	5.41	3.70	0.40	-	0.40	11.7

a. [4-methylphenol] = 7.42×10^{-2}M

b. [4-methylphenol] = 7.56×10^{-2}M

2.2.5: Effect of Nitrite Concentration, Light and Oxygen:

Addition of copper(II) ions to reaction mixtures produced a pink/mauve colouration due to 2-nitroso-4-methylphenol copper(II) chloride. Comparison with authentic material by spectrophotometry gave the results of Table 10. It appears that the amount of 2-nitroso-4-methylphenol remaining in solution was dependent upon the nitrite concentration, the yield being halved on raising (NO_2^-) above 10^{-4}M. This suggested that NO_2^- or HONO was involved in the oxidation of the 2-nitroso product to its nitro counterpoint, as mentioned in Section 2.1.

The effects of light and oxygen upon the system were examined briefly but the rates of reaction (measured *via* residual (NO_2^-) remained essentially unchanged, see Table 11.

Table 11: Effect of light and oxygen upon the nitrosation of of 4-methylphenol at pH *ca.* 5.5 and 25°.

$[AcO^-]$ = 0.30 M

Run	pH	$10^7[HONO]$ /M	$10^6 \bar{k}_1$ s^{-1}	$10^3 k_2$ /l $mol^{-1}s^{-1}$	Condition
390	5.47	2.88	1.60	7.48	light
391	5.47	2.88	1.55	7.27	dark
396	5.48	2.81	1.58	7.59	oxygenated
397	5.48	2.81	1.73	8.32	deoxygenated

Table 10: Amount of nitrosated product for the nitrosation of 4-methylphenol in aqueous acetate buffers at pH ca. 5.5 and 25°. Determined as 2-nitroso-4-methylphenol copper(II) chloride.

Run	pH	$10^4(NaNO_2)$ /M	$[AcO^-]$ /M	Reaction time /minutes	No. of $t_{\frac{1}{2}}$	$10^4(NO_2^-)$ consumed [a] /M	10^5[complex] /M	% nitrosated product
378	5.50	2	0.20	14534	1.84	1.46	2.20	15
379	5.47	2	0.30	14693	1.36	1.34	2.01	15
392	5.47	10	0.30	8663	1.11	5.5	7.67	14
390	5.47	1	0.30	8485	1.17	0.58	1.76	30
391 [b]	5.47	1	0.30	8515	1.14	0.58	1.83	32

a. Determined by Shinn's method.

b. Light excluded from reaction.

2.2.6: Validity of Results from the Two Experimental Methods:

Both of the methods by which the nitrosation of 4-methylphenol was investigated gave some unexpected results. In the case of the spectrophotometric method, the half order dependence upon $[H_3O^+]$ at high pH and the absence of both acetate ion catalysis and a kinetic hydrogen isotope effect suggested that the results were not related to the rate determining step of the nitrosation reaction. In the case of the residual nitrite method, the unusual features were the constancy of the acetate ion catalytic constant at acidities where the 'water' rate was both pH independent and dependent and the absence of chloride ion catalysis.

Despite the unusual features of the latter results it was felt that they reflected the pertinent aspects of the nitrosation reaction more correctly, viz. the rate determining process, for the reasons given in Section 2.1.4. Further discussion refers to results obtained by the residual nitrite method.

2.3: Consideration of the catalytic cofficients:

Study of the nitrosation of phenol and 2-naphthol in aqueous acetate buffers showed that the bases present in the system, i.e. acetate ions and water, exhibited general base catalysis. Application of the Brønsted relationship[64], defined by equations (2.8) and (2.9), to the data obtained for these two bases gave $\beta = 0.37$ and $\beta = 0.27$ for phenol at pH 4.0 and

2-naphthol at pH 2.91, respectively. These pH values are on the pH independent part of the rate constant vs. pH profile in both cases.

$$k_B = G_B K_B^{\beta} \qquad (2.8)$$

$$\log k_B = -\beta pK_B + \log G_B \qquad (2.9)$$

For nitrosation of 4-methylphenol under similar conditions at pH 4.3 (on the pH independent part of the pH profile) catalytic constants for the bases water and acetate ion are, $k_w = 1.07 \times 10^{-4}\ l^2 mol^{-2} s^{-1}$ and $k_A = 2.98 \times 10^{-2}\ l^2 mol^{-2} s^{-1}$, where $k_w = k_{H_2O}/55.6$. The values for k_{H_2O} and k_A are taken from Table 8. The base pK_B values for water and acetate ion are taken to be 15.75 and 9.25, respectively, as in the previous study[48]. Application of equation (2.9) gives $\beta = 1.2$. This value lies outside the generally accepted upper limit for β of unity for a general base catalysed rate determining step.

The Brønsted exponent for this reaction therefore suggests that acetate ion and water are catalysing the reaction in different ways. This observation may be compared with the finding reported in Section 2.2.3 concerning the participation of acetate ion and water in the removal of the 2-position proton during the rate determining step of the reaction.

2.4: Rationalisation of the Experimental Results:

Only the results obtained by the residual nitrite method (Section 2.2) will be considered.

4-methylphenol showed different kinetic features to 2-naphthol despite the apparent similarity of the substrate structures. The experimental results are rationalised best in terms of Scheme 2.1, where k_b is rate determining for water as the base at pH < 4.6, k_b' is rate determining for acetate throughout and k_a is rate determining for water at pH > 4.6. The rate constants, k_{H_2O} and k_A, for water and acetate catalysis, respectively, determined as described in Section 2.2.3, are given by rate equations (2.10) and (2.11).

$$k_{H_2O} = \frac{k_a k_b K_e [H_2O][H_3O^+]}{k_{-a}[H_3O^+] + k_b[H_2O]} \qquad (2.10)$$

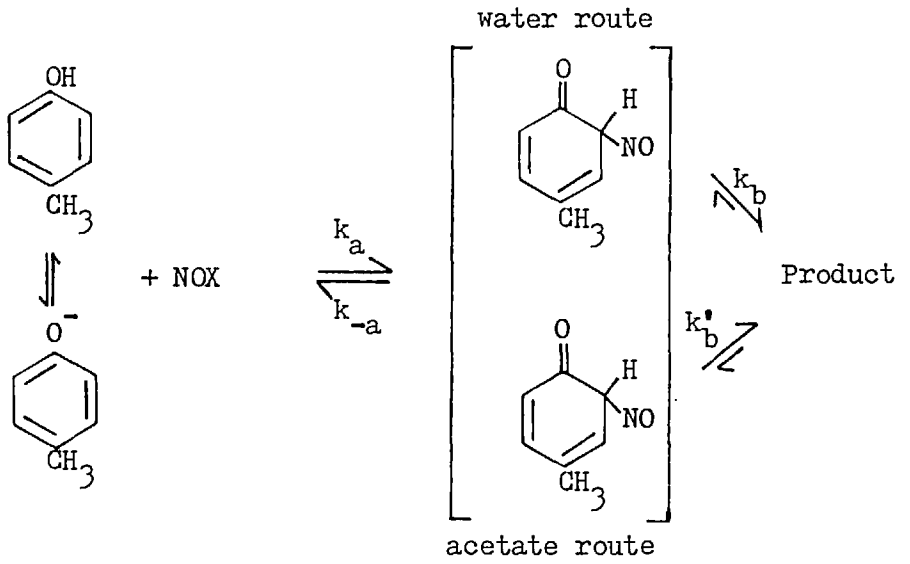
$$k_A = \frac{k_a k_b' K_e [AcO^-][H_3O^+]}{k_{-a}[H_3O^+] + k_b'[AcO^-]} \qquad (2.11)$$

where, $K_e = [H_2O^+NO] \,/\, [HONO][H_3O^+]$

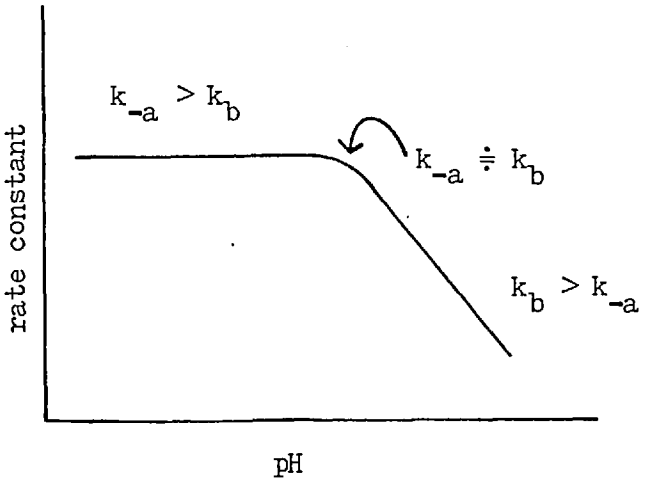
The key factor in determining the acidity dependence is the relative values of the k_{-a}/k_b and k_{-a}/k_b' ratios. For the water reaction, k_{-a}/k_b can be deduced from the turn-over point on the pH profile, see Scheme 2.2.

(i) For 2-naphthol, the turn-over occurs at pH ca. 2.5.

Hence, if $[H_2O]$ = 55.6 M, then at pH 2.5,



Scheme 2.1: Rate determining steps for the nitrosation of 4-methylphenol



Scheme 2.2: Relationship of rate constants to the pH profile

$$k_{-a}[H_3O^+] \doteqdot k_b[H_2O]$$

$$k_{-a} \times 10^{-2.5} \doteqdot k_b \times 55.6$$

$$k_{-a}/k_b \doteqdot 55.6 \times 10^{2.5}$$

$$\doteqdot 1.75 \times 10^4$$

(ii) For 4-methylphenol, the turn-over occurs at pH ca. 4.6.

Hence,

$$k_{-a}/k_b \doteqdot 55.6 \times 10^{4.6}$$

$$\doteqdot 2.2 \times 10^6$$

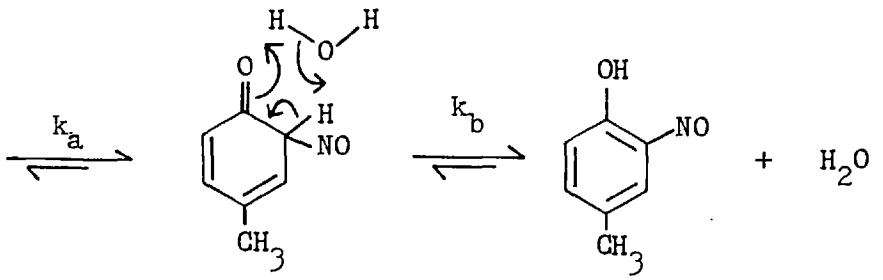
This shows, unambiguously, that the intermediate for 4-methylphenol is ca. 100 times more likely to return to reactants than is the intermediate for 2-naphthol.

For the acetate reaction for 4-methylphenol, at pH ca. 6 the reaction is still pH independent. This implies that $k_{-a}[H_3O^+] > k_b'[AcO^-]$ at pH 6. Thus, for $[AcO^-] = 0.1$ M,

$$k_{-a}/\ k_b > 0.1 \times 10^6$$

$$> 1 \times 10^5$$

This shows that although acetate is a stronger base than water the reaction intermediate is still only 10 times (maximum) more likely to partition to products than it was with water as base. Thus, it is necessary to suggest that water effects removal of the proton from the intermediate via a special mechanism enabling it to be particularly effective. It is proposed that water acts simultaneously as an acid and a base, removing the proton from the intermediate but also furnishing a proton to the oxygen atom of the intermediate, see Scheme 2.3.

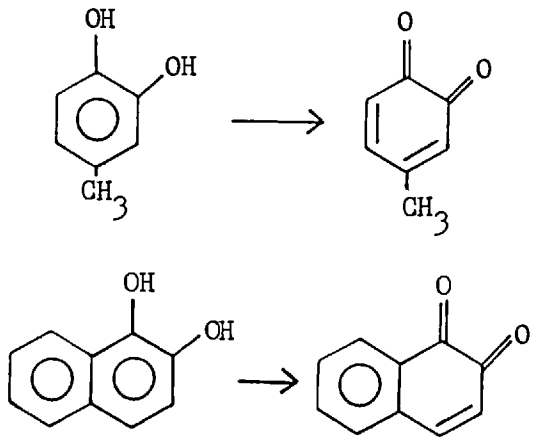


Scheme 2.3: Special mechanism proposed for water catalysis

It was shown above that the k_{-a}/k_b ratio was 100 times greater for 4-methylphenol than 2-naphthol. This implies that either k_{-a}(naphthol) < k_{-a}(4-methylphenol) or k_b(naphthol) > k_b(4-methylphenol) or both inequalities exist. There is some indication that both of these inequalities may indeed exist:

(i) Naphthol has pK_a 9.5, 4-methylphenol has pK_a 10.2. This means that naphthol is a weaker base than 4-methylphenol, which may imply that the intermediate from 4-methylphenol will be more basic than the intermediate from 2-naphthol . Hence one might expect k_{-a}(naphthol) < k_{-a}(4-methyphenol).

(ii) The initially formed nitrosophenols may exist as the oximino tautomers or the quinone monoximes during the intermediate stage of the reaction. The quinone monoximes would probably have similar stabilities to the related 1,2-quinones. An indication of the relative stabilities of the 1,2-quinones is given by the standard electrode potentials (E_o) for the oxidation of the 1,2-dihydroxy analogues. These potentials are given in Scheme 2.4.



E_o (V)

0.78

0.56

Scheme 2.4: Standard oxidation electrode potentials

The standard potentials indicate that 1,2-naphthoquinone is more stable than 4-methyl-1,2-benzoquinone. Hence, one might expect k_b(naphthol) > k_b(4-methylphenol).

CHAPTER 3

RESULTS AND DISCUSSION FOR THE NITROSATION OF DL-TYROSINE, N-ACETYL-L-TYROSINE AND URINE

3.1: Nitrosation of DL-tyrosine:

Via the determination of residual nitrite, reaction rate constants were obtained for reaction solutions with $[HClO_4]$ = 0.05 M to 8 M, as given in Table 12. The data were discontinuous but appeared to indicate that the overall, stoichiometric rate constant, $\bar{k}_2$, was independent of acidity for $[H_3O^+] > 0.2$ M. $\bar{k}_2$ increased rapidly to a maximum at $[H_3O^+]$ = ca. 7 M (note the temperature change). This dependence was similar to that reported for 4-methylphenol[51]. The rate was shown to be first order in substrate by the results of Table 13. Confirmation of

Table 12: Variation of $\bar{k}_2$ with acidity for the nitrosation of DL-tyrosine in perchloric acid at 25°/0°.

$[\text{tyrosine}] = 10^{-2}$M; $(NaNO_2) = 10^{-4}$M

$[HClO_4]$ / M	$10^3 \bar{k}_2$ / l mol^{-1}s^{-1}	Temperature/°C
0.05	1.10	25
0.1	0.93	25
0.2	0.90	25
2.1	2.91	25
	2.93	25
7.0	42.2	0
	34.7	0
8.0	13.9	0

a first order dependence upon $(NaNO_2)$ was given by the results of Table 14.

Table 13: Kinetic order in substrate for the nitrosation of DL-tyrosine in perchloric acid at 25°.

$[HClO_4] = 10^{-1}M$; $(NaNO_2) = 10^{-4}M$

10^3[tyrosine] / M	$10^5 \bar{k}_1/s^{-1}$	$10^3 \bar{k}_2/l\ mol^{-1}s^{-1}$
5	0.45	0.90
10	0.93	0.93
20	1.67	0.84

Table 14: Kinetic order in nitrite for the nitrosation of DL-tyrosine in perchloric acid at 25°.

[Substrate] = 2 x $10^{-2}M$; $[HClO_4] = 10^{-1}M$

$10^4(NaNO_2)/M$	$10^3 \bar{k}_1/s^{-1}$
1	1.00
2	0.96
4	0.93

These results may be compared with those reported by Cantoni[34], obtained by the same experimental method. Results are summarised in Table 15. It is evident that there is a large difference in the observed rates of reaction, as indicated by the half-lives. The results in Tables 13 and 14 suggest that this work is reliable and perhaps casts doubt upon the accuracy of the earlier work. However, the earlier results were from reactions performed in hydrochloric acid whereas this work employed perchloric acid. Chloride ion catalysis may explain

Table 15: Comparison of results for the nitrosation of Tyrosine

Source	$10^5(NaNO_2)$/M	10^2[tyrosine]/M	$t_{\frac{1}{2}}$/min
Cantoni[34]	8.5	5.13	ca. 40 (20°)
This work	10.0	2.0	ca. 693 (25°)
Projected from this work	10.0	5	ca. 277 (25°)

the rapidity of the reported reactions, assuming that this applies for tyrosine nitrosation, as it does for 4-methylphenol nitrosation (Section 2.2.4).

Single determinations of $\bar{k}_1$ and $\bar{k}_2$ at 25° and 38° *via* complexation of the nitrosated product with Cu^{2+} and monitoring spectrophotometrically, gave the results of Table 16.

Table 16: Rate constants for the nitrosation of DL-tyrosine at pH 4.6 in the presence of copper (II) ions at 25° and 38°.

$[\text{tyrosine}] = 5.53 \times 10^{-3}$M; $(NaNO_2) = 6.25 \times 10^{-2}$M;
$[AcO^-] = 0.25$ M; $(CuSO_4) = 6.08 \times 10^{-3}$M

Temperature/°C	$10^6 \bar{k}_1/s^{-1}$	$10^3 \bar{k}_2/1\ mol^{-1}s^{-1}$
25	3.4	1.4
38	3.2	1.3

The rate constants appear to show very little variation with temperature and are, therefore, probably inaccurate. However, $\bar{k}_2$ is of the same order of magnitude as was obtained by the residual nitrite method, see Table 13.

3.2: Nitrosation of N-acetyl-L-tyrosine:

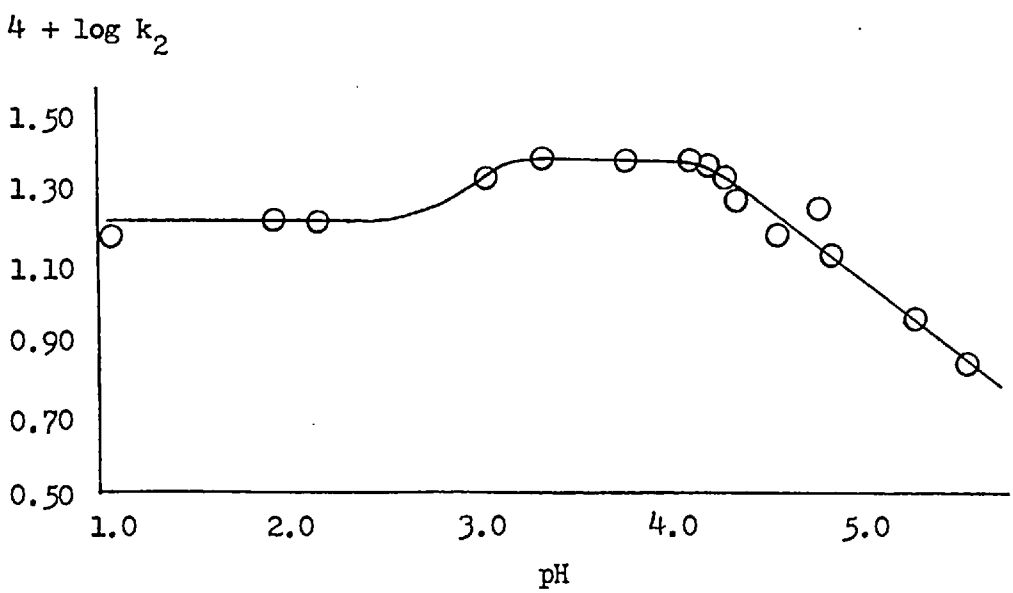
Via the determination of residual nitrite, the first order, stoichiometric rate constant, $\bar{k}_1$, was derived as

in Section 2.2. The overall second order rate constant was derived also, based upon molecular concentrations, k_2. It was assumed that the rate was first order in [substrate] and [HONO] as for 4-methylphenol nitrosation. The variation of log k_2 with pH was generally similar to that obtained for 4-methylphenol (Section 2.2.1). The results are presented as Figure 3. The rate constant was independent of pH for pH < 2.8 but rose to a second pH independent value for pH ca. 3.1 to 4.2. There was then a pH dependent region for pH ca. 4.2 to 5.6. It was found possible to maintain the pH of the reaction solutions simply by addition of sodium hydroxide, presumably due to ionisation of the substrate to form tyrosinate ions, which exerted a buffering effect. It is suggested that the rise in k_2 at pH ca. 3 resulted from tyrosinate ions acting as a base, either intramolecularly or intermolecularly, effecting proton removal from the nitrosated intermediate. The pH dependent region has a slope of -0.4. The discrepancy between this value and that of unity for 4-methylphenol (Section 2.2.1) may be attributed to k_2 being the composite rate of tyrosinate ion and water catalysis, whereas the 4-methylphenol plot used the rate constant isolated for water only acting as base.

It may be concluded that the interactions of nitrite with N-acetyl-L-tyrosine and 4-methylphenol are reasonably similar kinetically and, hence, probably similar mechanistically.

Figure 3: Variation of log k_2 with pH for the nitrosation of N-acetyl-L-tyrosine at 25°

[Substrate] = 8 x 10^{-2}M; μ = 0.4 M; ($NaNO_2$) = 10^{-4}M



3.3: Nitrosation of Urine:

Reaction of nitrite with human urine proceeded very rapidly at pH 5.24 (the 'natural' pH) and 25°. The reaction was followed *via* determination of residual nitrite. After 3 minutes only 16 % of the initial ($NaNO_2$) remained. A sample of the same urine appeared to have an inherent nitrite concentration = 8 % of the added initial nitrite. After *ca.* 200 minutes the reaction solution showed 14 % of the initial ($NaNO_2$) remaining whilst the urine sample showed 6 %.

The rapid consumption of *ca.* 84 % of the added nitrite thus appeared to be followed by a very slow (relatively) loss of further nitrite.

The positive reaction of the urine sample to the analytical method (Shinn's Method) suggested that either ther were nitrosating species in the urine which were capable of diazotising the sulphanilamide or there were diazotised species capable of coupling with the NED to give a product absorbing at 541 nm, see Section 7.1.1a.

The results suggest that phenolic material is unlikely to compete successfully with other, more reactive, compounds present in the urine, *e.g.* urea. With [4-methylphenol] = 7.56×10^{-2} M and [AcO^-] = 0.3 M at pH 5.24 and 25°, only 8 % of the initial ($NaNO_2$) had been consumed after 260 minutes compared with *ca.* 90 % of the same initial ($NaNO_2$) reacting with urine under similar conditions.

CHAPTER 4

RELATION OF THE EXPERIMENTAL RESULTS TO THE NITROSATION OF SECONDARY AMINES

4: Relation of the Experimental Results to the Nitrosation of Secondary Amines:

This work was initiated to assess the relative importance of the nitrosation of phenolic materials and secondary amines present in the diet[33]. Tyrosine residues are present in animal and milk proteins. 4-methylphenol was considered to be a suitable model compound for tyrosine.

Phenol was shown to react ca. 10^4 times more rapidly than dimethylamine with nitrous acid at pH 1.5 and 25°. It was anticipated that this reactivity ratio would persist up to pH 5 [33]. Table 17 gives rates for phenol, 4-methylphenol and N-acetyl-L-tyrosine at pH 4.6 and 25°. The differences in

Table 17: Rate constants for the nitrosation of phenols at pH 4.6 and 25°

Substrate	$10^2 k_{H_2O}$/1 mol^{-1}s^{-1}	$10^2 k_2$/1 mol^{-1}s^{-1}
Phenol	0.84	1.35 [a]
4-methylphenol	0 59	1.04 [a]
N-acetyl-L-tyrosine	0.19 [b]	0.19 [c]

a. $[AcO^-]$ = 0.15 M

b. From smoothed data (Figure 3)

c. No acetate

reactivities of the 3 substrates are insignificant compared with the 10^4 times difference between phenol and dimethylamine. Tyrosine residues should, therefore, compete successfully with

secondary amines for available nitrite.

This conclusion concurs with that of a recent report[108] which showed that 4-methylphenol significantly reduced the rate of N-nitrosation of pyrrolidine in aqueous and model (soybean protein) systems of pH 5.25 at 37°. However, the report[38] from the same laboratory that nitrosophenols may catalyse the N-nitrosation of pyrrolidine illustrates that reaction conditions are critical and that extrapolation of *in vitro* results to *in vivo* situations is likely to be of limited utility.

CHAPTER 5

LITERATURE REVIEW OF ELECTROPHILIC SUBSTITUTION REACTIONS OF KETONES

5.1: Keto-enol Tautomerism:

Keto-enol tautomerism refers to the equilibrium which exists between a ketone(I) and its enol(II) as a result of a 1,3-proton shift. Tautomerism is therefore a prototropic equilibrium.

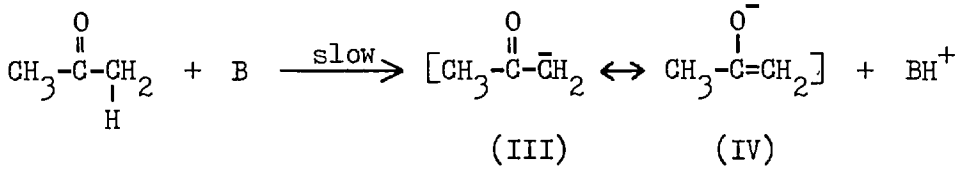
(I) (II)

R = H, alkyl or aryl

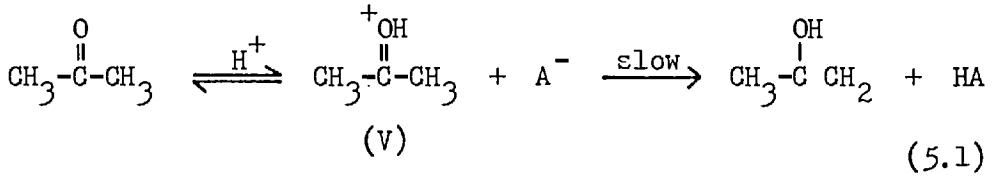
The phenomenon has been widely investigated and extensively reviewed, including accounts by Ingold[67] and more recently Buncel[68]. It is not intended to present a detailed chronology of the development of the ideas concerning keto-enol equilibria but to consider the more pertinent aspects of the relationship between rates of enolisation and rates of electrophilic substitution of ketones.

5.1.1: The mechanism of enolisation:

It has been shown that enolisation of a ketone can be effected by either general base or general acid catalysis. In general base catalysis an α-hydrogen, i.e. a hydrogen atom bonded to a carbon atom juxtaposed to the carbonyl function, is abstracted to give a delocalised anion with the canonical forms (III) and (IV), a carbanion and enolate ion, respectively. Rapid O-protonation of the anion ensues to give the enol tautomer.

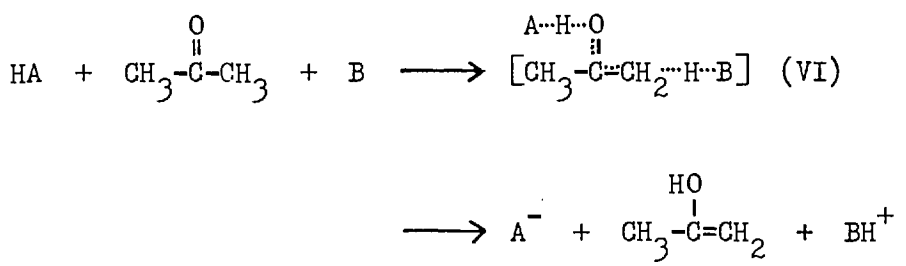


In general acid catalysis there is considered to be a pre-equilibrium O-protonation of the ketone to give the oxonium ion (V) which reacts with base to give the enol, equation (5.1).



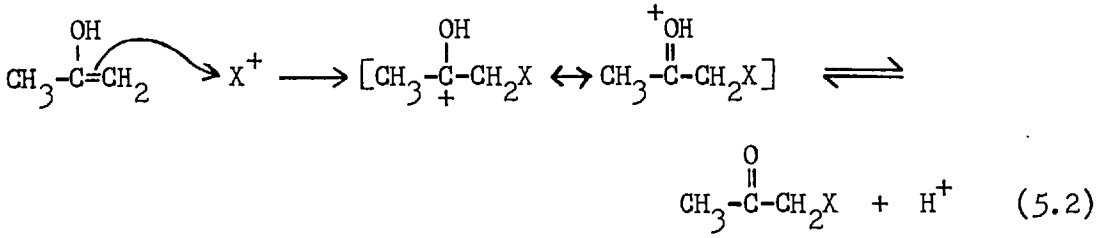
(5.1)

Another possible mechanism involves concerted action of acid and base leading to enol formation via an intermediate of type (VI). The requirement of a termolecular rate law has been fulfilled for up to 20 % of the extent of some enolisation reactions.



The discovery by Lapworth[69] that the rate of bromination of acetone in acidic aqueous solution was directly proportional to the acetone and acid concentrations but independent of the bromine concentration together with later findings[70] that the rate of iodination of acetone in basic aqueous solution was proportional to the acetone and base

concentrations but not to that of the iodine led to the conclusion that the halogenation was a fast process in both cases and that the enolisation of acetone was the rate determining step of the reaction. Howsoever the enol is formed, the subsequent halogenation, or more generally, electrophilic substitution, proceeds as for an alkene, equation (5.2).



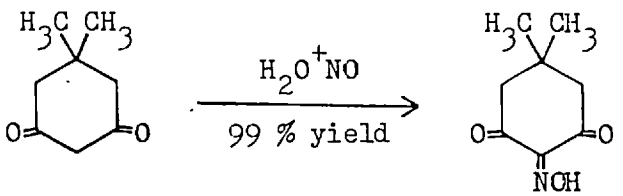
(5.2)

5.2: Hydrogen Isotopes as the Electrophile:

If the above deductions about the rate determining step of the reaction were correct then the rate of deuterium exchange with a deuteriated solvent should be equal to the rate of halogenation under similar conditions. Reitz[71] showed this to be true for the acid catalysed bromination and deuteriation of acetone. This has been confirmed with many other substrates although some exceptions have been found[72]. These have been explained in terms of increased reactivity towards the halogenating electrophile by the monohalogenated reaction product relative to the original substrate[73].

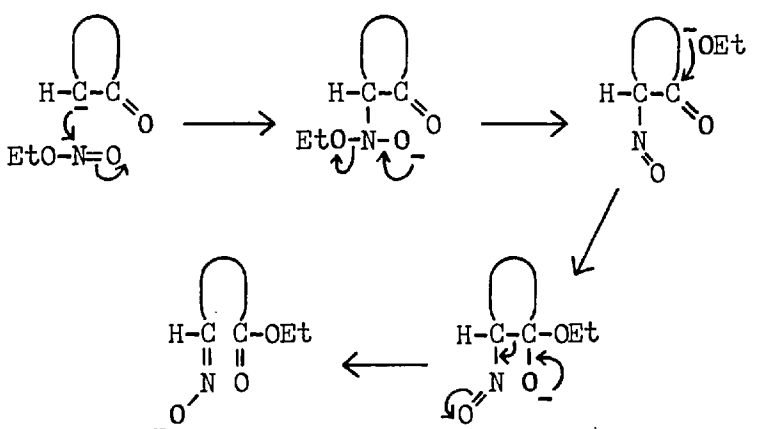
5.3: Nitrosating Agents as the Electrophile:

Early literature was reviewed by Touster[74]. The general feature of ketone nitrosation reported was that the substitution proceeded *via* the enol form of the ketone. β-diketones were reported to give good, occasionally excellent yields of the oximino derivative, equation (5.3). The oximino group is a tautomer of the nitroso group.



(5.3)

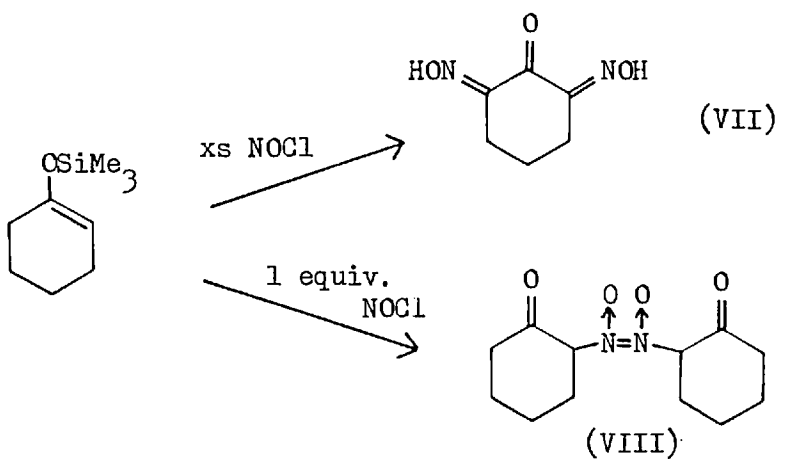
Woodward and Doering[75] proposed base catalysed nitrosation of cyclic ketones proceeding *via* the carbanion, equivalent to the enol pathway, followed by cleavage of the cyclic structure, equation (5.4).



(5.4)

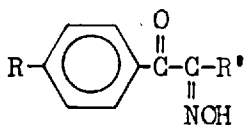
This type of reaction, nitrosation followed by carbon/carbon bond cleavage, is termed nitrosolysis. Nitrosolysis of cyclohexanone is particularly interesting as a synthetic route to nylon-6 polymer *via* ω-cyanovalerate esters[76]. Reaction is at -70° with nitrosyl chloride as the nitrosating agent, liquid sulphur dioxide as solvent and methanol or ethanol present as a nucleophile to trap the initial nitrosocarbonium ion (*cf.* equation (5.2) with X = nitroso). Depending upon the presence of nucleophile, catalysing acid or excess ketone many exotic products may result from this reaction[76,77].

Trimethylsilyl enol ethers, *e.g.* the enol ether of cyclohexanone, were nitrosated with NOCl in dichloromethane at -15° to give 2,6-bis-oximinocyclohexanone (VII) with excess NOCl and a mononitroso dimer (VIII) with one equivalent of NOCl[78].



Nitrosyl chloride has also been used in photochemical nitrosations of cycloalkanes[79,80] giving the cycloalkanone oximes as products. The reagents were the nitrosyl radical and the alkane and therefore the reaction did not involve a keto-enol equilibrium despite the nature of the product.

Cyclohexanone was nitrosated by methyl nitrite[81,82] in anhydrous ethyl ether containing hydrogen chloride. The earlier paper[81] reported 2,6-bis-oximinocyclohexanone as the product whilst the other paper[82] reported 2-oximinocyclohexanone in 62 % yield together with the 2,6-disubstituted product. A similar procedure, except for the absence of solvent, was used for the nitrosation of aceto- and propiophenones[83] giving products (IX) showing insertion of an oximino group into the acyl function of the substrate.

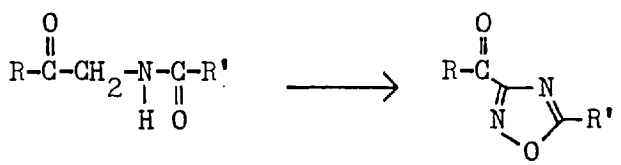


(IX)

R = H, Me, OMe, OEt

R' = H, Me

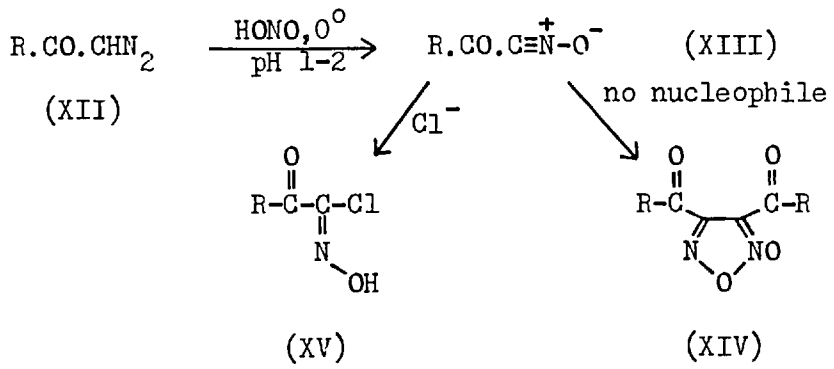
Reaction of N-acyl- and N-ethoxycarbonyl- α-amino-ketones (X) with sodium nitrite/acetic acid or isopropyl nitrite/hydrochloric acid gave heterocyclic products (XI), presumably via nitrosation (oximation) of the methylene group followed by intramolecular condensation[84].



(X) (XI)

The action of nitrous acid upon primary aliphatic diazocarbonyl compounds (XII) gave furoxans (XIV)[85]. The product was a dimer of the intermediate (XIII) and was only obtained when

nucleophiles were absent. If Cl^- was present, for example, then the hydroximoyl chloride (XV) resulted.



(XV) (XIV)

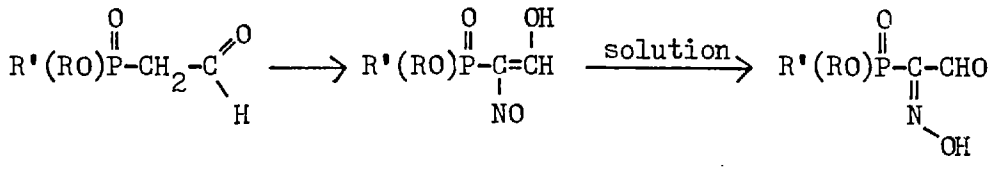
R = EtO, Me, Ph, p-MePh

Treatment of acetophenone (XVIa), acetofuranone (XVIb) and acetothiophenone(XVIc) with an inorganic nitrite in 4 M hydrochloric acid led to oxidation of the methyl group giving the keto-acid (XVII)[86]. Acetofuranone gave the product in 66 % yield in KNO_2 /4 M HCl at 66°. The reaction may be compared with that described above[83] performed under anhydrous conditions with methyl nitrite/HCl which gave substitution products (IX). The substitution product probably forms under the aqueous conditions also but the more vigorous reaction conditions cause hydrolysis to the acid.

Nitrosation of 2-butanone and 3-pentanone by $LiNO_2$ in EtOH/HCl [87] gave 2-oxo-3-oximinobutane (65 %) and 2-oximino-3-oxopentane (2-oximinopentan-3-one, 55 %), respectively, the oximino tautomers of the nitrosation products of the enol forms of the substrates.

Reaction of phosphorylated aldehydes (XVIII) with nitrous acid produced phosphorylated nitrosoenols (XIX) in moderate yield (31 - 57 %)[88]. The nitrosoenols were reported

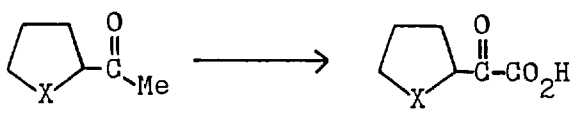
to exist as dimers and to rearrange in solution to the oximes (XX).



(XVIII) (XIX) (XX)

The stability of the nitrosoenol structure is unusual; the expected product is the nitroso tautomer of (XX).

.....



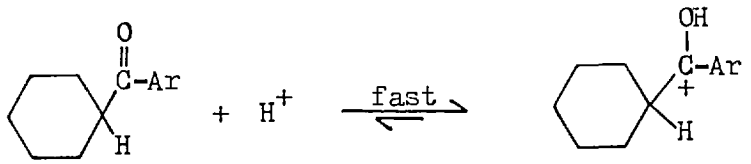
(XVI) (XVII)

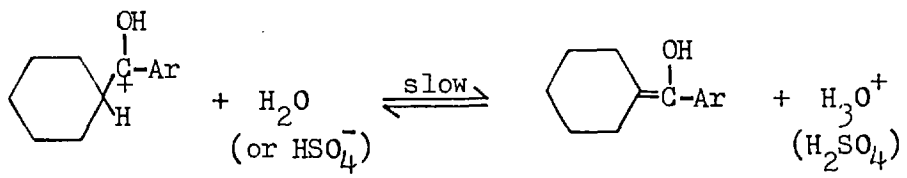
a, X = CH CH
b, X = O
c, X = S

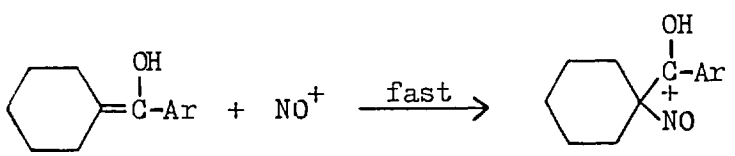
.....

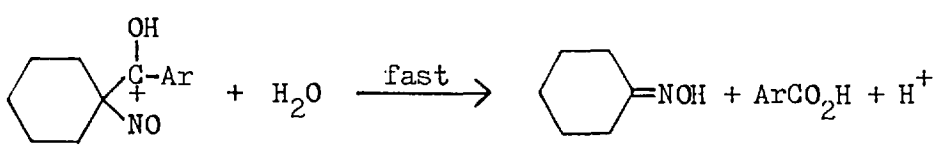
The foregoing discussion of ketone nitrosation has dealt only with 'non-kinetically' studied systems. Indeed, little work has been performed from a kinetic viewpoint. Ogata[89] studied the reaction of cyclohexyl aryl ketones with nitrous acid in sulphuric acid (84 - 95 %) at 60°. Under these conditions the nitrosating species would probably be the nitrosonium ion. Ogata concluded that the rate determining step of the reaction was deprotonation of the conjugate acid of the ketone to form the enol which reacted rapidly with the nitrosating species.

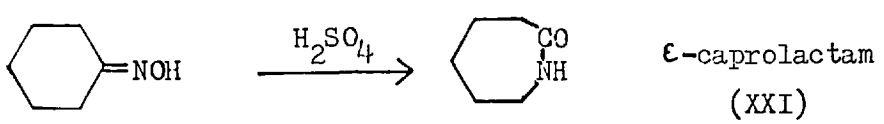
The final product was ε-caprolactam (XXI) formed *via* cyclohexanone oxime.











ε-caprolactam
(XXI)

Consideration of this reaction led Rogic[76] to suggest that "...the nitrosation of ketones...is a special case of electrophilic addition to a carbon-carbon double bond" (sic). The suggestion that ketone nitrosation is a 'special' case as a result of Ogata's work is curious since the mechanism proposed by Ogata, i.e. rate limiting enolisation followed by addition of the electrophile, is that which is generally accepted, cf. equation (5.2).

However, earlier work by Singer[90] gave results which did suggest that ketone nitrosation is a 'special' case of electrophilic substitution. Study of the nitrosation of aliphatic ketones, e.g. acetone and ethyl methyl ketone, by nitrous acid in aqueous sulphuric or perchloric acid (ca. 0.1 - 3 M) at 25° gave the result that "... the rate of nitrosation is greater than the acid catalysed rate of enolisation under similar conditions" (sic). For example, acetone was found to nitrosate seven times faster than it enolised in mineral acid of $[H_3O^+] = 0.15$ M. It was proposed that nitrosation occurred at the carbonyl oxygen, the point of greatest electron density. The electron releasing effect of the ketone substituents was found to affect the nitrosation rate as required by this proposal, i.e. the greater the release of electrons by the substituent (+I inductive effect) the greater the rate of nitrosation. It was also stated that "..the rate determining step in the acid catalysed nitrosation of ketones does not appear to involve loss of a proton" (sic). This was based on the evidence that there was no catalysis by added sulphate ion and that a linear relationship existed between log $(k - k_o)$

and H_o (rather than log $[H_2SO_4]$), where the rate constant for the nitrosation was determined to be, $k = k_o + k_H [H^+]$, and H_o is the Hammett acidity function. Absence of sulphate ion catalysis was not too surprising since the ion is not a very effective base.

Although Rogic[76], unlike most workers in this field of study, chose not to ignore Singer's findings the significance of them appears to have eluded him. Bell[91], reporting results for the chlorination of acetone by chlorine in strong acids, noted that when hypochlorous acid was present in the reaction medium the results appeared to indicate the involvement of the keto form of acetone rather than the enol form. He attributed no great relevance to the observation, dismissing it by saying that the major part of the reaction involved enolisation followed by reaction with chlorine, i.e. the generally accepted mechanism for electrophilic substitution of ketones.

The present work was performed in order to reassess the findings of Singer[90] that nitrosation of ketones (and, possibly, other electrophilic substitution reactions of ketones) does not proceed via the enol tautomer of the ketone.

CHAPTER 6

RESULTS AND DISCUSSION FOR THE NITROSATION OF ALICYCLIC KETONES

6.1: Nuclear Magnetic Resonance Spectrometric Method:

If a ketone is enolised in deuteriated solvent, e.g. deuteriosulphuric acid in deuterium oxide, the labile hydrogen atoms of the substrate are replaced by deuterium atoms. Since deuterium nuclei give no response in the proton nuclear magnetic resonance spectrum, the rate of enolisation of a ketone may be determined by observing the diminution of the spectrum signal given by the labile hydrogen atoms of the ketone. The area of the signal is directly proportional to the amount of undeuteriated substrate present and may be evaluated by electronic integration of the signal. The observed signal is normalised by reference to a signal corresponding to protons not involved in the enolisation. These protons may be located within the substrate molecule or be present in another compound purposely added to the reaction mixture.

6.1.1: The Enolisation and Nitrosation of Cyclopentanone:

A few preliminary reactions were performed using cyclopentanone as substrate with a tert.-butanol internal reference and deuteriosulphuric acid (ca. 0.4 - 1 M) as solvent. These reactions gave familiarity with the experimental technique but yielded little pertinent information.

6.1.2: The Enolisation and Nitrosation of 4-methylcyclohexanone:

It was expected that the intramolecular reference signal provided by the methyl group would give greater accuracy in the normalisation of the enolisable proton signal.

A linear relationship was established between the rate of enolisation and the deuteriosulphuric acid concentration (Table 18, Figure 5). It was then attempted to measure the rate of loss of the 2,6 protons (those juxtaposed to the ketone function, i.e. the enolisable protons) in the presence of sodium nitrite. The nitrite would be converted to the nitrous acidium ion under the reaction conditions. Addition of nitrite to the reaction mixture produced erratic integrals from which it was impossible to deduce rates of reaction. Comparison of the recorded spectrum and the integrals of the signals in the spectrum gave the paradoxical finding that whilst the spectrum appeared to indicate exchange of substrate protons with the solvent the integral of these protons remained essentially constant, apparently indicating that no reaction was occurring. It was considered, tentatively, that these findings may have resulted from reaction of the substrate with the nitrosating agent subsequent to the enolisation, although it was not understood how such an interaction could restore the integral to the value given by the original ketone. In an attempt to obviate this possibility it was decided to employ a substrate with substituents at the α-positions (relative to the ketone function) leaving only one exchangeable proton at each of these positions.

6.1.3: The Enolisation and Nitrosation of 2,5-dimethylcyclopentanone:

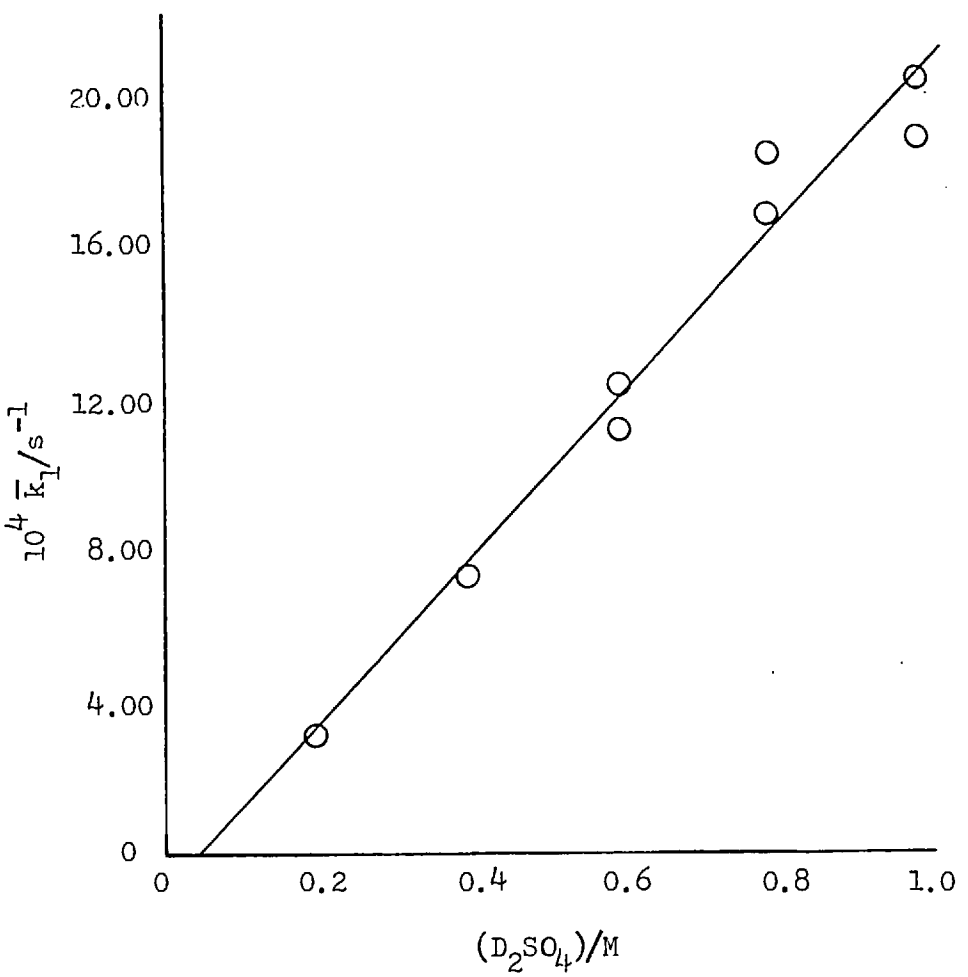
Poor results were obtained, even when nitrite was

Table 18: Variation of the rate constant with acid concentration for the enolisation of 4-methylcyclohexanone at 50.8°

[substrate] = 0.1 M

Run	(D_2SO_4)/M	$10^4\ \bar{k}_1/s^{-1}$
427	0.2	3.05
422b	0.4	7.22
423a/b	0.6	11.00/12.18
418/420	0.8	16.75/18.33
419/424	1.0	18.73/20.42

Figure 5: Graphical representation of the data of Table 18



absent, probably due to the small change in the integral of the ring protons relative to the reference integral provided by the two methyl groups. The change in the integral was only one-sixth of the total initial integral, even for complete enolisation, introducing a significant error into the normalisation procedure. The results thought to be most reliable for the enolisation (deuteriation) of the substrate are given in Table 19 and Figure 6. Addition of nitrite did not produce the unusual feature given by 4-methylcyclohexanone apparently justifying the use of an α-substituted substrate. Indeed, reaction rates were calculated for the enolisation in the presence of nitrite and are included in Table 19 and Figure 6. The data clearly show that the observed rate constant for the deuteriation of the substrate was increased by the addition of nitrite to the reaction mixture.

A linear relationship was observed between $[D_2SO_4]$ and the experimental rate constant, $\bar{k}_1$, when nitrite was absent and also when it was present, see Figure 6. The data for the latter condition was not of as good quality as when nitrite was absent.

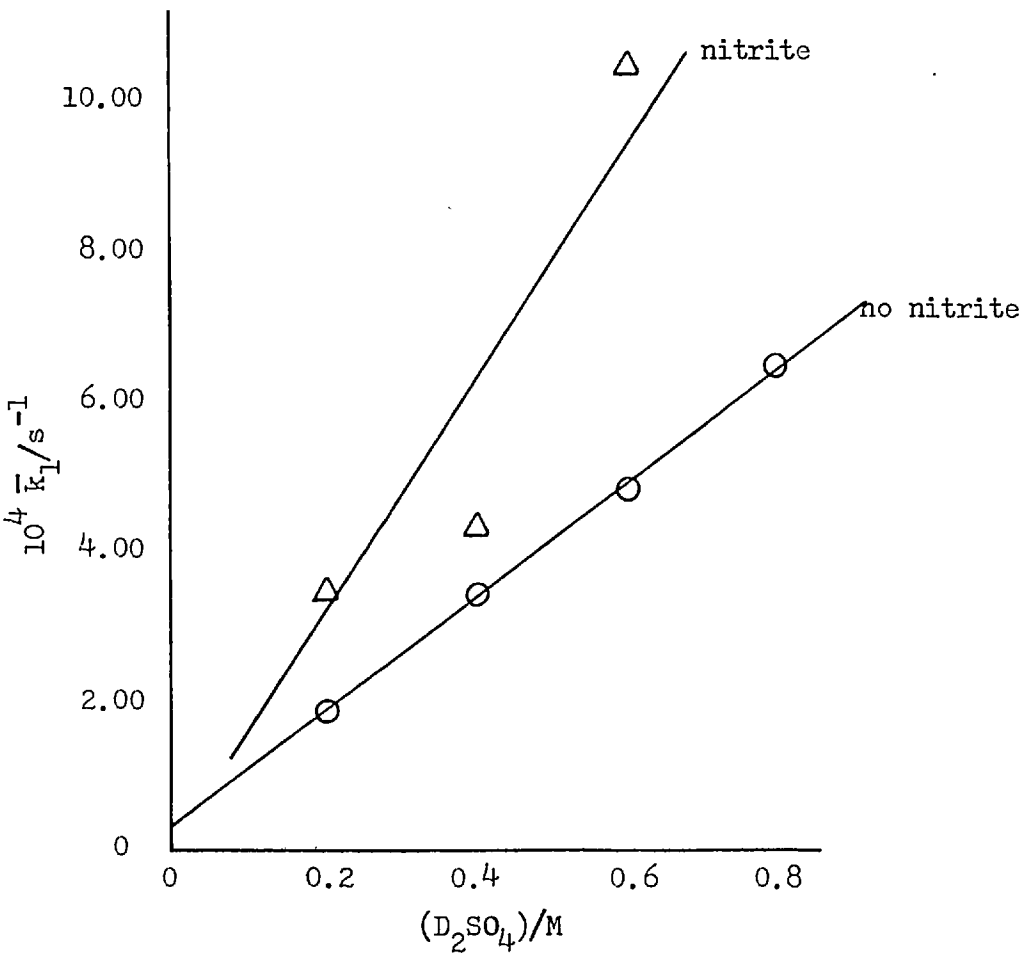
Thus it was found that the rate of deuteriation of 2,5-dimethylcyclopentanone was increased on addition of nitrite. This supported the conclusions of Singer[90] since the rate of deuteriation with no nitrite was a measure of the rate of enolisation wheras the rate of deuteriation with nitrite was a measure of the rate of nitrosation. However, the experimental method did not yield data readily with any of the substrates and was replaced by a second technique.

Table 19: Rate constants for the deuteriation of 2,5-dimethylcyclopentanone at 50.8° in the absence and presence of sodium nitrite.

[Substrate] = 9.6 x 10^{-2}M; ($NaNO_2$) = 10^{-2}M

Run		(D_2SO_4)/M	$10^4\ \bar{k}_1/s^{-1}$	
NO_2^- absent	NO_2^- present		NO_2^- absent	NO_2^- present
445	438	0.2	1.88	3.43
436/442	449	0.4	3.35/3.42	4.25
430/444	433	0.6	4.80/4.75	10.3
447	-	0.8	6.42	-

Figure 6: Graphical representation of the data of Table 19



6.2: Radioactivity Scintillation Counting Method:

Replacement of the labile hydrogen atoms of a ketone with tritium atoms renders the compound radioactive via β-particle emission. Enolisation of the tritiated ketone in protic medium results in protodetritiation of the ketone. Since the concentration of the tritiated species is directly proportional to the amount of radioactivity it possess the enolisation reaction may be monitored by the decrease in radioactivity of the reaction solution during the course of the reaction. However, the radioactivity produced by the tritium atom is of low energy and is difficult to measure directly. Compounds are utilised, therefore, which absorb the radiation and re-emit it as photons which may be accurately quantified by application of the photoelectric effect. Each β-particle absorbed by such a compound (a scintillator) leads to emission of one photon. Measurement of the number of photons produced gives the number of β-particles emitted by the ketone and provides a simple representation of the ketone concentration to be obtained.

6.2.1: The Enolisation and Nitrosation of [$^3H_1$]-2,5-dimethylcyclopentanone:

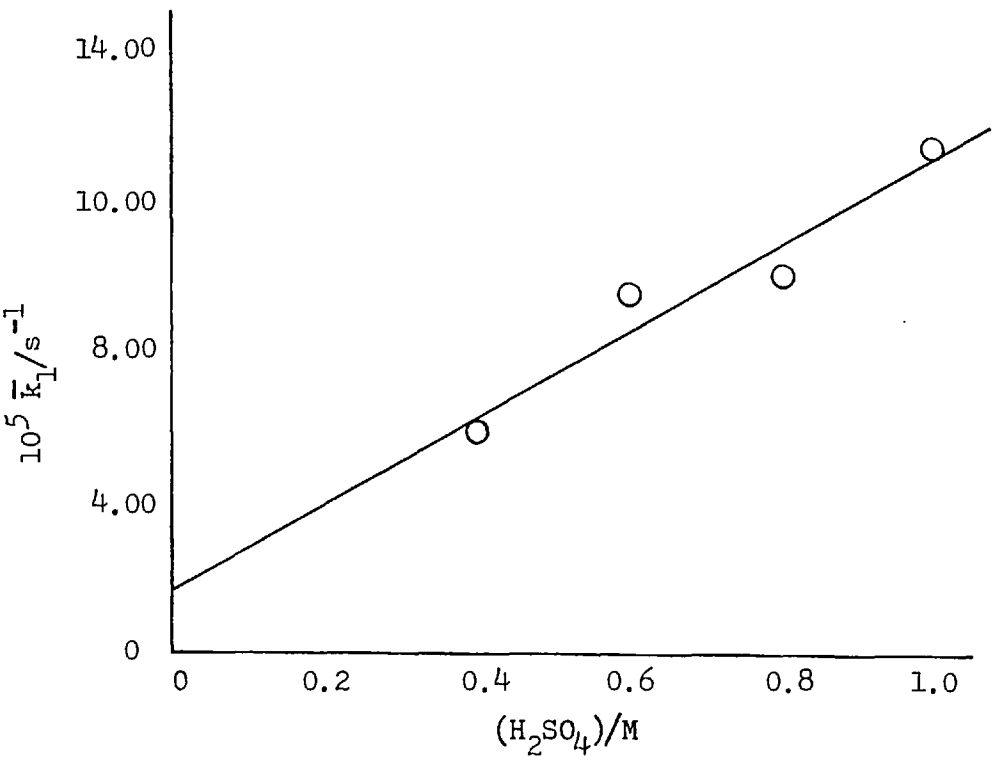
The rate of enolisation in aqueous sulphuric acid at 50° was shown to be linearly dependent upon (H_2SO_4) over the concentration range studied ($[H^+]$ = 0.4 - 2.0 M), see Table 20, Figure 7.

Table 20: Rate constants for the enolisation *via* proto-detritiation of [$^3H_1$]-2,5-dimethylcyclopentanone in sulphuric acid at 50°

[substrate] = 1.6×10^{-3} M

Run	(H_2SO_4)/M	$10^5 \bar{k}_1$
460	0.4	5.93
459	0.6	9.45
455	0.8	9.87
456	1.0	13.3

Figure 7: Graphical representation of the data of Table 20



Addition of sodium nitrite to the system caused an increase in the rate of protodetritiation. An excess of nitrite was used so that the reaction was pseudo-first order in substrate. Individual runs showed a deviation from first order behaviour after ca. 40 % reaction compatible with unproductive loss of nitrite via decomposition. The rate of protodetritiation showed a linear dependence upon the initial nitrite concentration, see Table 21, Figure 8.

Since nitrite decomposition caused deviation in the kinetic plots the study was repeated at 0° to minimise the nitrite loss. The enolisation rate was reduced also but this allowed more frequent sampling during the early stages of the reaction.

6.2.2: The Enolisation and Nitrosation of $[^{3}H_{1}]$-2,5-dimethylcyclopentanone at 0°:

The rate of enolisation (protodetritiation) was linearly dependent upon (H_2SO_4) and was increased by the addition of sodium nitrite, see Table 22 and Figure 9.

A first order dependence of rate upon both substrate and nitrite concentrations was verified by the data of Tables 23 and 24, respectively. The data of Table 24 is shown graphically on Figure 10. The constancy of $\bar{k}_1$ in Table 23 confirmed that the reaction was pseudo-first order with respect to ketone and the constancy of k_2, the second order rate constant for the reaction, in Table 24 confirms that the reaction

Table 21: Psuedo-first order rate constants for the proto-detritiation of [$^3H_1$]-2,5-dimethylcyclopentanone in the presence of nitrite at 50°

[Substrate] = 1.6×10^{-3}; (H_2SO_4) = 0.4 M

Run	$10^2(NaNO_2)/M$	$10^5\ \bar{k}_1/s^{-1}$
460	-	5.93
454/461	1.0	19.6/25.5
457	1.5	34.0
458	2.0	38.3

Figure 8: Graphical representation of the data of Table 21

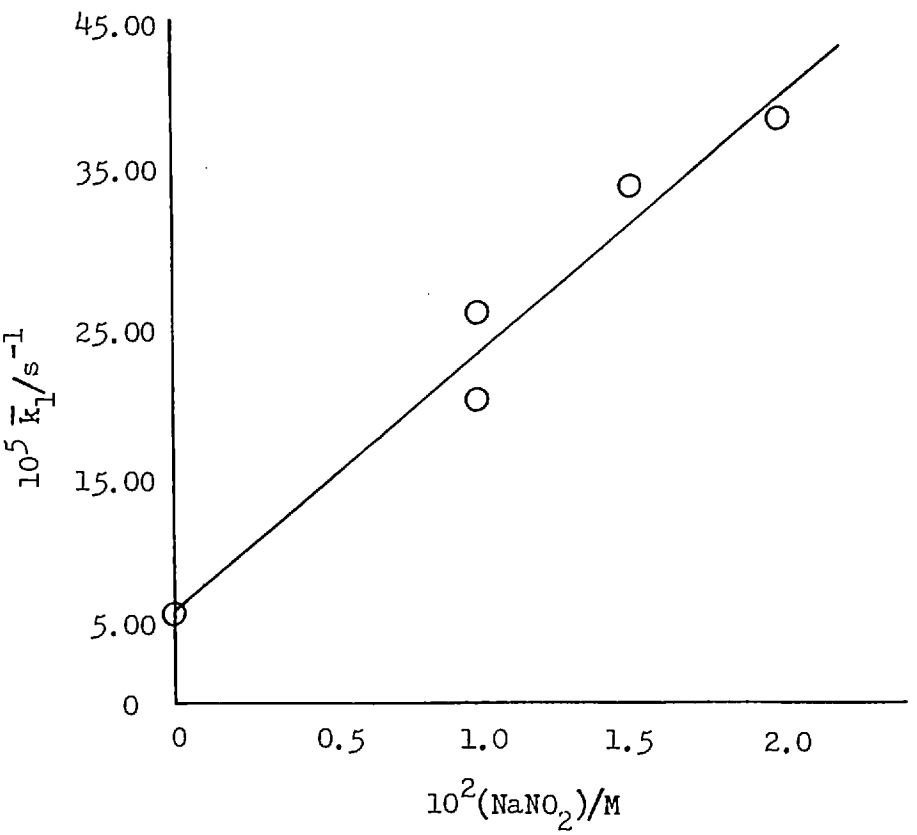
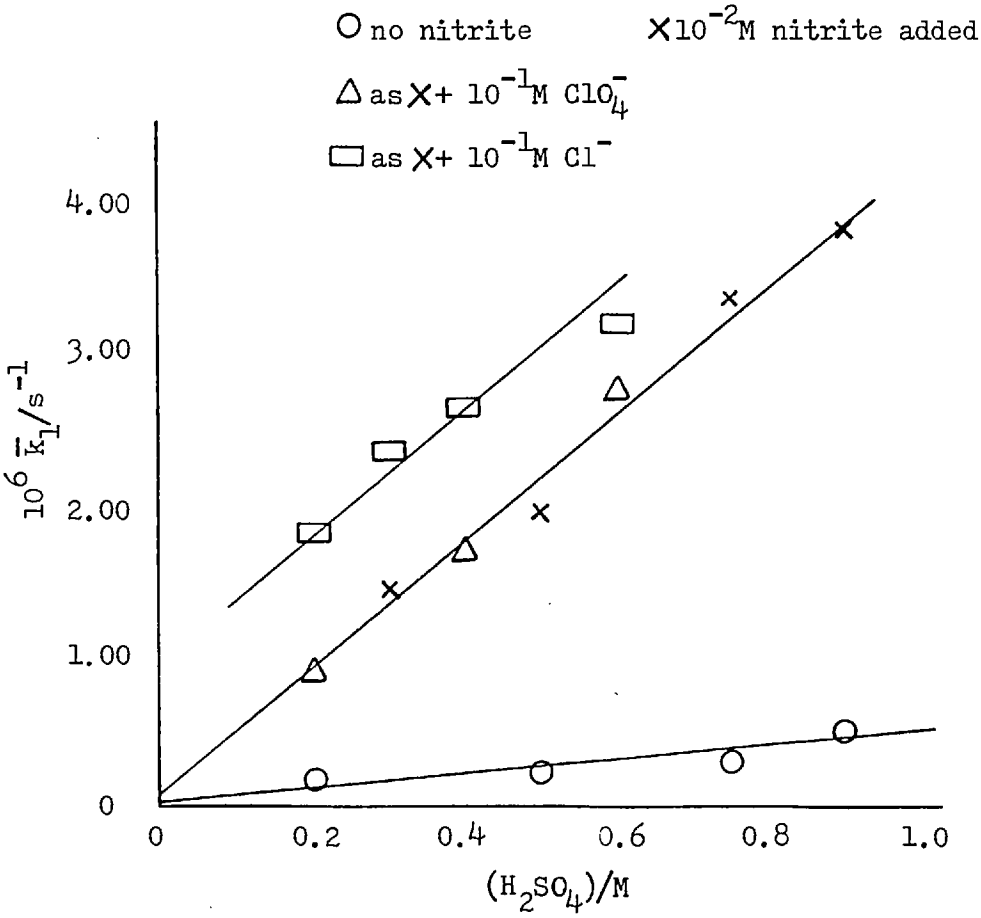


Table 22: Rate constants for the protodetritiation of [$^3H_1$]-2,5-dimethylcyclopentanone with and without sodium nitrite at 0°

[Substrate] = 1.6 x 10^{-3}M

Run	(H_2SO_4)/M	$10^2(NaNO_2)$/M	$10^6\ \bar{k}_1/s^{-1}$
476	0.50	-	0.19
479	0.50	1.0	1.92
477	0.75	-	0.29
480	0.75	1.0	3.32
472	0.90	-	0.52
470	0.90	1.0	3.78

Figure 9: Variation of $\bar{k}_1$ with (H_2SO_4) and the effect of added nitrite, perchlorate and chloride ions for the protodetritiation of [$^3H_1$]-2,5-dimethylcyclopentanone at 0°.



that the reaction was first order with respect to nitrite.

Table 23: Kinetic order in substrate for the protodetritiation of [$^3H_1$]-2,5-dimethylcyclopentanone with added $NaNO_2$ at 0°

$(NaNO_2) = 10^{-2}$M; $(H_2SO_4) = 0.75$ M

Run	10^3[Sub]/M	$10^6 \bar{k}_1/s^{-1}$
488	0.32	3.10
487	0.80	3.44
480	1.6	3.32

Table 24: Kinetic order in nitrite for the protodetritiation of [$^3H_1$]-2,5-dimethylcyclopentanone at 0°

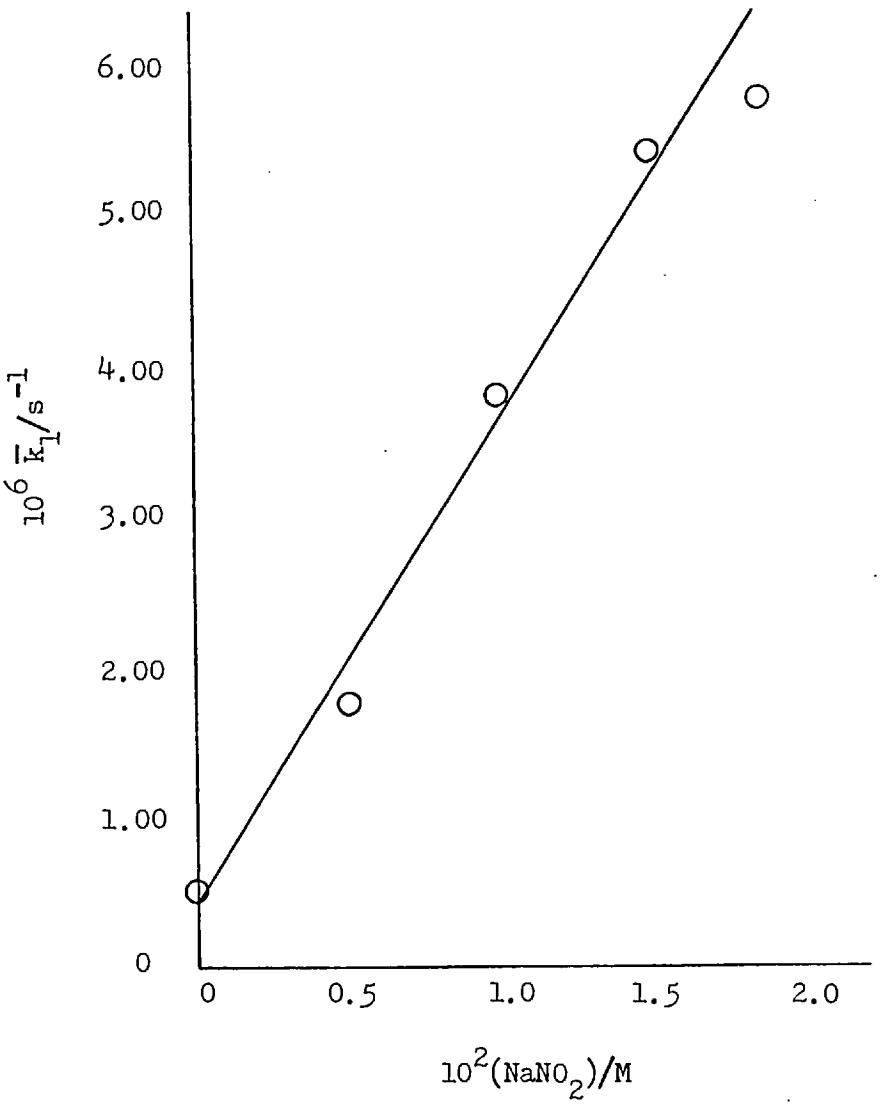
[Substrate] = 1.6×10^{-3}M; $(H_2SO_4) = 0.9$ M

Run	$10^2(NaNO_2)$/M	$10^6 \bar{k}_1/s^{-1}$	$10^4 k_2$/l mol^{-1}s^{-1} [b]
473	0.50	1.73	3.46
470	1.00	3.78	3.78
474	1.50	5.38	3.59
471	1.74 [a]	5.72	3.29

a. Intended $(NaNO_2) = 2.00 \times 10^{-2}$, Shinn's method estimation of $(NaNO_2)$ gave 1.74×10^{-2}M.

b. $k_2 = \bar{k}_1/(NaNO_2)$

Figure 10: Variation of $\bar{k}_1$ with ($NaNO_2$) for the nitrosation of [$^3H_1$]-2,5-dimethylcyclopentanone at 0°.



6.2.2a: Effect of added salt:

In the runs reported above (Section 6.2.2) the ionic strength was not maintained constant. Certain runs were repeated with added sodium perchlorate to ascertain the effect of ionic strength on the reaction rate. The rate constants obtained are plotted on Figure 9. It is seen that the values agree closely with those expected in the absence of added salt indicating that ionic strength did not affect the rate of the reaction noticeably.

6.2.2b: Effect of added chloride ion:

Addition of chloride ions to nitrosation mixtures markedly enhanced the rate of protodetritiation. Results are shown on Figure 9. Variation of $[Cl^-]$ at a fixed acid concentration gave an increased but constant rate relative to the rate in the absence of chloride ions. This was attributed to a pre-equilibrium conversion of nitrous acid to nitrosyl chloride by the excess chloride ions producing an effectively constant [NOCl] which then acted as an improved nitrosating agent. Results are given in Table 25.

6.2.2c: Calculation of catalytic rate constants:

Consideration of the data of Figures 9 and 10 allowed calculation of the individual rate constants for

Table 25: Rate constants for the protodetritiation of [$^3H_1$]-2,5-dimethylcyclopentanone in the presence of nitrite and chloride ions at 0°

[Substrate] = 1.6×10^{-3}M; (H_2SO_4) = 0.3 M

$(NaNO_2) = 10^{-2}$M; $[NaClO_4] + [NaCl]$ = 1.0 M

Run	10[NaCl]/M	$10^6 \bar{k}_1/s^{-1}$
478	-	1.40
495	0.5	2.24
491	1.0	2.30
496	1.0	2.31
492	3.0	2.04
493	6.0	2.29
494	10.0	2.54
497	10.0	2.32

catalysis by hydroxonium and nitrous acidium ions. The observed rate constant, $\bar{k}_1$, is related to the catalytic rate constants by equations (6.1) and (6.2).

$$\text{Rate} = \bar{k}_1[\text{Sub}] = k_{H_3O^+}[H_3O^+][\text{Sub}] + k_{HONO}[H_3O^+][HONO][\text{Sub}] \quad (6.1)$$

$$\underline{\text{i.e.}}\ \bar{k}_1 = (k_{H_3O^+} + k_{HONO}[HONO])\ [H_3O^+] \quad (6.2)$$

From Figure 9,

(i) no nitrite, slope = $k_{H_3O^+} = 2.28 \times 10^{-7}$ l mol^{-1}s^{-1}

(ii) with nitrite, slope = $k_{H_3O^+} + k_{HONO}[HONO]$

$= 2.10 \times 10^{-6}$ l mol^{-1}s^{-1}

but, $[HONO] = 10^{-2}M$,

$$\therefore k_{HONO} = 1.87 \times 10^{-4}\ l^2 mol^{-2} s^{-1}$$

From Figure 10,

(i) slope = $k_{HONO}[H_3O^+] = 3.16 \times 10^{-4}$ l $mol^{-1} s^{-1}$

but, $[H_3O^+] = 1.8$ M,

$$\therefore k_{HONO} = 1.76 \times 10^{-4}\ l^2 mol^{-2} s^{-1}$$

(ii) intercept = $k_{H_3O^+}[H_3O^+] = 4.30 \times 10^{-7}\ s^{-1}$

but, $[H_3O^+] = 1.8$ M,

$$\therefore k_{H_3O^+} = 2.40 \times 10^{-7}\ l\ mol^{-1} s^{-1}$$

The two sets of data gave a check on the consistency of the experimental results aince each set led to values for both catalytic rate constants. It is seen that the values from the two sets of data agree closely.

6.3: Hydrogen Isotope Effect for the Nitrosation of Cyclopentanone:

Cyclopentanone and 2,5-[$^2H_1$]-cyclopentanone were reacted with sodium nitrite in sulphuric acid and deuteriosulphuric acid, respectively. Substrate was in excess and the reactions were monitored <u>via</u> Shinn's method. Results are presented in Table 26 and show the experimental value for

k^H/k^D to be 0.50. However, account must be taken of the relative concentrations of the nitrosating species, viz. $[H_2O^+NO]$ and $[D_2O^+NO]$. Earlier studies of diazotisation by nitrous acidium ion[92] suggested that $[D_2O^+NO] / [H_2O^+NO] = 2.2$. Application of this factor gives $k^H/k^D = 1.1$. This indicates that there was no significant primary isotope effect.

Table 26: Kinetic hydrogen isotope effect for the nitrosation of cyclopentanone at 25°.

$(H_2SO_4$ or $D_2SO_4) = 0.2$ M ; $(NaNO_2) = 10^{-4}$ M

[Substrate] = 0.4 M

Run	∝-substituent	$10^5 k$ a/s^{-1}		k^H/k^D
500	$^1H_1$	3.14	3.07	0.50
503	$^1H_1$	3.00		
504	$^2H_1$	6.16		

a. Corrected for unproductive loss of nitrite.

6.4: The Enolisation and Nitrosation of 1,2-cyclohexanedione:

1,2-cyclohexanedione enolises to the mono-enol via a rapidly formed monohydrate giving an equilibrium mixture of approximately equal amounts of diketone and mono-enol[93] in aqueous medium. In 0.5 M sulphuric acid at 25° the rate constant

for enolisation was found to be ca. 4×10^{-6} s^{-1}, in good agreement with a reported value of ca. 7×10^{-6} s^{-1} [94]. Addition of nitrite (effectively nitrous acidium ion) to an otherwise identical reaction mixture completely inhibited the enolisation. A similar pair of reactions in 0.9 M acid gave the same result, i.e. no enolisation with nitrite in the system.

6.5: Diazotisation of Aniline in the presence of Cyclopentanone:

The rate of diazotisation of aniline by nitrite in sulphuric acid was measured to be ca. 2×10^{-4} s^{-1}. Addition of cyclopentanone to the reaction system reduced the rate constant to ca. 1×10^{-4} s^{-1}. The ketone appeared to have inhibited the diazotisation by a factor of two, although this deduction was based on a single experiment.

6.6: The Enolisation of [$^3H_1$]-2,5-dimethylcyclopentanone in the presence of Electrophilic Chlorine:

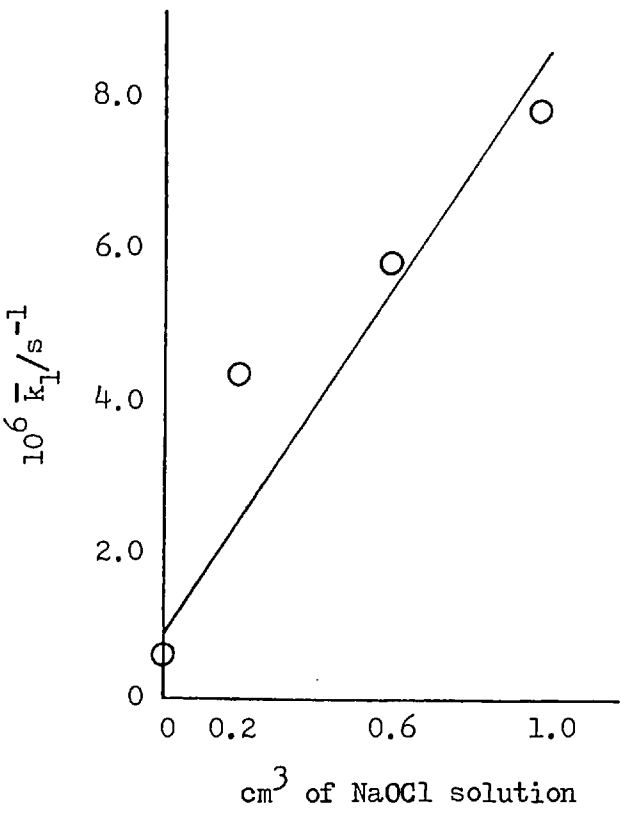
Having found that nitrous acidium ion increased the rate of enolisation (protodetritiation) of [$^3H_1$]-2,5-dimethylcyclopentanone it was decided to ascertain whether another electrophile, viz. hypochlorous acidium ion (H_2O^+Cl), would give the same effect. The electrophile was furnished by addition of sodium hypochlorite to the reaction system.

Results within a run were rather erratic but allowed calculation of approximate rate constants for the protodetriation. These results are shown on Figure 11. The rate of protodetritiation increased as the concentration of electrophile increased.

Figure 11: Variation of $\bar{k}_1$ with volume of added NaOCl solution for the protodetritiation of [$^3H_1$]-2,5-dimethyl-cyclopentanone at 0°.

[Substrate] = 1.6 x 10^{-3}M; (H_2SO_4) = 0.9 M

NaOCl given as cm^3 of 10 - 14 % w/v solution per 10 cm^3 of reaction mixture



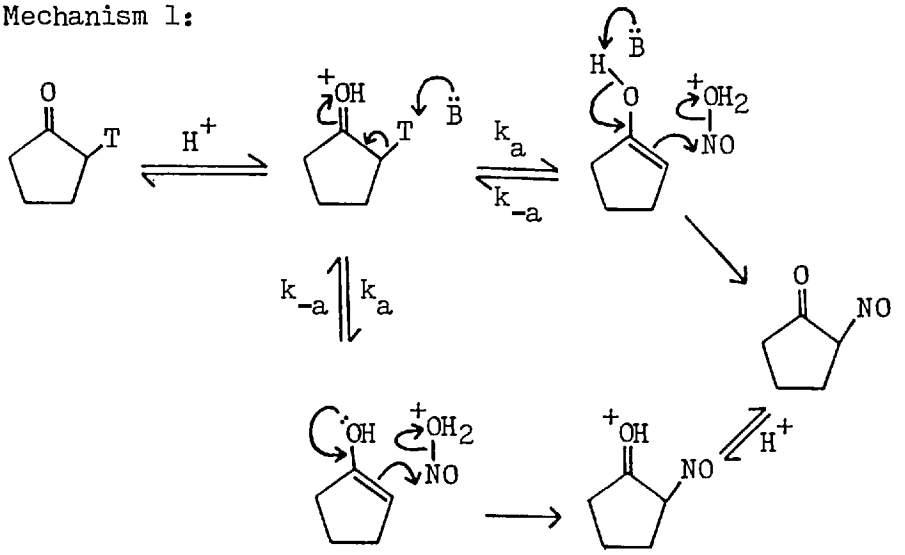
6.7: Discussion of the Results:

The rate of enolisation of 2,5-dimethylcyclopentanone was increased by addition of nitrite ion, effectively nitrous acidium ion under the conditions employed. This was shown by both deuteriation and protodetritiation procedures and supports the findings of Singer and Vamplew[90] who employed the measurement of nitrite uptake and u.v. spectrophotometry as their experimental procedures. Comparison of the catalytic rate constants $k_{H_3O^+}$ and k_{HONO} derived from protodetritiation data at 0° suggested that the nitrosating agent was the more effective catalyst by a factor of *ca.* 10^3. The rate of protodetritiation was increased further by the addition of chloride ions.

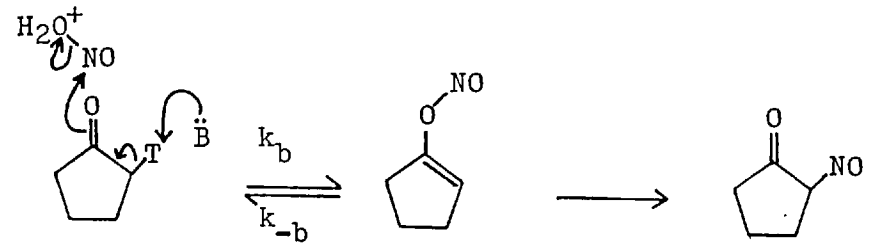
The finding that k^H/k^D = 1.1 for enolisation of cyclopentanone in the presence of nitrite indicated that the removal of the enolisable hydrogen atom was not the rate determining step, contrary to previous ideas. Ketone enolisation normally shows k^H/k^D = *ca.* 7 [95]. Three mechanisms are envisaged whereby the ketone (shown as 2-[$^3H_1$]-cyclopentanone in Scheme 6.1, for clarity) can lose its enolisable hydrogen atom in the presence of nitrous acidium ion, see Scheme 6.1.

If k_a were rate determining then the reaction would be the usual enolisation followed by reaction of the enol with the electrophile. In this case the rate of protodetritiation would be unaffected by the presence of nitrous acidium ion. For protonated and deuteriated substrates, k^H/k^D = *ca.* 7 would be expected if k_a were the rate determining step.

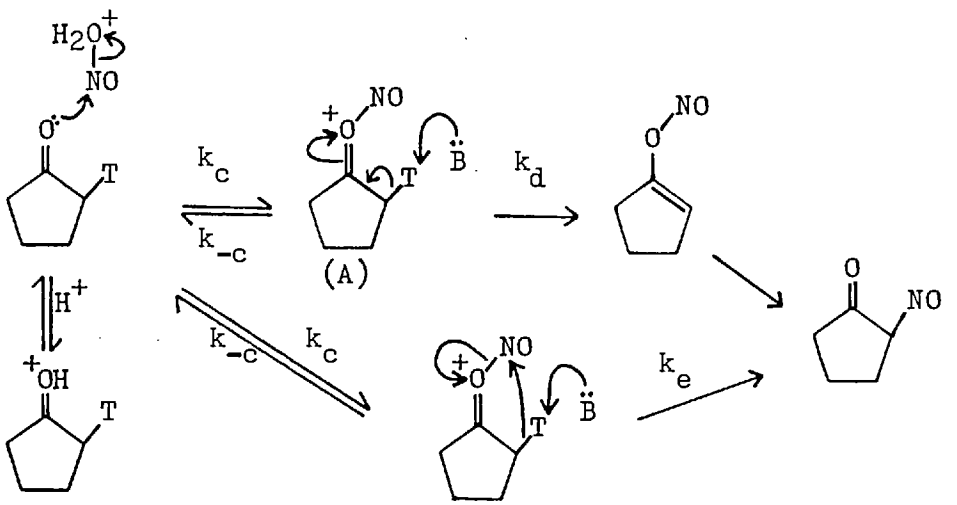
Mechanism 1:



Mechanism 2:



Mechanism 3:



Scheme 6.1: Proposed mechanisms for the reaction of an alicyclic ketone with nitrous acidium ion.

If the process represented by k_b were rate determining then a termolecular process, detected for some enolisations (Section 5.1.1) would be operative. Since addition of chloride ions increased the rate by a fixed amount and not in a manner proportional to the $[Cl^-]$ it may be concluded that chloride ions are not effecting the removal of the labile hydrogen (tritium) atom.

Consideration of k_c as the rate determining step allowed an explanation of the experimental results. With formation of the intermediate (A) being rate determining, i.e. k_d or k_e fast, then no primary isotope effect would be observed. If $k_c > k_a$ (interaction of substrate with nitrosating agent faster than interaction with base) then $k_d > k_a$ and the presence of H_2O^+NO would cause an increase in the rate of protodetritiation. Addition of chloride ions to a system reacting according to this mechanism could have two effects; either the nitrosating agent becomes nitrosyl chloride in a rapid pre-equilibrium reaction or Cl^- acts as a base effecting removal of the tritium label from the intermediate. The latter effect would not be observed since k_d (k_e) would not be rate determining. As Cl^- did catalyse the reaction the conclusion to be drawn is that nitrosyl chloride was a more effective electrophile than nitrous acidium ion under the conditions of the reaction. The finding that increasing the concentration of chloride ions did not further increase the rate supports the conclusion that they were forming nitrosyl chloride and not acting as a base.

The observations that nitrite completely

inhibited the enolisation of 1,2-cyclohexanedione and that cyclopentanone appeared to inhibit the diazotisation of aniline suggested that the nitrite and ketone interacted to form stable intermediates, possibly similar to that shown as (A) in Scheme (6.1). It is not understood why such intermediates should be stable. No product analysis was attempted for any of the reactions described above and those indicated in Scheme (6.1) are hypothetical. Determination of the actual products may allow further explanation of the experimental results.

Hypochlorous acidium ion increased the rate of protodetritiation, probably in a similar manner to nitrous acidium ion, i.e. by the postulated rate determining reaction of the electrophile with the ketone forming an intermediate chlorinated on oxygen, in this case. As mentioned previously (Section 5.3), Bell suggested that chlorination of acetone proceeded via the keto form of the ketone for a small amount of the reaction[91].

6.8: Conclusion:

This work has substantiated the suggestion by Singer and Vamplew[90] that ketone nitrosation does not proceed via a rate determining enolisation of the ketone. The rate of nitrosation was found to be greater than the rate of enolisation and there was no significant primary hydrogen isotope effect. The mechanism of the nitrosation is thought to proceed via

rate determining O-nitrosation of the ketone followed by rapid hydrogen abstraction from the O-nitrosated intermediate. The mechanism also appeared to operate for ketone chlorination. These findings challenge the generally accepted mechanism for the reaction of ketones with electrophiles which supposes that the ketone undergoes a rate determining enolisation prior to the electrophilic reaction.

CHAPTER 7

EXPERIMENTAL DETAILS

Instrumentation:

Ultra-violet spectra were recorded on Pye-Unicam SP800A or SP1800 spectrophotometers. Kinetic runs were monitored on SP1700 or SP1800 spectrophotometers. Samples from the residual nitrite method were estimated on an SP1800 fitted with an SP40 automatic sampler and flow cell. Nuclear magnetic resonance specctra were recorded on a Varian T60 spectrometer. Mass spectral analyses and microanalyses were performed by the appropriate laboratories in the Department of Chemistry, Imperial College. Acidities were measured using an EIL 7050 pH meter and EIL combined electrodes. Refractive indices were taken on a Hilger & Watts M46-MK18 refractometer. Reactions were thermostatted in the cell block of an SP1800 spectrophotometer connected to a Grant thermostatic circulator ($\pm$ 0.5°) or in a thermostatted bath ($\pm$ 0.5°) of polyethylene glycol. Melting points were recorded on a Kofler hot-stage and are reported uncorrected.

7.1: Kinetic Methods for the Nitrosation of 4-methylphenol, N-acetyl-L-tyrosine and DL-tyrosine:

7.1.1: Analysis of reaction mixtures for unreacted nitrite:

7.1.1a: Colourimetric Assay:

For reactions with an excess of substrate relative to nitrite the progress of the reactions was followed by estimating the unreacted nitrite at any given time. A deficit of nitrite was desirable to produce a large, measurable change in its concentration.

Shinn[96] and other workers[97,52] developed methods whereby the unreacted nitrite is rapidly consumed by an aromatic amine to produce a stable aromatic diazonium ion which is then coupled with a naphthol to give an azo dye, estimated spectrophotometrically. The concentration of the dye is directly proportional to that of the nitrite.

In this work the aromatic amine used was sulphanilamide and the coupling agent was N-1-naphthyl-etylenediamine di-hydrochloride (NED).

Typically, an aliquot (1.0 cm^3) of the reaction mixture, containing ca. 10^{-4}M nitrite, was added to a volumetric flask (10 cm^3) containing acidic sulphanilamide solution (1.0 cm^3; 1.25 g/250 cm^3 5M HCl) and the solutions were mixed by shaking the flask. After a minimum of 2 minutes a quantity of NED solution (1.0 cm^3; 0.25 g/250 cm^3 water) was added and the flask again shaken. The solution was made up to volume with deionised water. The azo dye does not form immediately but in moderately acidic solutions formation is

complete after ca.15 minutes. At least 15 mins. were allowed, therefore, before the solution was estimated spectrophotometrically.

The dye has a broad absorption in the region of 540 nm. Solutions were always measured at 541 nm where the dye was found to have $\varepsilon = 5.00 \times 10^{-4}$ l $mol^{-1} cm^{-1}$ at room temperature (ca. 22°). Thus, for nitrite solutions of 10^{-4}M concentration the absorbance measured in a 1 cm cell was 0.500. It has been shown that the absorbance of the dye is directly proportional to the nitrite concentration[98].

7.1.1b: Kinetic Runs:

Requisite amounts of all reagents, except nitrite, were placed in a volumetric flask (25 cm^3) and water was added so that sufficient volume remained for the addition of the nitrite solution. The flask was placed in a thermostatted bath for a minimum of 10 mins. It was removed and a measured quantity of a stock solution of aqueous sodium nitrite was added rapidly. The flask was quickly made up to volume with a small amount of deioised water, thoroughly shaken and transferred to a thermostatted conical flask (25 cm^3, Quickfit) to allow removal of aliquots (1.0 cm^3) with an automatic pipette using a clean dispensing tip for each aliquot. Aliquots taken at timed intervals were added to sulphanilamide solution as described in Section 7.1.1a.

7.1.1c: Sources of Error:

(i) Side Reactions:

(a) Decomposition of nitrite *via* nitrous acid and dinitrogen trioxide is a potential source of error in nitrosation reactions. Blank runs were performed under reaction conditions to ascertain the importance of this nitrite loss relative to the reaction under study. At acidities of pH < *ca.* 4 it was necessary to allow for the decomposition in the final rate calculation but not at pH > 4.

(b) It is possible for nitrous acid to be consumed by reaction with the *C*-nitroso compound initially formed to produce a diazonium ion which may couple with the aromatic substrate to form an azo dye[99]. However, for the substrates and reaction conditions employed, this complication was not apparent from inspection of the u.v./visible spectra of the reaction mixtures.

(c) For DL-tyrosine and *N*-acetyl-*L*-tyrosine (NALT) reaction of nitrous acid with the amino moiety may be envisaged. No evidence for this was observed spectrophotometrically or detected in the kinetic plots. This is in agreement with earlier work[57] conducted under similar conditions.

(ii) Errors in the Analytical Method:

(a) The method produces the usual errors associated with volumetric procedures.

(b) No interference in the analysis was encountered

from absorption at 541 nm by reagents or products, or by reaction of reagents or products with the sulphanilamide diazonium ion or the NED to form a species (other than the required azo dye) giving an absorption at 541 nm.

(c) The azo dye was unstable on prolonged exposure to light. If the absorption could not be measured within 2 h of coupling the solution was shielded from light until measurement. If measurement were not possible until the following day the vessels were placed in a refrigerator (ca. 4°) overnight. No deterioration of the dye resulted from this procedure. However, if the concentration of the dye was greater than ca. 1.5×10^{-4} M precipitation occurred on cooling. Such solutions were allowed to return to room temperature and shaken to redissolve the dye before measurement. This procedure produced no change in the absorption.

7.1.1d: Derivation of Rate Constants from the Data:

Since the substrate was in large excess over the initial stoichiometric concentration of nitrite a pseudo-first order, stoichiometric rate constant (coefficient), $\bar{k}_1$, was applicable, as defined by equation (7.1) and calculated from equation (7.3).

$$\frac{-d(NaNO_2)}{dt} = \bar{k}_1(NaNO_2) \qquad (7.1)$$

On integration,

$$\bar{k}_1 t = - \ln (NaNO_2) + \text{a constant} \qquad (7.2)$$

which is equivalent to,

$$\bar{k}_1 t = - \ln A_t + \text{a constant} \qquad (7.3)$$

where A_t is the absorbance of the azo dye solution resulting from an aliquot taken after time t.

The modulus of the slope of the linear plot of $\ln A_t$ *vs.* t thus gives the value of the rate constant.

When the decomposition of nitrite was negligible during a reaction the observed rate constant, $\bar{k}_o$, was equal to to $\bar{k}_1$. When the decomposition of nitrite was significant then $\bar{k}_o$ was the sum of the first order rate constants for substrate nitrosation ($\bar{k}_1$) and for nitrite decomposition (k'). Equations (7.4) to (7.7) give the derivation of $\bar{k}_1$ in this case.

$$\text{Decomposition,} \quad \frac{-d(NaNO_2)}{dt} = k'(NaNO_2) \qquad (7.4)$$

$$\text{Nitrosation,} \quad \frac{-d(NaNO_2)}{dt} = \bar{k}_1(NaNO_2) \qquad (7.5)$$

$$\text{Total } NaNO_2 \text{ loss,} \quad \frac{-d(NaNO_2)}{dt} = \left\{k' + \bar{k}_1\right\}(NaNO_2) \qquad (7.6)$$

$$= \bar{k}_o(NaNO_2) \qquad (7.7)$$

The second order, stoichiometric rate constant for the reaction, $\bar{k}_2$, was derived from $\bar{k}_1$ using equations (7.8) and (7.9).

$$\frac{-d(NaNO_2)}{dt} = \bar{k}_2(NaNO_2)(S) \qquad (7.8)$$

$$\bar{k}_2 = \bar{k}_1/(S) \qquad (7.9)$$

where (S) = concentration of substrate.

Since the pK_a of nitrous acid is ca. 3 (dependent upon temperature and ionic strength) for reactions at pH > 0 the actual (molecular) concentration of nitrous acid available for reaction will be less than the stoichiometric concentration of nitrite added to the reaction mixture. It is therefore necessary to define a new rate constant in terms of the molecular nitrous acid concentration, [HONO]. This is denoted as k_2 and is related to $\bar{k}_1$ by the equations below.

$$\frac{-d[HONO]}{dt} = k_2[HONO](S) \qquad (7.10)$$

Since, $\frac{-d[HONO]}{dt} = \frac{-d(NaNO_2)}{dt}$

comparing equations (7.8) and (7.10),

$$k_2 = \bar{k}_2(NaNO_2)/[HONO] \qquad (7.11)$$

thus, from equation (7.9),

$$k_2 = \bar{k}_1(NaNO_2)/[HONO](S) \qquad (7.12)$$

All the substrates have $pK_a > 9$ (ionisation of aromatic hydroxyl) and therefore exist as the neutral molecules at the acidities used. Their stoichiometric concentrations therefore equal their molecular concentrations (assuming

reaction via the neutral molecule). This meant that,

$$k_2 = \bar{k}_1(NaNO_2)/[HONO][S] \qquad (7.13)$$

N.B. Equation (7.4) described a first order rate constant, k', for the decomposition of HONO. The decomposition is really second order in HONO but, even at pH 2, the rate of decomposition was sufficiently slow for the approximation of a first order decomposition to be applied. This facilitated the calculation of the rate constant for the nitrosation using equations (7.6) and (7.7).

7.1.1e: Typical Kinetic Runs:

Reaction rate constants were calculated from plots of ln A_t vs. time (t). Examples of such kinetic plots are given by Figures 12 and 13. Run 294, a blank run corresponding to run 293 (Figure 12) showed that the nitrite decomposition was very slow relative to the rate of loss of nitrite during the nitrosation, i.e. run 293. The half-life of run 294 was calculated to be $t_{\frac{1}{2}}$ = 2002 minutes compared with $t_{\frac{1}{2}}$ = 51.5 minutes for run 293.

7.1.1f: Precision of the Measured Rate Constants:

When the graph of ln A_t vs. t was plotted co-ordinates deviant from the straight line coincident with most of the data were ignored when estimating the "best

Figure 12: Kinetic plot of ln A_t *vs.* time for the nitrosation of 4-methylphenol at 25°. RUN 293.

[Substrate] = 7.56×10^{-2}M; $(NaNO_2) = 10^{-4}$M

$(NaClO_4)$ = 0.4 M; $(HClO_4) = 10^{-3}$M; pH = 3.06

The plot represents *ca.* 75 % loss of initial $(NaNO_2)$

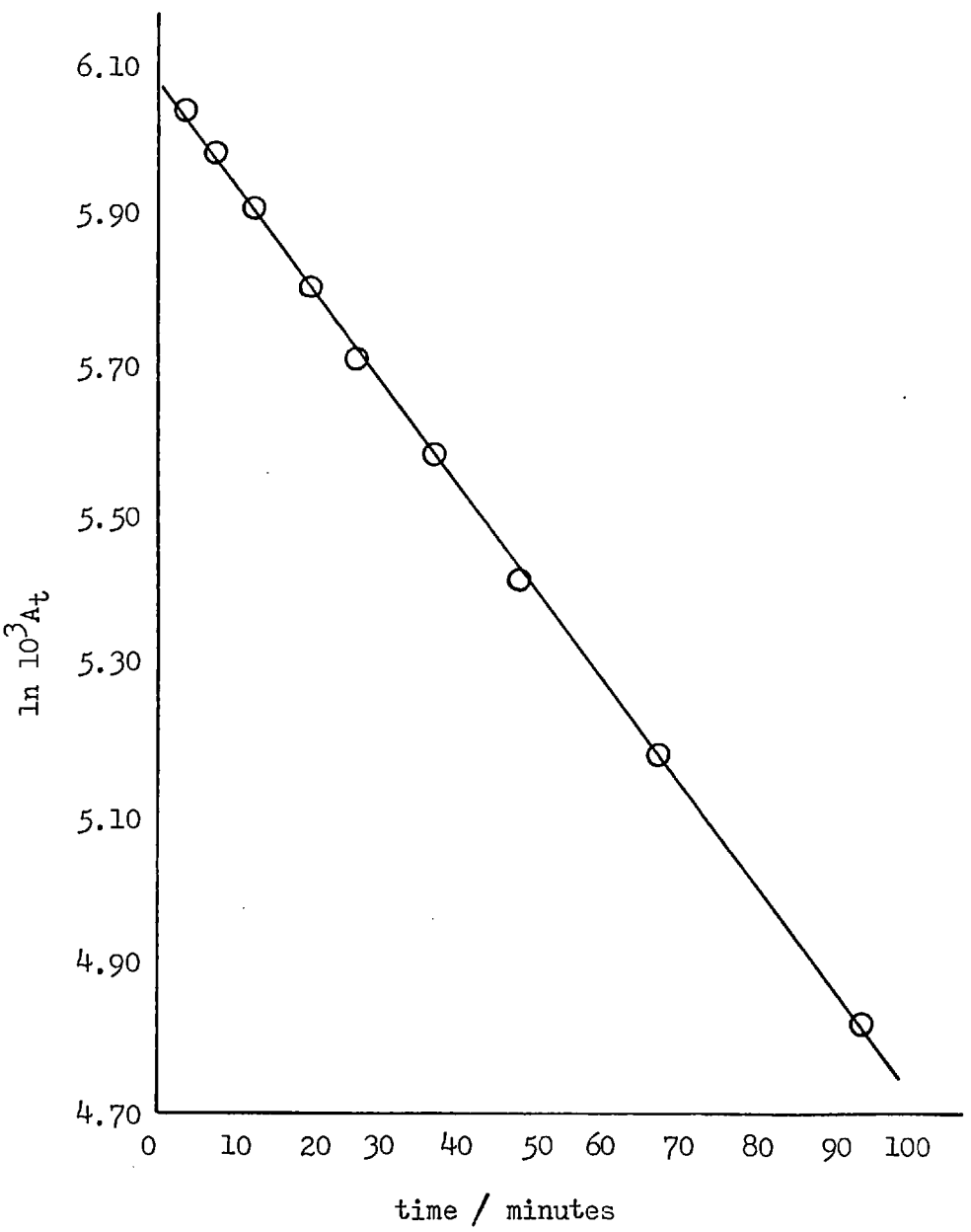
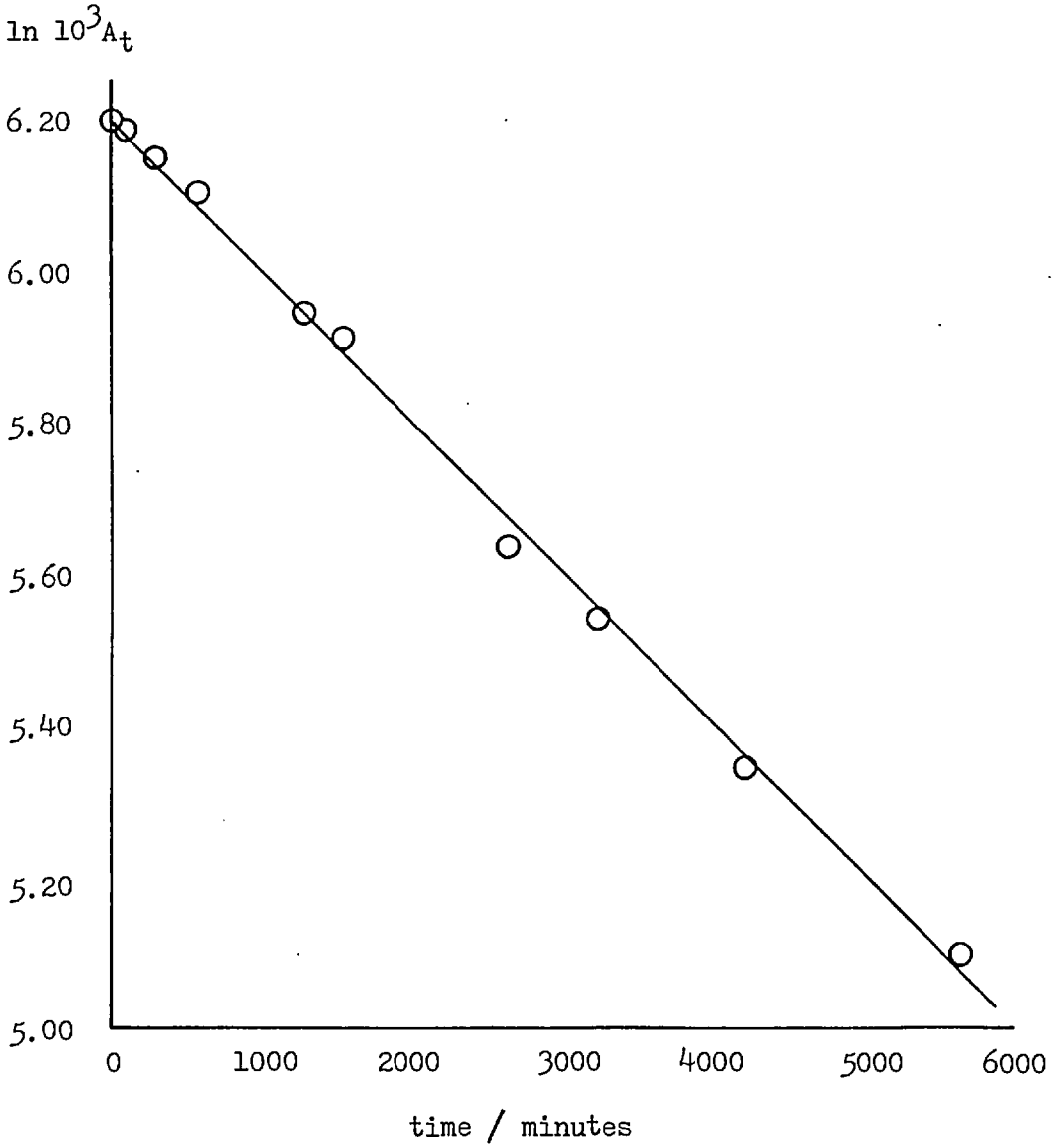


Figure 13: Kinetic plot of ln A_t *vs.* time for the nitrosation of 4-methylphenol at 25°. RUN 280.

[Substrate] = 7.56×10^{-2}M; $(NaNO_2) = 10^{-4}$M

(NaOAc) = 0.2 M; (AcOH) = 4.75×10^{-2}M; pH = 5.19

The plot represents *ca.* 67 % loss of initial $(NaNO_2)$



straight line" visually or when calculating the linear least squares (LLS) fit of the data. For most runs the observed rate constant was calculated *via* the LLS programme of a CBM SR5190R calculator.

Such treatment of the data usually showed reproducibility of kinetic runs to within ± 10 % for at least 75 % reaction. Occasionally, the discrepancy was as high as ± 20 %. It is considered reasonable to place a maximum error of ± 20 % upon all the data reported with the qualification that results are often well within such an error.

Considering the exacting conditions of many of the experiments it was often surprising to observe the consistency of the rate constant throughout a run. For example, run 280 (Figure 13) had a half-life of *ca.* 57 h yet exhibited good first order kinetics for at least 67 % reaction, *i.e.* 4 days of reaction.

7.1.2: Direct Spectrophotometric Method:

By using an excess of nitrite over 4-methylphenol, so that the substrate was of a suitable concentration (*ca.* 10^{-4}M) for accurate spectrophotometric estimation, it was possible to follow the reaction by the change in the ultra-violet absorption of the reaction mixture. Since the product (2-nitro-4-methylphenol) showed a much greater

absorbance than the substrate at 285 nm, the increase in the absorption at this wavelength was monitored.

Although both substrate and product absorb at 285 nm rate constants can be obtained directly from the change in absorption of the reaction solution. Assuming that A = absorbance at 285 nm, ε_s and ε_p = the molar extinction coefficients of substrate, S, and product,P, respectively:

$$\text{at time} = 0, \quad A_o = \varepsilon_s[S]_o \qquad \text{(i)}$$

$$\text{at time} = t, \quad A_t = \varepsilon_s[S]_t + \varepsilon_p[P]_t$$

$$\text{but,} \quad [S]_o = [S]_t + [P]_t$$

$$A_t = \varepsilon_s[S]_o - \varepsilon_s[P]_t + \varepsilon_p[P]_t$$

$$\text{using (i)}, \quad A_t = A_o + [P]_t(\varepsilon_p - \varepsilon_s)$$

$$[P]_t = \frac{(A_t - A_o)}{(\varepsilon_p - \varepsilon_s)}$$

Since ε_s and ε_p are constants, $[P]_t$ is directly proportional to $(A_t - A_o)$.

7.1.2a: Kinetic Procedure:

Requisite volumes of stock solutions of substrate and buffer or acid were placed in a volumetric flask and water was added so that sufficient volume remained for the addition of the nitrite solution. The flask was placed in a thermostatted bath for at least 10 minutes. It was removed, a measured volume of a stock solution of nitrite added to start the reaction, made to volume with water, shaken and a portion

transferred to a silica cell previously themostatted at the same temperature as the flask contents in the cell block of the spectrophotometer. The cell was returned to the cell block and the reaction was monitored at 285 nm, the absorbance being recorded after known time intervals.

7.1.2b: Absorption Spectra of the Materials under Study:

	$\lambda_{max}^{pH\ 2}$	log ε	$\lambda_{max}^{pH\ 4.6}$	log ε	$\lambda_{max}^{pH\ 14}$	log ε
4-methylphenol	277	3.26	277	3.26	295	3.47
2-nitro-	282	3.82	282	3.82	285	3.66
4-methylphenol	368	3.47	368	3.46	438	3.69

7.1.2c: Sources of Error:

Apart from the inherent volumetric and spectrophotometric errors the only other significant source of error was side reactions. Product analysis suggested that the ultimate product was the monitored species and so further reaction of the product did not occur. Loss of nitrite due to decomposition was insignificant at the acidities used and did not seriously interfere with the kinetics of the reaction, as reported previously[98].

7.1.2d: Derivation of Rate Constants from the Data:

Application to equation (7.15) of a similar argument to that presented in Section 7.1.1d leads to the

$$\frac{d[P]}{dt} = \bar{k}_1[P] \qquad (7.15)$$

conclusion that a graph of $\ln (A_\infty - A_t)$ *vs.* time will give a linear plot, the modulus of the slope being the value of the pseudo-first order rate constant, $\bar{k}_1$. This plot actually represents the rate of loss of substrate with respect to time, readily derived from the relationship given in equation (7.14). For most runs the infinity value, A_∞, was obtained from a plot of A_t *vs.* $A_{t + \Delta t}$, where $\Delta t > t_{\frac{1}{2}}$. The intercept of the resulting linear plot and of the line $A_t = A_{t + \Delta t}$ gave the adopted infinity value.

The other relevant rate constants, $\bar{k}_2$ and k_2, were derived from $\bar{k}_1$ (*cf.* equations (7.8) to (7.12)) and have the same meanings as the similarly denoted constants in Section 7.1.1d.

7.1.2e: Typical Kinetic Runs:

Reaction rate constants were calculated from plots of $\ln (A_\infty - A_t)$ *vs.* time. An example of such a kinetic plot is given by Figure 14.

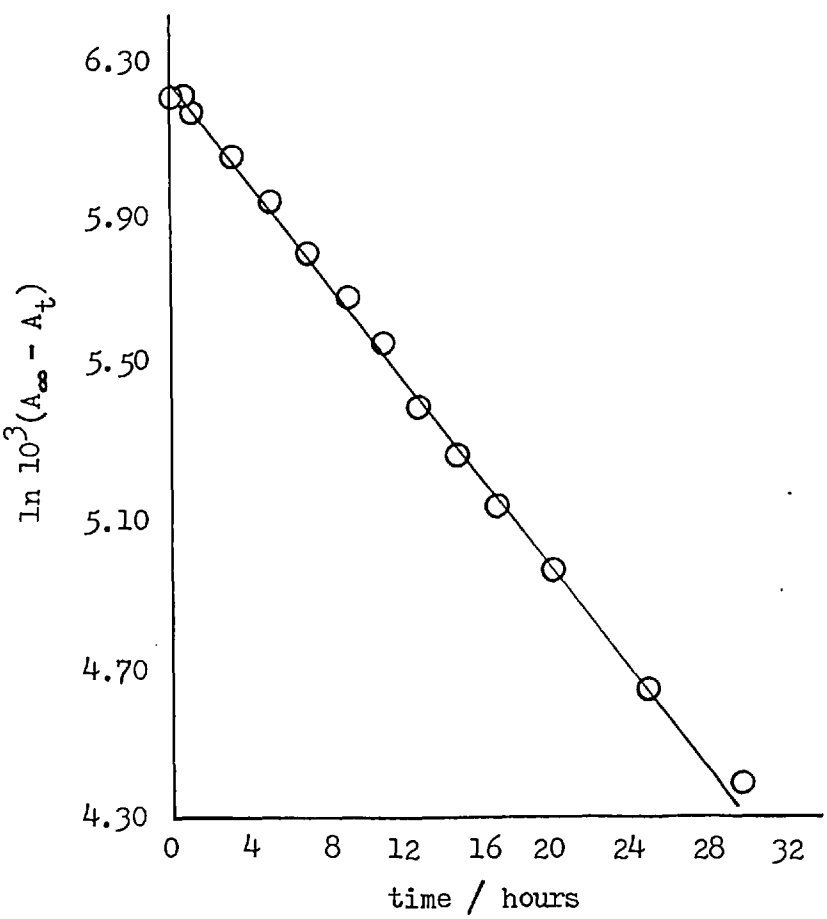
Figure 14: Kinetic plot of ln $(A_\infty - A_t)$ *vs.* time for the nitrosation of 4-methylphenol at 38°. RUN 153.

[Substrate] = 10^{-4}M; $(NaNO_2) = 10^{-2}$M

$[AcO^-] = 0.15$ M; $(AcOH) = 6.4 \times 10^{-2}$M

pH = 5.11

The plot represents *ca.* 89 % consumption of initial [Substrate]



7.1.2f: Precision of the Measured Rate Constants:

In certain cases kinetic runs were exactly reproducible and in all cases a maximum error of ± 5 % may be placed upon individual runs.

7.1.3: Spectrophotometric Determination of the Nitrosated Product *via* the Copper (II) Complex:

Reactions of phenols and nitrite in acidic aqueous solution containing copper (II) yield a complex of the nitrosophenol. If copper(II) is in deficit of the nitrosophenol then a 1:2 copper (II):nitrosophenol complex is obtained which precipitates from solution. However, if an excess of copper (II) is available then a water-soluble 1:1 complex is formed. This has a characteristic absorption at 500 - 550 nm, depending upon the particular phenol. By employing the method described in Section 7.1.2 with solutions containing excess copper (II) it was therefore possible to follow the reaction by monitoring the production of the complex at the pertinent wavelength.

7.1.3a: Kinetic Procedure:

The procedure was that described in Section 7.1.2a modified to ensure that reaction solutions contained an excess of copper (II) chloride relative to the substrate concentration.

7.1.3b: Absorption Spectra of the Materials under Study:

	λ_{max}	log ε
4-methylphenol (pH 4.6)	277	3.26
2-nitroso-	261	3.74
4-methylphenol	340	3.88
copper (II) chloride	ca. 437	3.22
	530	3.40
2-nitroso-	339	4.27
4-methylphenol	436	3.56
copper (II) chloride	527	3.77
in 0.1 M $CuCl_2$(aq)		

7.1.3c: Sources of error:

The method was applied successfully only to DL-tyrosine. When attempted with 4-methylphenol precipitation occurred during the reaction. For DL-tyrosine the product was stable and thus sources of error were volumetric and spectrophotometric. However, since similar runs at 25° and 38° gave similar values for the

rate constants one may consider that an unidentified source of error was present.

7.1.3d: Derivation of Rate Constants from the Data:

Rate constants were derived in a similar manner to that described in Section 7.1.2d.

7.1.3e: Typical Kinetic Run:

Reaction rate constants were calculated from plots of ln $(A_\infty - A_t)$ *vs.* time. An example is given by Figure 15.

7.1.3f: Precision of the Measured Rate Constants:

Very few runs were performed by this method and reproducibility was not tested. For 40 % reaction (*ca.* 40 h) using an infinity value taken after 12 days the reactions followed good pseudo-first order kinetics. The precision of $\bar{k}_1$ may be expected to be within $\pm$ 5 % of the determined value but the peculiarity associated with temperature (Section 7.1.3c) may cast doubt upon this.

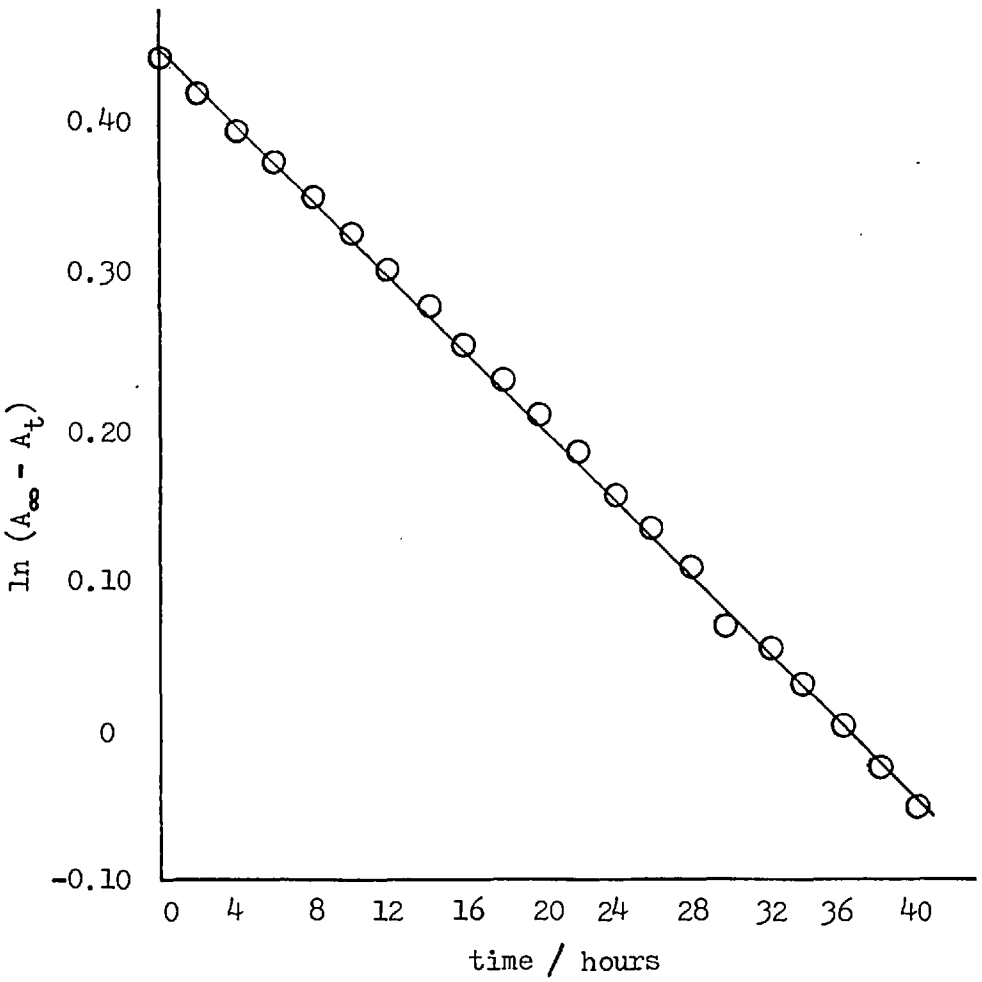
Figure 15: Kinetic plot of ln $(A_\infty - A_t)$ vs. time for the nitrosation of DL-tyrosine in the presence of copper (II) ions at 25°. RUN 101.

[Substrate] = 5.53×10^{-3}M; $(NaNO_2) = 6.3 \times 10^{-2}$M

$[Cu^{2+}] = 6.08 \times 10^{-3}$M; $[AcO^-]$ = 0.5 M; pH = 4.6

Reaction monitored at 515nm.

The plot represents ca. 40 % consumption of initial [Substrate]



7.2: Analysis of the Products:

7.2.1: Nitrosation of 4-methylphenol:

7.2.1a: With excess nitrite:

Examination of the reaction mixtures by quantitative u.v. spectrophotometry and by thin layer chromatography indicated that the reaction product was 2-nitro-4-methylphenol from comparisons with the authentic material.

(i) Spectral Data:

	$\lambda_{max}^{pH\ 2}$	logε	$\lambda_{max}^{pH\ 4.6}$	logε	$\lambda_{max}^{pH\ 14}$	logε
Run 123			283	3.80	435	3.80
Run 124					434	3.48
Run 125			282	3.76	434	3.57
Reference 48 [a]	275	3.70			430	3.45
Authentic [a,c]	283	3.73			435	3.66
Reference 100 [b]	313	3.58			480[d]	3.77

a. 2-nitro-4-methylphenol

b. 2-nitroso-4-methylphenol

c. Not pH 2, sample dissolved in water

d. pH 12

(ii) Thin layer chromatograms were obtained with silica as the adsorbent and chloroform/ethanol (1:1) as elutant.

7.2.1b: With excess 4-methylphenol:

U.v. spectra of reaction mixtures indicated the presence of both 2-nitro and 2-nitroso-4-methylphenol by comparison with authentic spectra. Alkalinisation of reaction solutions increased the intensity of the yellow colouration of the solutions, producing new absorbances at wavelengths greater than 400 nm corresponding to both of the above products. Previous reports[51] that the nitroso product was fairly readily oxidised to the nitro product on dissolution in water makes the above observation rather surprising. However, addition of Cu^{2+} to the reaction solutions gave a pink/mauve colouration with a visible spectrum characteristic of the complex of 2-nitroso-4-methylphenol (Section 7.1.3). Extraction of reaction solutions with petroleum ether ($60^{o}/80^{o}$) or dichloromethane and re-extraction with aqueous copper (II) chloride gave a red colouration in the aqueous phase. The spectrum of this chromophore corresponded with that of authentic 2-nitroso-4-methylphenol copper (II) chloride. Raising the pH to 14 with NaOH destroyed the complex and the solution then gave a spectrum identical with authentic 2-nitroso-4-methylphenol under similar conditions.

7.2.2: Nitrosation of DL-tyrosine:

7.2.2a: In aqueous perchloric acid:

The only product detected by u.v./visible spectrophotometry and thin layer chromatography was 3-nitro-

DL-tyrosine by comparison with authentic material. This result was independent of the relative initial concentrations of substrate and nitrite.

7.2.2b: In aqueous acetate buffer with added copper(II) ions:

The substrate was in excess of nitrite and the only product was 3-nitroso-DL-tyrosine copper (II) sulphate (Cu^{2+} added as $CuSO_4$), identified from the u.v./visible spectrum.

7.2.3: Nitrosation of N-acetyl-L-tyrosine:

All reactions were performed with substrate in excess of nitrite. Addition of Cu^{2+} to the reaction solutions gave quite intense pink/mauve colouration indicating relatively large amounts of 3-nitroso product by comparison with solutions of the 4-methylphenol analogue. U.v./visible spectra of reaction solutions before and after basification to pH 14 suggested that the nitroso compound, N-acetyl-3-nitroso-L-tyrosine, was the sole product.

7.3: Preparation and Purification of Materials:

Reagent grade 4-methylphenol was fractionally distilled and the middle fraction (b.p. 82.5° - 82.8°, 9 mmHg) vacuum dried over P_2O_5. $n_D^{22.5}$ 1.5389, adjusted to n_D^{20} 1.5397 (lit.[101] n_D^{20} 1.5395).

A mixture of 2-[$^2H_1$] and 2,6-[$^2H_1$]-4-methylphenol was prepared by the method of Kirby and Ogunkoya[102]. The products (an inseparable mixture) were fractionally distilled and the middle fraction (b.p. 79.5°, 8 mmHg) was vacuum dried over P_2O_5. Mass spectral analysis indicated that deuterium was incorporated at the 2-position (98 %) and the 2,6-positions (76 %).

DL-tyrosine (Aldrich), as supplied, was dried *in vacuo* over P_2O_5 prior to use, m.p. 265° - 275° (slow heating)(lit.[103] 295°, slow heating, 340°, rapid heating).

N-acetyl-L-tyrosine (Sigma) was stored at low temperature (*ca.* -20°) *in vacuo* over silica gel and used as supplied, m.p. 152-3° (lit.[104] 153-4°).

3-nitro-L-tyrosine (Aldrich), as supplied, was dried *in vacuo* over P_2O_5 before use. 2-nitro-4-methylphenol (Aldrich) was recrystallised from ethanol/water and dried *in vacuo* over P_2O_5, m.p. 31.5° - 32.5°(lit.[103] 36.5°).

Bis(2-nitroso-4-methylphenol)copper was prepared by the method of Cronheim[105]. Recrystallisation from ethanol/water and ethanol/chloroform (2:3) gave deep purple needles, m.p. > 360°. The crystals were dried *in vacuo* over P_2O_5. Microanalysis:

Found: C, 49.75 %; H, 3.64 %; N, 8.27 %. Calculated for

$C_{14}H_{12}N_2O_4Cu$: C, 50.07 %; H, 3.60 %; N, 8.24 %.

2-nitroso-4-methylphenol copper (II) chloride was prepared, in aqueous solution, by adding bis(2-nitroso-4-methylphenol)copper (0.01679 g, 5 x 10^{-5}mol) to an aqueous solution of copper (II) chloride (50 cm^3, 10^{-3}M, AnalaR) and stirring for ca. 10 h. The intense red solution was filtered to give a solution of the required product (10^{-3}M).

AnalaR grade sodium nitrite, sodium chloride and sodium perchlorate was dried over P_2O_5 in vacuo and used without further purification.

7.4: Kinetic Methods for the Enolisation and Nitrosation of Alicyclic Ketones:

7.4.1: Nuclear Magnetic Resonance Spectrometry:

7.4.1a: Experimental Procedure:

A quantity of the substrate, e.g. 18 μl of cyclopentanone, empirically determined to give a suitable signal under the reaction conditions, was placed in a n.m.r. tube. The reaction was initiated by adding 0.50 cm^3 of deuteriosulphuric acid of the required concentration, e.g. 0.4 M. The tube was capped, shaken to dissolve the substrate and placed in a thermostatted bath. At timed intervals, the tube was removed and the spectrum taken. Signals were electronically integrated 3 - 5 times. In the case of cyclopentanone a small volume of tert.-butanol was added to provide a reference signal for the normalisation procedure.

When the reaction was particularly rapid, the tube was cooled in ice/water as the reaction solution was composed. The reaction was effectively stopped in the same way for the spectra to be recorded. The recording period was included in the elapsed time of the reaction.

Reactions with nitrite were prepared in a cooled tube to minimise decomposition. The nitrite was added by weighing a calculated quantity (ca. 0.017 g) into the tube as the first reagent or, preferably, by adding a small volume of a concentrated stock solution in deuterium oxide, e.g. 5 μl of 1 M solution, as the final reagent. The tube was then sealed in a flame and the above procedure continued.

7.4.1b: Sources of Error:

(i) Side reactions:

For enolisation in the absence of nitrite there are no possible side reactions for the ketones studied. With nitrite present various reactions may occur subsequent to the enolisation process. However, such reactions should not introduce error into the kinetic method. Interaction of substrate with nitrite in positions other than the α- positions was not indicated by the spectra. No account was taken of nitrite decomposition in the sealed tube. This was likely to be minimal due to the rapid establishment of an equilibrium between nitrite in solution and decomposition products above the solution.

(ii) Errors in the Analytical Method:

The Varian T60 instrument allowed integrals to be assessed $\pm$ 5 %. The usual volumetric errors were also present. The removal of the tube from the thermostatted bath to record spectra introduced errors into both the timing of the reaction and the extent of reaction recorded since some reaction would occur during the recording period even when the tube temperature was reduced. The latter source of errer was least for reactions at 50° since the temperature change was greatest. For 2,4-dimethylcyclopentanone the total decrease in the recorded integral was only one-sixth of the initial integral introducing potentially large errors into the normalisation procedure.

7.4.1c: Derivation of Rate Constants from the Data:

The integral of the enolising proton signal was normalised by dividing it by the integral of the reference signal. A 'theoretical' infinity was obtained by multiplying the initial normalised integral by the ratio of the number of labile protons to the total number of protons in the undeuteriated substrate.

If the normalised integral at time $t = X_t$ and at time $\infty = X_\infty$, then,

$$(S)_t \propto (X_t - X_\infty)$$

where $(S)_t$ denotes the concentration of undeuteriated substrate at time t.

An argument similar to that given in Section 7.1.1d may be used to obtain the rate constants, viz.,

$$\frac{-d(S)}{dt} = \bar{k}_1(S)$$

$$\text{i.e.} \quad \bar{k}_1 t = -\ln (S) + \text{a constant}$$

$$\bar{k}_1 t = -\ln (X_t - X_\infty) + \text{a constant}$$

Thus, a graph of $\ln (X_t - X_\infty)$ vs. time gave a linear plot, the modulus of the slope being $\bar{k}_1$.

7.4.1d: Typical Kinetic Runs:

Examples of plots of $\ln (X_t - X_\infty)$ vs. time are given by Figures 16, 17 and 18.

Figure 16: Kinetic plot of ln $(X_t - X_\infty)$ vs. time for the enolisation of cyclopentanone at 50.8°. RUN 407.

[Substrate] = 0.41 M; (D_2SO_4) = 0.97 M

Theoretical infinity calculated as $X_\infty = 1.10$

The plot represents 85 % loss of labile protons

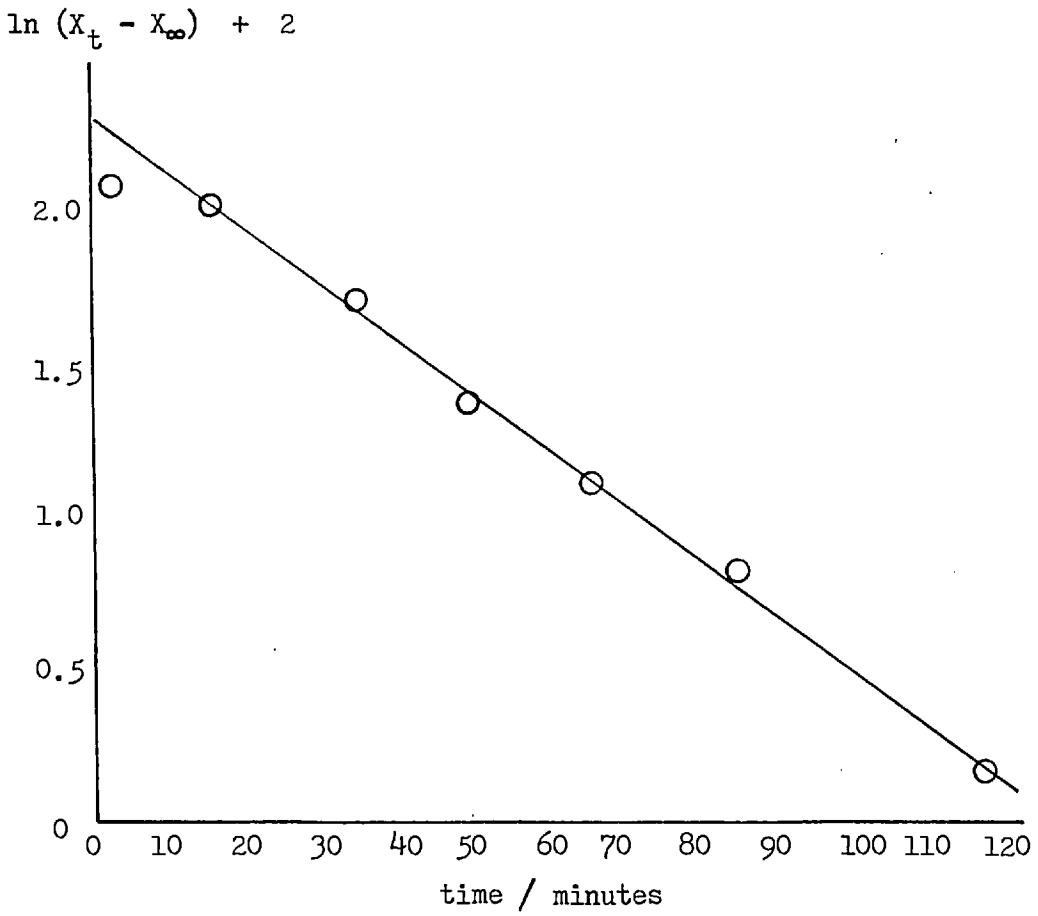


Figure 17: Kinetic plot of ln $(X_t - X_\infty)$ vs. time for the enolisation of 4-methylcyclohexanone at 50.8°.

RUN 415

[Substrate] = 0.1 M; (D_2SO_4) = 0.8 M

The plot represents 98.4 % reaction

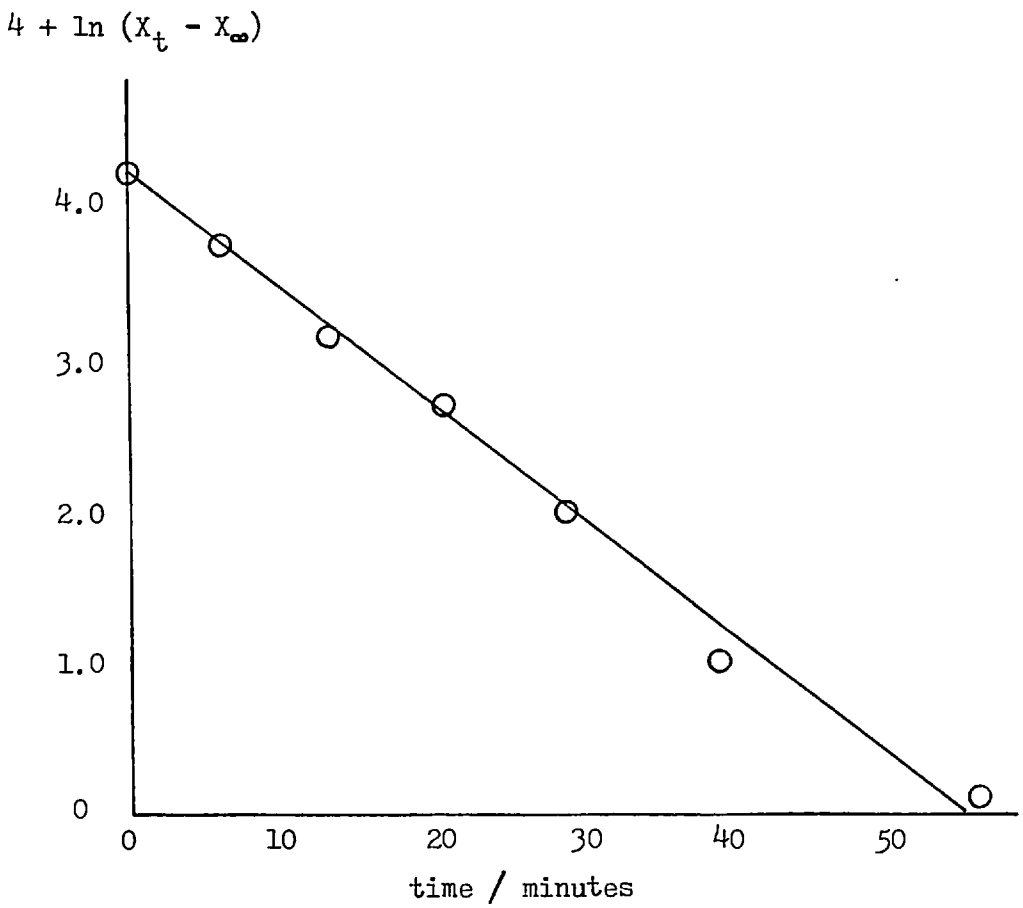


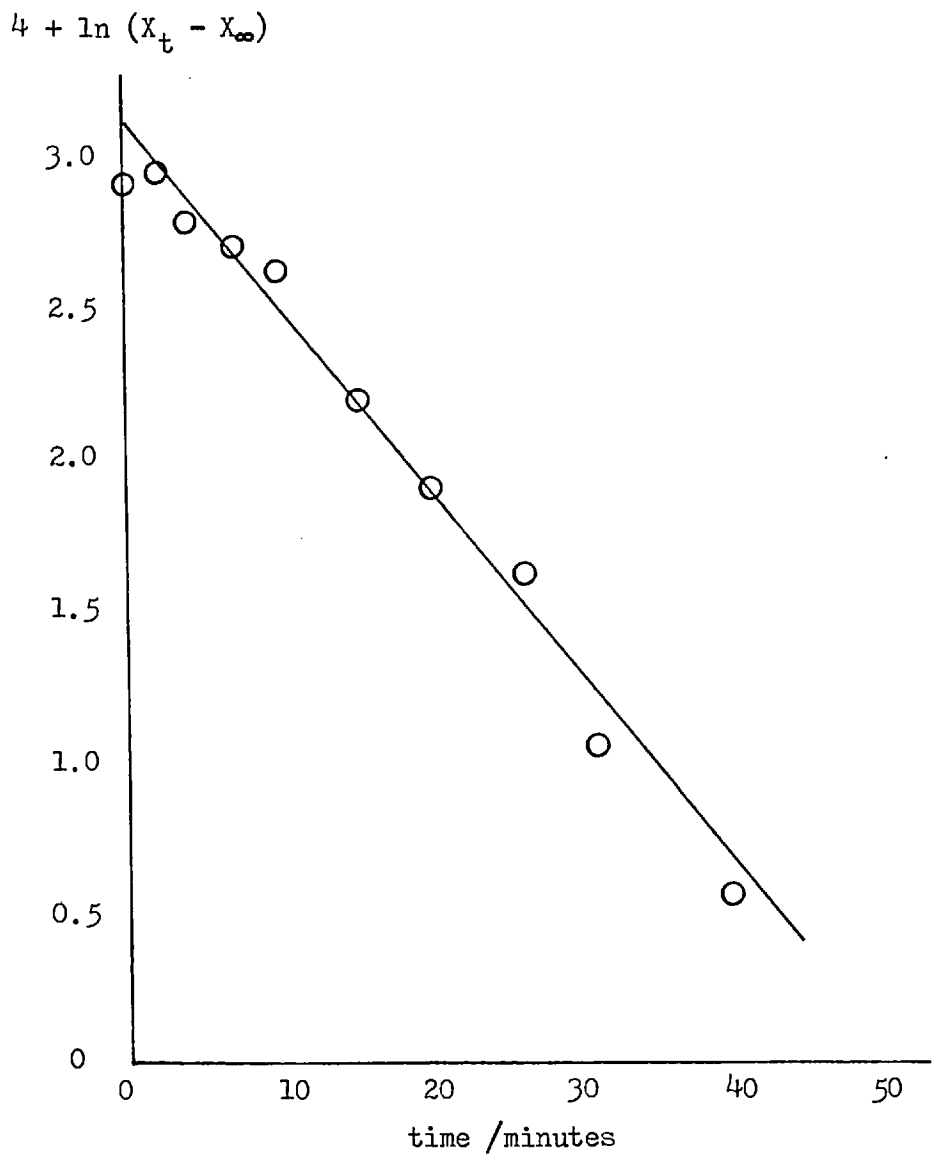
Figure 18: Kinetic plot of ln $(X_t - X_\infty)$ vs. time for the nitrosation of 2,5-dimethylcyclopentanone at 50.8°.

RUN 433

[Substrate] = 9.6×10^{-2}M; (D_2SO_4) = 0.6 M

$(NaNO_2) = 10^{-2}$M

The plot represents 89 % loss of labile protons



7.4.1e: Precision of the Measured Rate Constants:

In certain cases exact reproducibility was obtained but usually an error of ± 10 % was apparent. Considering that the T60 gave an inherent error of ± 5 % the overall error was quite acceptable. For 2,4-dimethylcyclopentanone runs at low acid concentrations rate constants were found to vary by as much as a factor of 2. Results quoted in Section 6.1.3 were therefore critically selected as the most reliable of those obtained.

7.4.2: Protodetritiation Measured by Liquid Scintillation Counting:

7.4.2a: Experimental Procedure:

To a volumetric flask (10 cm^3) was added the required volume of a stock solution of sulphuric acid, e.g. 3.0 cm^3 of 1 M solution to give (H_2SO_4) = 0.3 M, and water was added so that sufficient volume remained for addition of nitrite solution, if required. The flask was thermostatted for a minimum of 10 minutes. A quantity of neat tritiated ketone (ca. 2 μl, hence [ketone] = 1.6 x 10^{-3}M) was added from a syringe and, if required, a measured volume of nitrite stock solution. The volume was made up with water, the flask shaken and the flask replaced in the thermostatted bath. At timed intervals aliquots were removed and assayed.

7.4.2b: Radiochemical Assay:

Typically, an aliquot (1.0 cm^3) of the reaction solution containing the tritiated ketone was added to xylene (10 cm^3) in a stoppered bottle and shaken for 1 minute to extract the ketone. The bottle contents were allowed to separate and an aliquot (5.0 cm^3) of the upper xylene layer was taken and added to a quantity (5.0 cm^3) of scintillator solution (0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazoly l)] benzene and 4 g 2,5-diphenyloxazole per dm^3 of benzene) in a scintillation counting vial. Scintillations were recorded in a liquid scintillation counter as counts per minute (c.p.m.) for a known period of time or until a predetermined accuracy was obtained for the count.

7.4.2c: Sources of Error:

The method was applied only to 2,5-[$^3H_1$]-2,5-dimethylcyclopentanone with the intention of removing the possibility of further reaction of the C-nitroso function, if formed, to give α-oximino-ketone or 1,2-quinone products. Side reaction of this nature should not interfere with the kinetics, therefore. 1,2-bond cleavage was a possibility.

Some decomposition of nitrite occurred but since the nitrite was in ca. 8-fold excess the pseudo-first order kinetics would be preserved. Kinetic plots did not indicate deviation from such kinetic order for at least nine hours of reaction at 0°.

The usual volumetric errors were present in the preparation and sampling of the reaction solutions but, in addition, there would be further errors in the extraction procedure. However, tests of the extraction procedure (by taking 3 samples from an aqueous solution of the ketone) showed agreement of counts as ± 1 %. The reliability of a particular count was determined by the pre-set error of the counter. The minimum error obtainable was ± 0.2 %.

7.4.2d: Derivation of Rate Constants from the Data:

The method of Section 7.4.1c was applied to the data. In this case, X_t and X_∞ were the c.p.m. of samples taken at times t and ∞, respectively.

7.4.2e: Typical Kinetic Runs:

Examples of plots of ln $(X_t - X_\infty)$ vs. time are given by Figures 19 and 20. The plots show data obtained with and without nitrite in the reaction solutions.

Figure 19: Kinetic plots of ln $(X_t - X_\infty)$ vs. time for the protodetritiation of 2,5-[$^3H_1$]-2,5-dimethyl-cyclopentanone with and without nitrite at 50°

$$[\text{Substrate}] = 1.6 \times 10^{-3}\text{M}$$

$$[H_2SO_4] = 0.4\ \text{M}$$

The plots represent 77 % protodetritiation without nitrite and 84 % with nitrite.

○ RUN 460, no nitrite

△ RUN 458, $[NO_2^-] = 2 \times 10^{-2}$M

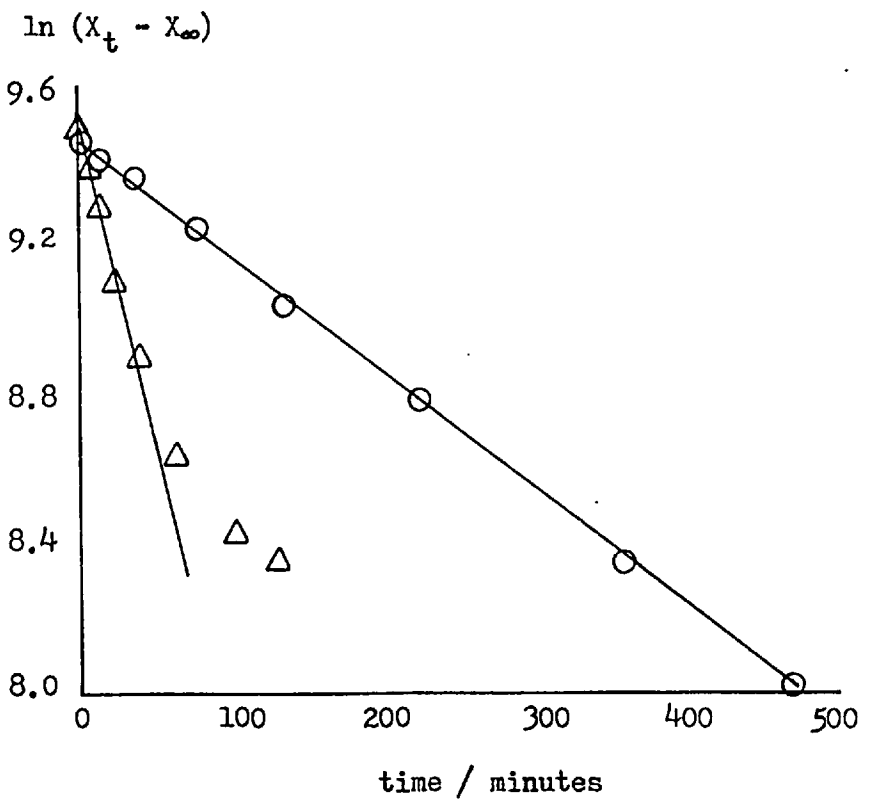


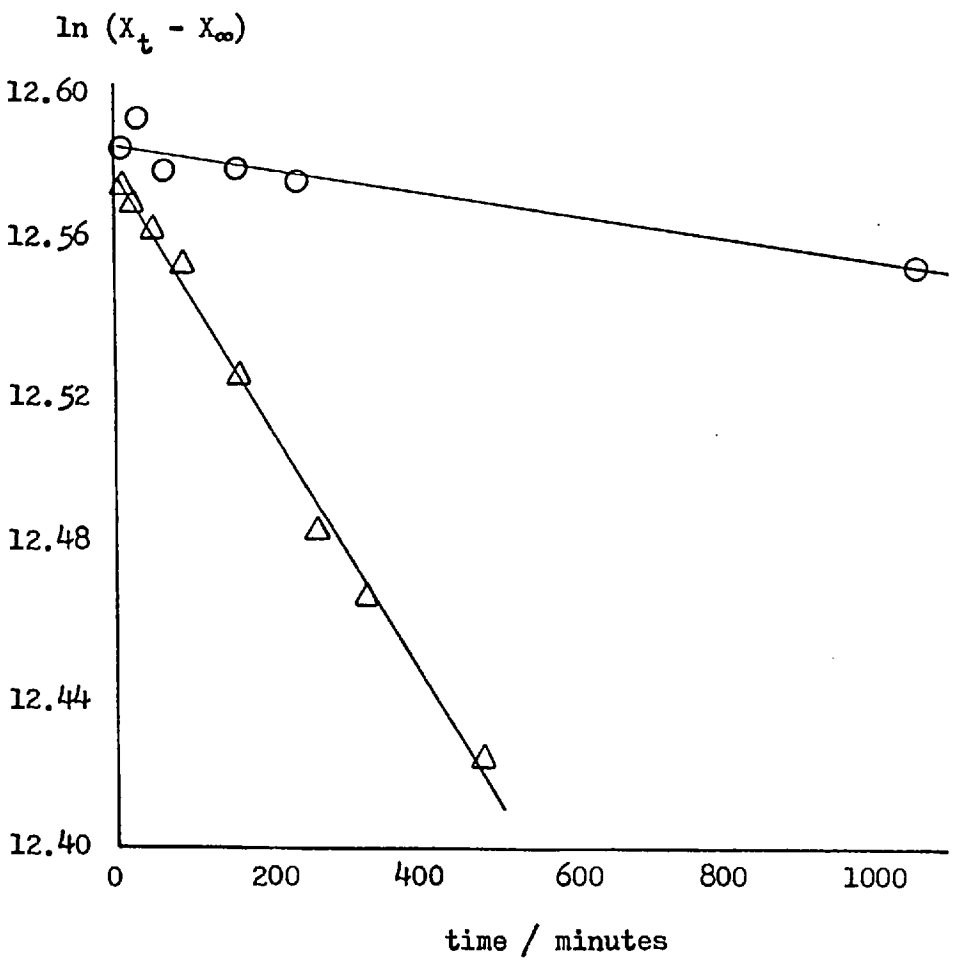
Figure 20: Kinetic plots of ln $(X_t - X_\infty)$ vs. time for the protodetritiation of 2,5-[$^3H_1$]-2,5-dimethylcyclopentanone with and without nitrite at 0°

[Substrate] = 1.6×10^{-3}M; $[H_2SO_4]$ = 0.9 M

The plots represent 3 % protodetritiation without nitrite and 14 % with nitrite.

RUN 472, no nitrite

RUN 474, $[NO_2^-] = 1.5 \times 10^{-2}$M



7.4.2f: Precision of the Measured Rate Constants:

Some runs gave an error $< \pm 1$ % but the usual error was within ± 5 %.

For runs in which hypochlorite replaced nitrite, repeat runs were not performed so that errors in reproducibility can not be given. The absolute value of the rate constants for such runs was not important but their magnitude relative to those of the enolisation without catalyst other than H_3O^+.

7.4.3: Analysis of Reaction Mixtures for Unreacted Nitrite:

The method was applied to cyclopentanone and 2,2,5,5-[$^2H_1$]-cyclopentanone to investigate the kinetic hydrogen isotope effect for the nitrosation reaction. The account of the method given in Section 7.1.1 pertains to this reaction system .

7.4.3a: Typical Kinetic Run:

Rate constants were calculated from plots of ln A_t *vs.* time. Figure 21 gives a plot for nitrite decomposition and ketone nitrosation.

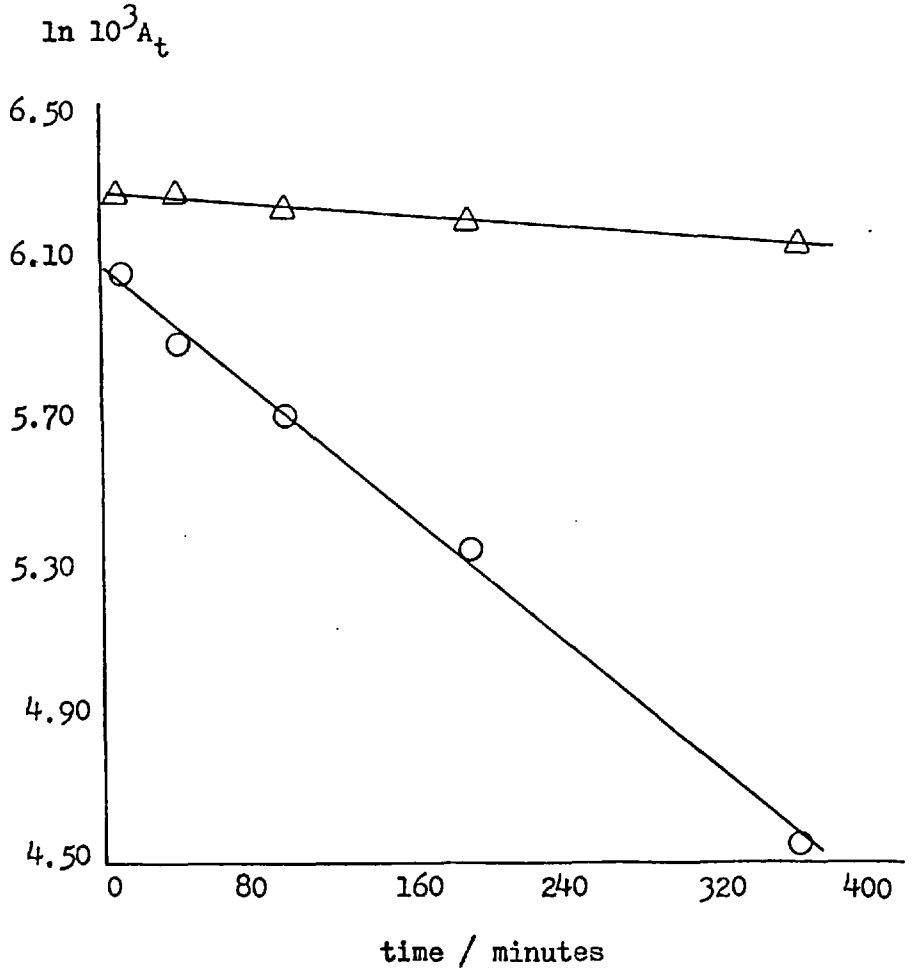
Figure 21: Kinetic plot of ln A_t *vs.* time for the nitrosation of 2,2,5,5-[$^2H_1$]-cyclopentanone in deuteriosulphuric acid at 25°.

RUNS 504 and 505

△ Nitrite decomposition (505)

○ Nitrosation (504)

The plot represents 81 % loss of nitrite for the nitrosation



7.5: Preparation and Purification of Materials:

Reagent grade cyclopentanone was shaken with aqueous $KMnO_4$ and separated. The cyclopentanone was dried with anhydrous Na_2SO_4, filtered and dried overnight in contact with molecular sieve (Linde 4A). The ketone was fractionally distilled the fraction of b.p. 129° being retained. n_D^{23} 1.4355 (lit.[106] n_D^{23} 1.4352).

2,2,5,5-[$^2H_1$]-cyclopentanone was prepared by stirring cyclopentanone (5 cm^3), deuteriosulphuric acid (1.5 cm^3) and deuterium oxide (20 cm^3) for 2 h at 55°. The mixture was neutralised with anhydrous K_2CO_3, extracted with diethyl ether (Na dried) and the extracts dried with anhydrous K_2CO_3, filtered and evaporated. The residue was fractionally distilled from molecular sieve, the fraction of b.p. 128° being retained and stored in contact with molecular sieve.

4-methylcyclohexanone was fractionally distilled, the fraction of b.p. 169° being retained (lit.[103] b.p. 170, 761 mmHg).

2,5-dimethylcyclopentanone (Aldrich) was fractionally distilled, the fraction of b.p. 145° being retained (lit.[107] 147-9°). The material was stored in contact with molecular sieve. n_D^{23} 1.4305 (lit.[107] n_D^{25} 1.4279).

2,5-dimethylcyclopentanone was tritiated in acid or base as follows:

(i) Acid: 2,5-dimethylcyclopentanone (0.5 cm^3), THO (2.0 cm^3, 200 C cm^{-3}), ethanol (2.0 cm^3) and concentrated H_2SO_4 (0.2 cm^3) were stirred for 2.5 h. THO (5 drops, 10 C / 50 cm^3) was added and the mixture stirred for a further 2 h. The mixture was

extracted with diethyl ether, the extracts dried over anhydrous $MgSO_4$ and evaporated. The residue was fractionally distilled, the fraction of b.p. 140° being retained. n_D^{23} 1.4305 (lit.[107] n_D^{25} 1.4279). Activity, 0.5 l in 5 cm^3 scintillator solution gave a count of ca. 5.8×10^4 c.p.m.

(ii) Base: 2,5-dimethylcyclopentanone (0.5 cm^3), sodium hydroxide (0.5 cm^3, 1 M aqueous), THO (10 drops, 10 C/50 cm^3) and ethanol (10 drops) were shaken for ca. 20 minutes. The mixture was worked up as above. The material retained was of b.p. $140\text{-}2^{\circ}$, $n_D^{22.5}$ 1.4305 (cf. lit. value above). Activity, 0.5 l in 5 cm^3 scintillator solution gave a count of ca. 6×10^5 c.p.m.

Deuterium oxide (Merck, Sharp and Dohme, Canada, Ltd., 99 atom % D) was used as supplied.

Deuteriosulphuric acid (Merck, Sharp and Dohme, Canada, Ltd., 99 atom % D) was used as supplied.

Tritium oxide (Radiochemical Centre, Amersham) of activity 10 C cm^{-3} was used as supplied.

AnalaR grade sodium nitrite, sodium chloride and sodium perchlorate were dried in vacuo over P_2O_5 and used without further purification.

REFERENCES

1. P.N. Magee and J.N. Barnes, Brit. J. Cancer, 1956, 10, 114.
2. idem, Adv. Cancer Res., 1967, 10, 163.
3. P.A.S. Smith and R.N. Loeppky, J. Amer. Chem. Soc., 1967, 89, 1147.
4. R. Doll, Nature, 1977, 265, 589.
5. C.D. Bartlett, Ph.D. Thesis, University of London, 1977, pp. 2-9.
6. C.L. Walters, Chem. Brit., 1977, 13, 140.
7. G. Hawksworth, M.J. Hill, G. Cordillo, C. Cuello, "N-nitroso Compounds in the Environment", I.A.R.C., Lyon, I.A.R.C. Scientific Publication No. 9 (1975), pp.229-234.
8. M.J. Hill and G. Hawksworth, ibid, pp. 220-222.
9. A. Wild, Nature, 1977, 268, 197.
10. A. Mirna and K. Coretti, Fleischwirtschaft, 1974, 54, 507.
11. L.F. Larkworthy, M.H. Turnbull and A.Yavari, Chem. Ind., 1977, 401.
12. J.A. Perigo, et al., J. Food Technol., 1967, 2, 377 and 1968, 3, 91.
13. J. Nordal and R. Gudding, Acta Vet. Scand., 1975, 16, 537.
14. P.L. Haust, J.W. Spence and M. Miller, Environ. Sci. Technol., 1977, 11, 403.
15. N.D. Kramer, J. Am. Med. Assn., 1977, 237, 1693.
16. S.S. Mirvish, Toxic. app. Pharmacol., 1975, 31, 325.
17. L.K. Keefer and D.P. Roller, Science, 1973, 181, 1245.
18. B.C. Challis and S.A. Kyrtopoulos, Brit. J. Cancer, 1977, 35, 693.
19. A.J. Haagen-Smit, M.F. Brunelle and J. Hara, A.M.A. Arch. Ind. Health, 1959, 20, 399.
20. V. Norman and C.H. Keith, Nature, 1965, 205, 915.
21. T.A. Gough and K. Goodhead, J. Sci. Food Agric., 1975, 26, 1473.
22. T.A. Gough et al., J. Sci. Food Agric., 1977, 28, 345.
23. B.E. Wells, R. Walker and C.L. Walters, J. Sci. Food Agric., 1974, 25, 1048.
24. H. Marquardt, T. Rufino and J.H. Weisburger, Science, 1977, 196, 1000.
25. J.P. Seiler, Mutation Res., 1977, 48, 225.
26. J. Sandler and F. Schweinberg, Abl. Bakt. Hyg., 1972, B156, 299.
27. D.H. Fine et al., Nature, 1977, 265, 753.
28. P.N. Sen et al., J. Agric. Food Chem., 1976, 24, 397.

29. D.S. Mottram and R.L.S. Patterson, J. Sci. Food Agric., 1977, 28, 352.
30. G. Woolford and R.G. Casseus, J. Food Sci., 1977, 42, 586.
31. E.A. Walker, B. Pignatelli and M. Castagnaro, Nature, 1975, 258, 176.
32. B.C. Challis and C.D. Bartlett, Nature, 1975, 254, 532.
33. B.C. Challis, Nature, 1973, 244, 466.
34. C. Cantoni et al., Ind. Aliment. (Italy), 1974, 13, 118.
35. M.E. Knowles et al., Nature, 1974, 247, 288.
36. M.E. Knowles et al., Nature, 1974, 249, 672.
37. M.E. Knowles et al., J. Sci. Food Agric., 1975, 26, 267.
38. R. Davies and D.J. McWeeny, Nature, 1977, 266, 657.
39. J.W. Wallace and W.S. Caughey, Biochem. Biophys. Res. Commun., 1975, 62, 561.
40. R. Bonnett et al., J. Chem. Soc. Chem. Commun., 1975, 884.
41. B.C. Challis et al., I.A.R.C. Scientific Publication No. 9 (1975), pp. 94-99.
42. T. Marshall and L.R. Dugan, J. Agric. Food Chem., 1975,23,975.
43. P.B.D. de la Mare and J.H. Ridd, "Aromatic Substitution, Nitration and Sulphonation", Butterworths, London, 1959.
44. R.O.C. Norman and R. Taylor, "Electrophilic Substitution in Benzenoid Compounds", Elsevier, 1965.
45. R. Taylor, "Aromatic and Heteroaromatic Chemistry", ed. C.W. Bird and G.W.H. Cheeseman, Specialist Periodical Report, The Chemical Society, London, 1973, 1, 176.
46. H. Zollinger, Adv. Phys. Org. Chem., 1964, 2, 163.
47. E. Berliner, Prog. Phys. Org. Chem., 1964, 2, 253.
48. R.J. Higgins, Ph.D. Thesis, University of London, 1972.
49. B.C. Challis and R.J. Higgins, J. Chem. Soc. Perkin II, 1973, 1597.
50. B.C. Challis, R.J. Higgins and A.J. Lawson, J. Chem. Soc. Perkin II, 1972, 1831.
51. B.C. Challis and R.J. Higgins, J. Chem. Soc. Perkin II, 1972, 2365.
52. B.C. Challis and A.J. Lawson, J. Chem. Soc. (B), 1971, 770.
53. L. Melander, Arkiv. Kemi, 1959, 2, 211.
54. K. Schofield et al., J. Chem. Soc. (B), 1968, 800.
55. J. Jahelka, V. Sterba and K. Valter, Coll. Czech. Chem. Commun., 1973, 38, 877.

56. D.A. Morrison and T.A. Turney, J. Chem. Soc., 1960, 4827 .
57. J.S.L. Philpot and P.A. Small, Biochem. J., 1938, 32, 534.
58. ibid., 1938, 32, 542.
59. A. Kurosky and T. Hofmann, Can. J. Biochem.,1972, 50, 1282.
60. R. Bonnett et al., J. Chem. Soc. Perkin II, 1975, 2261.
61. H. Shimura, J. Chem. Soc. Japan, 1961,82,641.
62. M.C. Archer et al., "Environmental N-nitroso Compounds Analysis and Formation", I.A.R.C. Scientific Publication No. 14 (1976), pp. 141-5.
63. P. Lumme and J. Tummavuori, Acta Chem. Scand., 1965, 19, 617.
64. J.N. Bronsted and K. Pederson, Z. Physik. Chem., 1923, A108, 185.
65. J.F. Bunnett, "Technique of Organic Chemistry", Vol. VIII, Part I, 2nd Edn., ed. A. Weissberger, Interscience, p. 239.
66. "Proton Transfer Reactions", ed. E. Caldin and V. Gold, Chapman and Hall, London, 1975, pp. 188-194 and refs. therein.
67. C.K. Ingold, "Structure and Mechanism in Organic Chemistry", G. Bell & Sons Ltd., London, 1953, pp. 535, 566.
68. E. Buncel, "Carbanions: Mechanistic and Isotopic Aspects", Elsevier, Amsterdam, 1975, pp. 69-90.
69. A. Lapworth, J. Chem. Soc., 1904, 85, 30.
70. H.M. Dawson and J.S. Carter, J. Chem. Soc., 1926, 2282. H.M. Dawson and C.R. Hoskins, ibid, 1926, 3166.
71. O. Reitz, Z. Physik. Chem., 1937, 179, 119.
72. C. Rappe, Acta Chem. Scand., 1967, 21, 1200; 1968, 22, 219.
73. Ref. 68, p. 81 and refs. therein.
74. O. Touster, Org. React., 1953, 7, 327.
75. R.B. Woodward and W.v.E. Doering, J. Amer. Chem. Soc., 1945, 67, 860.
76. M.M. Rogic et al., J. Amer. Chem. Soc., 1977, 99, 1156.
77. M.M. Rogic et al., J. Org. Chem., 1977, 42, 2748.
78. J.K. Rasmussen and A. Hassner, J. Org. Chem., 1974, 39, 2558.
79. G. Lucas, Ger. Offen., 2209626; CA, 1972, 77, 164141.
80. C.L. Osborn and M. R. Sandner, U.S. Patent, 3816285, CA, 1974, 81, 71041.
81. A.F. Ferris et al., J. Org. Chem., 1960, 25, 492.
82. R. Kikumoto and T. Koboyashi, CA, 1972, 76, 33859.
83. Farbenfabriken Bayer A.-G., Fr., 1603442; CA, 1972, 76, 59223.

84. H. Brachwitz, J. Prakt. Chem., 1971, 313, 667.
85. H. Dahn et al., Helv. Chim. Acta, 1973, 56, 457.
86. M.G. Johnson et al., Ger. Offen., 2528786; CA, 1976, 84, 150492.
87. M.F. Chen and S.F. MacDonald, Can. J. Chem., 1974, 52, 1760.
88. A.J. Razumov et al., Zh. Obsch. Khim. SSSR, 1977, 47, 1192; CA, 1977, 87, 135631.
89. Y. Ogata et al., J. Amer. Chem. Soc., 1963, 85, 3649.
90. K. Singer and P.A. Vamplew, J. Chem. Soc., 1957, 3052.
91. R.P. Bell and K. Yates, J. Chem. Soc., 1962, 1927.
92. B.C. Challis, L.F. Larkworthy and J.H. Ridd, J. Chem. Soc., 1962, 5203.
93. R. Bakule and F.A. Long, J. Amer. Chem. Soc., 1963, 85, 2309.
94. ibid, 1963, 85, 2313.
95. Ref. 65, pp. 393, 397, 414, and refs. therein.
96. M.B. Shinn, Ind. Eng. Chem. Anal., 1941, 13, 33.
97. N.F. Kershaw and N.S. Chamberlain, ibid, 1942, 14, 312.
98. A.J. Lawson, Ph. D. Thesis, University of London, 1970.
99. J.M. Tedder, J. Chem. Soc., 1957, 4003.
100. Ref. 61.
101. "Dictionary of Organic Compounds", ed. I. Heilbron, 1934, 60.
102. Kirby and Ogunkoya, J. Chem. Soc., 1965, 6914.
103. "Handbook of Chemistry and Physics", 55th Edn., 1974-5.
104. Sealock, J. Biol. Chem., 1946, 166, 1, 3.
105. G. Cronheim, J. Org. Chem., 1947, 12, 17.
106. D.D. Perrin, W.L.F. Armarego and D.R. Perrin, "Purification of Laboratory Chemicals", Pergamon, London, 1966, 122.
107. N.M. Bortnick, CA, 1959, 53, 9100.
108. R.C. Massey, C. Crews, R. Davies and D.J. McWeeny, J. Sci. Food Agric., 1978, 29, 815.