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ASPECTS OF THE DENITROSATION OF

NITROSO COMPOUNDS IN

ACIDIC SOLUTION

BY

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(Van Mildert College)

A thesis submitted for the degree of

Doctor of Philosophy in the

University of Durham

Department of Chemistry

1981

ACKNOWLEDGEMENTS

TO

My Parents,

M.R.J. and J.E.A.

"He writes as well as he climbs"

Anon.

DECLARATION

The work described in this thesis was carried out in the
University of Durham between October 1976 and June 1979 and has
not been submitted for any other degree. It is the original work
of the author except where acknowledged by reference.

ABSTRACT

The thesis seeks to provide a rationale for the behaviour of a wide range of N-nitroso compounds in acidic solution.

The work is introduced by a review of the various mechanisms proposed to date.

It is shown that the behaviour of the system towards certain catalytic agents is dictated by the relative rates of the protonation and denitrosation reactions. The effect of changing nitroso compound structure, changing nucleophile concentrations and changing solvents has been investigated and the results used to provide a common rationale for the denitrosation and N-nitrosation reactions.

Supporting data is provided in the form of investigations of the denitrosations of N-methyl-N-nitrosoaniline, N-nitrosodiphenylamine, N-methyl-N-nitrosourea and a series of p-substituted N-methyl-N-nitrosoanilines. The N-nitrosation of N-methylurea is also examined.

A comprehensive study of the action of sulphur containing nucleophiles such as the thioureas has led to the proposal of a reaction between N-methyl-N-nitrosoaniline and the naturally occurring nucleophiles, cysteine, S-methylcysteine, glutathione and methionine. The results may be important in the elucidation of cancer mechanisms.

Evidence is also provided for an interaction between N-methyl-N-nitrosoaniline and the ferrous ion.

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THE ACID CATALYSED REACTIONS OF N-NITROSO COMPOUNDS.

OBSERVATIONS FROM THE LITERATURE.

1.1 The Fischer-Hepp Rearrangement

Following the early work of Fischer and Hepp[1,2,3,4] in 1886, it

has long been known that aromatic N-nitrosamines (1) react readily

under the influence of selected mineral acids to yield the corresponding

C-nitrosamine (2).

R   NO   R   H   R   H
  N   N   +   N

(1)   (2)   (3)

H^+

NO
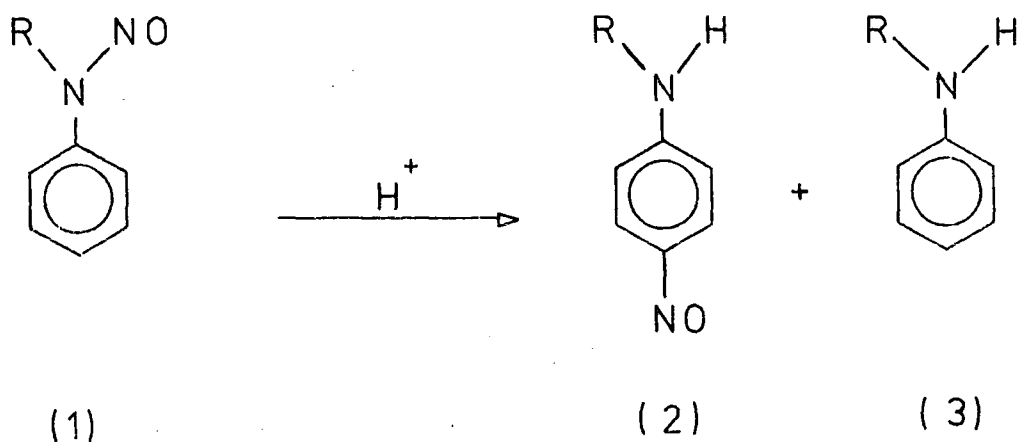
Under appropriate conditions substantial yields of the secondary

amine (3) may be obtained as a co-product. The rearrangement:

denitrosation product ratio is easily controlled by a judicious selection

of the reaction conditions and the reaction is able to provide a readily

accessible synthetic route towards either of the two possible products.

In ethanolic solutions of HCl production of the rearrangement product

predominates[5] and the reaction affords a convenient method of preparing

the para-nitrosamines[6]. Maximum yields of the denitrosation product are

favoured by the use of aqueous solutions of the mineral acid in the

presence of high concentrations of a nitrite trap such as urea[7] or CuCl[8].

In any discussion concerning the mechanism of the reaction the

question arises as to whether the rearrangement reaction is of an intramolecular nature, proceeding independently of the denitrosation reaction, or of an intermolecular nature with denitrosation to give a free nitrosating agent being followed by a nitrosation of the resultant secondary amine. In a historical context, attempts to provide an answer have revolved around the interpretation of four, key experimental observations.

(a) Yields of the rearrangement product are found to be largest when HCl is used as the acid catalyst. Use of sulphuric acid or nitric acid produces low yields and use of hydrobromic acid yields mainly the denitrosation product[9].

(b) Addition of sodium nitrite to the reaction mixture results in an increased overall yield of the rearrangement product[10].

(c) A number of trans-nitrosations are reported[10]. Thus the reaction of N-nitrosodiphenylamine (4) in the presence of N,N-dimethylaniline (5) leads to the formation of C-nitroso-N,N-dimethylaniline (6) in addition to the normal rearrangement product (7).
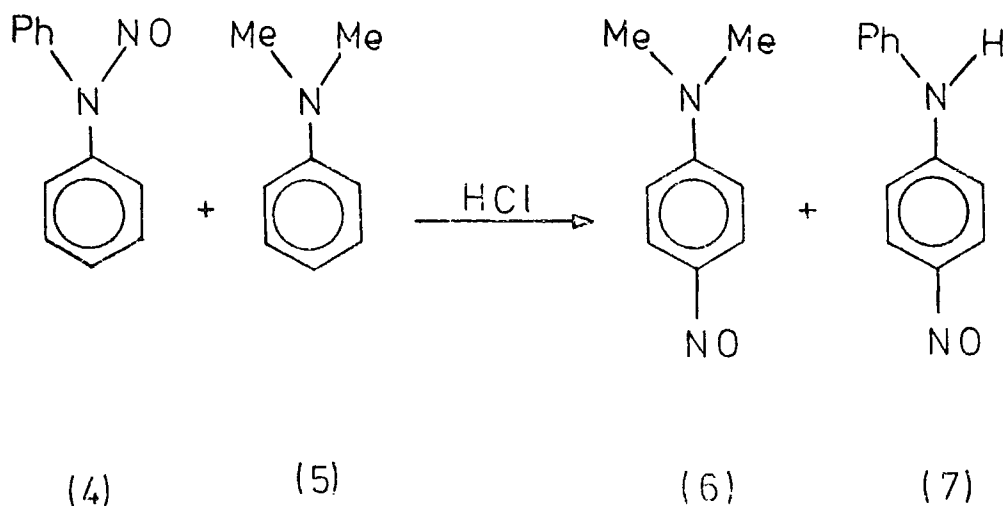
Similarly the reaction of N-methyl-N-nitrosoaniline (8) in the presence of the reactive olefin (9) gives the NOCl-adduct (10) amongst the products.

Me   N O
  \\ /
   N

   +

CH=CHMe

   +

OMe

HCl →

CHClCH(NO)Me

OMe

Me   H
  \\ /
   N

N O

(8)   (9)   (10)
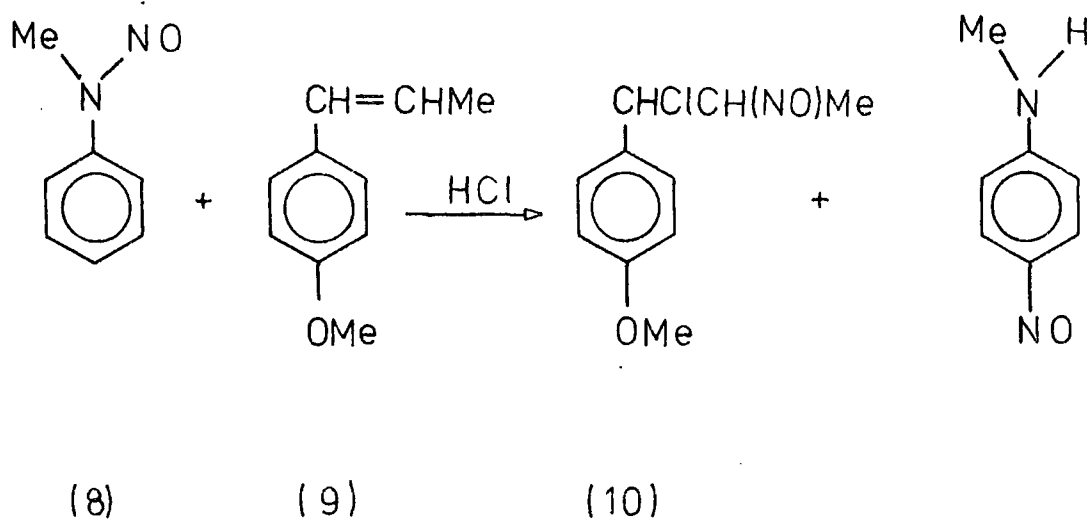
(d). Reaction of meta-nitro-N-methyl-N-nitrosoaniline (11) in the presence of a large excess concentration of urea is reported to give only the denitrosation product (12).

Me   N O
  \\ /
   N

NO$_2$

H$^+$/urea →

Me   H
  \\ /
   N

NO$_2$

(11)   (12)
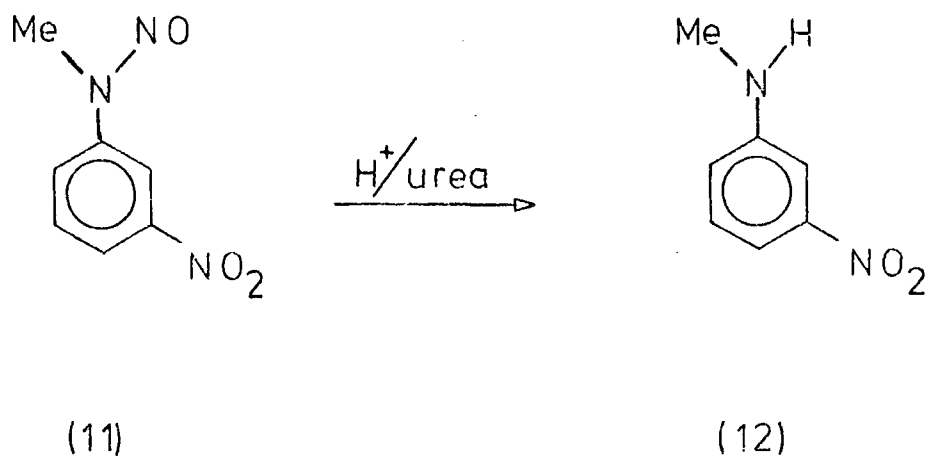
On the basis of these four points of evidence one may propose a series of plausible mechanisms.

1.2 The Intermolecular Mechanism

The intermolecular mechanism, as first proposed by Houben[11] in 1913, is depicted below.

X + NOY $\xrightarrow{k_3}$ Products
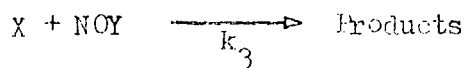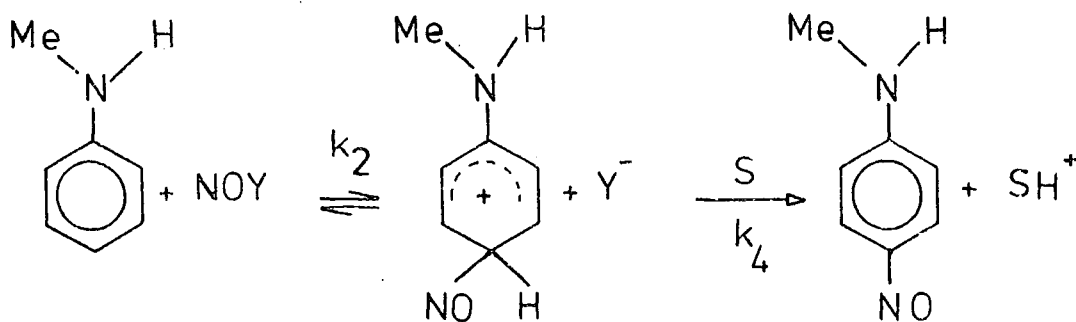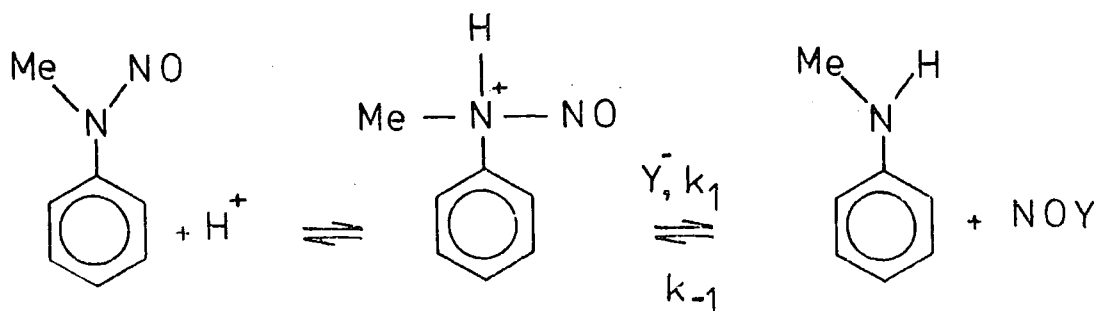
X represents a nitrite trap   e.g. urea or sulphamic acid

Y^- represents a nucleophile   e.g. bromide ion or chloride ion

S represents the solvent   e.g. ethanol or water.

An explanation of the system in terms of an intermolecular mechanism for the rearrangement therefore requires the postulation of an initial denitrosation reaction which may then be followed by a direct electrophilic substitution brought about by the action of the free nitrosating agent, NOY, at the para position of the amine. The nitrite trap, X, may take any one of a number of different identities; urea, sulphamic acid, hydrazoic acid, hydroxylamine, aniline, and hydrazine have all been employed with some success[11]. In the absence of an added nitrite trap the nitrosyl halide might be expected to undergo decomposition via reaction with the solvent.

It may be seen that the scheme proposed by Houben is entirely compatible with the four points of evidence presented in Section 1.1.

(a) The large yields of the rearrangement product obtained in the presence of HCl are taken as reflecting the important role of the halide ion in the reaction scheme. In sulphuric acid and in nitric acid, which can not provide the nitrosyl halide, very much lower yields of the rearrangement product are obtained. The production of the secondary amine as the main product in the presence of hydrobromic acid may be explained on the basis of the lower polarisation of the resultant NOBr compared with that of NOCl. The NOBr is thus insufficiently reactive to perform the required electrophilic attack at the para position of the aromatic amine.

(b) Addition of sodium nitrite to the reaction mixture might be expected to give increased yields of the rearrangement product since formation of the nitrosyl halide will be enhanced by the following reaction:-

$$NO_2^- + Cl^- + 2H_3O^+ \rightleftharpoons NOCl + 3H_2O$$
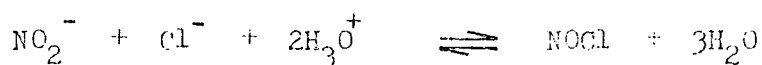
(c) The observations concerning the transnitrosation reactions
may be taken as being indicative of the existence of a free nitrosating
agent as required by the Houben mechanism.

(d) The presence of a large excess concentration of a nitrite trap
such as urea may be seen to promote the removal of free nitrosyl halide.
Under such conditions as these a significant yield of the rearrangement
product is not to be expected.

As a consequence of its apparent compatability with the known facts
the intermolecular mechanism has enjoyed a general acceptance and is
widely reproduced in the chemical literature[12,13]. Although the
intermolecular scheme is quite reasonable in terms of the experimental
observations detailed above it is by no means specifically demanded by
then and recent study has led to the postulation of a number of
alternatives[11,14,15,16,17]. The work of Williams and co-workers is
discussed below whilst a short review of the work of others is reserved
for Section 1.5.

1.3 The Intramolecular Mechanism

In 1968 the experimental work of Aslapovskaya et al.[14] led them to
believe that the mechanism of the Fischer Hepp rearrangement might best
be represented in terms of an intramolecular scheme. The experimental
results were subsequently confirmed by Williams and Morgan[18] who put
forward the scheme depicted over.

X + NOY $\xrightarrow{k_3}$ Products
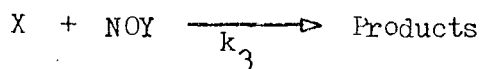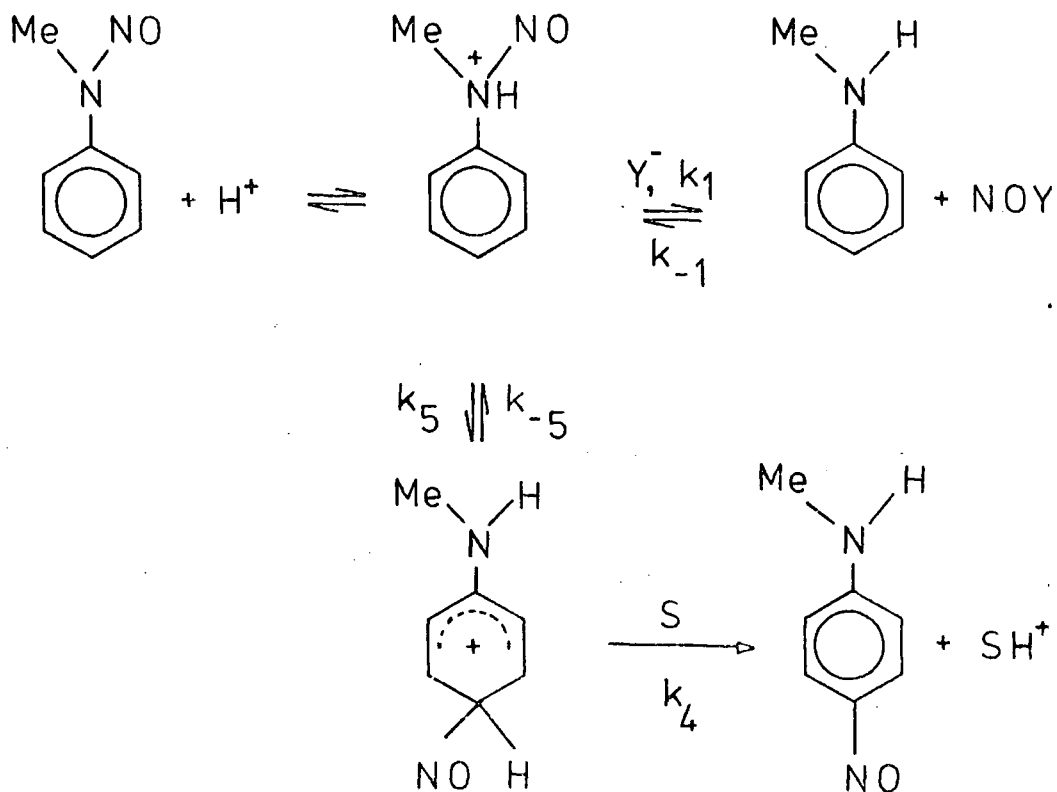
X represents a nitrite trap

Y represents a nucleophile

S represents the solvent

Rearrangement is thus proposed to occur concurrently with the denitrosation reaction via an independent, intramolecular pathway. If we consider the present scheme in the context of the four points of evidence which formed the basis of the intermolecular scheme's acceptance it becomes clear that the intramolecular scheme is capable of providing us with a series of alternative rationalisations.

(a) The maximisation of the yield of rearrangement product which occurs when HCl is used as the mineral acid is readily explained. In the case of HBr the greater nucleophilicity of the bromide ion, as compared with that of the chloride ion means that denitrosation may compete more effectively with the rearrangement reaction for the protonated nitrosamine species which is common to both routes. The recorded yield of rearrangement product in the HBr reaction is thus reduced compared with that obtained for the reaction with HCl. The low yields given by aq. H_2SO_4 and aq. HNO_3 when compared with those obtained in ethanolic HCl may well be attributed to the differing solvating properties of the solvents concerned; a detailed arguement is precluded here by our lack of knowledge regarding the intermediates and transition states of the rearrangement process.

(b) Addition of sodium nitrite results in an increase in the rate of the "reverse", N-nitrosation reaction governed by k_{-1}. The denitrosation reaction is suppressed and the increased stationary concentration of the protonated N-nitrosamine is reflected in an increased rate of intramolecular rearrangement. In the presence of sodium nitrite the rearrangement reaction is thus able to compete favourably with the denitrosation reaction, resulting in an increased yield of rearrangement product.

(c) Since the present scheme involves the production of a free nitrosating agent, NOY, explanation of the observation of cross-nitrosation reactions may be made on the same basis as for the intermolecular scheme.

(d) The addition of large concentrations of a recognised nitrite trap, such as urea, to the reaction mixture will result in the rapid removal of the free nitrosating agent, NOY, from the equilibrium mixture. The "reverse", N-nitrosation step governed by k_{-1} is thus suppressed and the yield of the denitrosation product is increased. On the basis of the

intramolecular scheme we might still expect to obtain significant yields

of the rearrangement product. At first sight this prediction is seen to

conflict with the observations of Macmillen and Reade[7] who reported that

reaction of meta-nitro-N-methyl-N-nitrosoaniline in the presence of urea

yields the denitrosation product exclusively. In general however it has

been shown[14,18] that small yields of the rearrangement product may be

obtained even in the presence of high concentrations of an added nitrite

trap, this evidence supporting the application of the intramolecular

scheme. Explanation of the observed lack of rearrangement product obtained

from meta-nitro-N-methyl-N-nitrosoaniline must have origins other than in

the removal of nitrosyl halide by urea since no rearrangement product is

observed even in the absence of a nitrite trap[19].

As both schemes are able to provide equally feasible theoretical

rationalisations for the observed result it is apparent that no decision

as to the relative applicabilities of the intermolecular and intramolecular

schemes may be made on the basis of the evidence presented thus far.

1.4 Intermolecular vs Intramolecular

The reports of Aslapovskaya et al.[14] and Williams[18] concerning the

observation of significant yields of the rearrangement product being

derived from the reaction of N-methyl-N-nitrosoaniline in the presence of

large excess concentrations of urea must be considered to generate

reservations in terms of our acceptance of the intermolecular scheme. Under

the conditions described urea, a recognised nitrite trap, might be expected

to react rapidly and irreversibly to remove free nitrosonium ion carriers

such as the nitrosyl halides. The observation of significant yields of

the rearrangement product is thus hard to rationalise in terms of the

intermolecular scheme.

The Fischer-Hepp rearrangement may be seen to bear a formal similarity to the Orton rearrangement of N-chloroanilides[20] which is depicted below
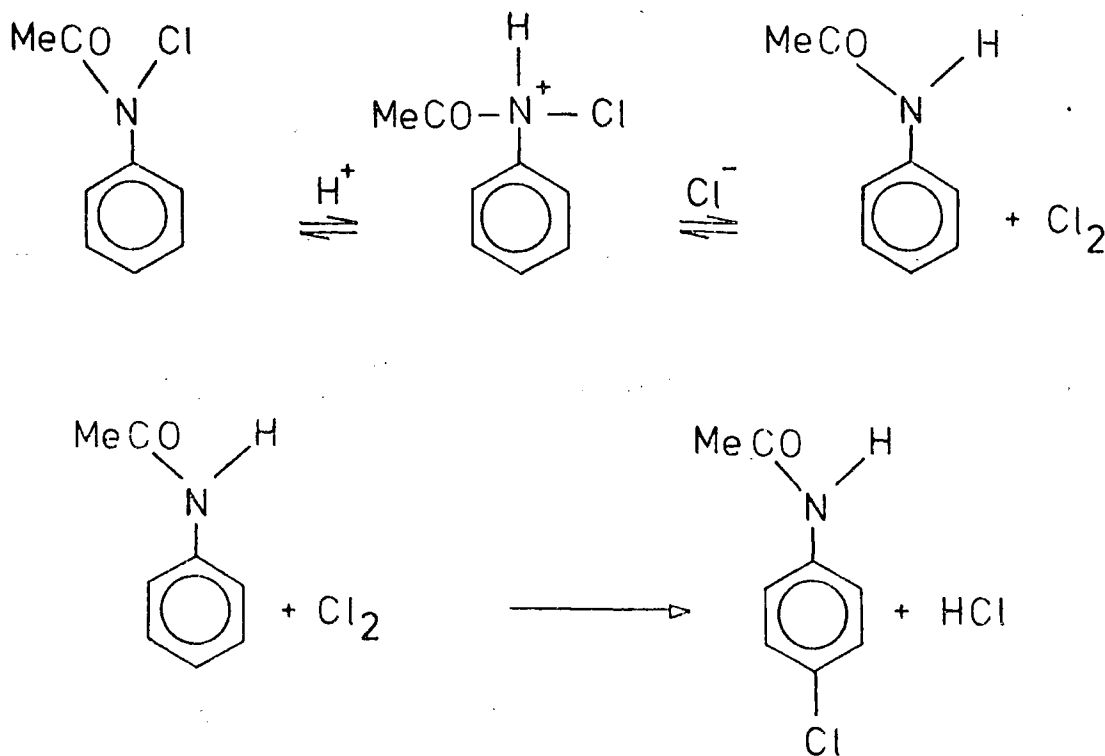
This mechanism has received general acceptance being amply supported by experimental evidence[21,22]. The kinetic observations of Soper and Pryde[23,24] have led them to formulate the following expression for the rate of disappearance of the reactant:-

$$\text{Rate} = k\,[\text{N-chloroacetanilide}]\,[\text{H}^+]\,[\text{Cl}^-]$$

Thus the rate of reaction is proportional to both chloride and hydrogen ion concentrations. In physical terms the expression is indicative of attack by chloride ion upon the protonated form of the N-chloroacetanilide. If the Fischer-Hepp Rearrangement were to occur via

an analogous intermolecular mechanism the rate of disappearance of the reactant might again be expected to be proportional to the product of the hydrogen ion and chloride ion concentrations. Williams[18] however has reported the rate of reaction of N-methyl-N-nitrosoaniline in aqueous HCl to be approximately proportional to h_o and not to the product $h_o[Cl^-]$.

Whilst these last observations cast some doubt as to the general applicability of the intermolecular scheme they can not, perhaps, be taken as conclusive proof of the scheme's inaccuracy.

The question as to the mechanism of rearrangement reactions may often be answered on the basis of information supplied by isotopic exchange experiments. Using unlabelled starting material the reaction is carried out in the presence of a labelled species which is chosen so as to promote isotopic exchange with fragments produced by a particular reaction scheme. The incorporation of the isotopic label into the rearrangement product is taken as being indicative of the existence of an intermolecular reaction mechanism. In the rearrangement of phenylhydroxyl-amine (13) to give para-aminophenol (14) the reaction proceeds with full uptake by the product of the ^{18}O label derived from the labeled water solvent[25]. Incorporation of the ^{18}O label into the reactant is not observed and the reaction must be regarded as being of an intermolecular nature.
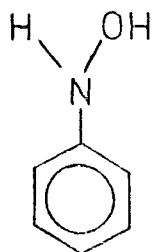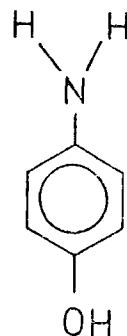
(13)   (14)

Conversely the lack of ^{15}N incorporation into the product in the case of the rearrangement of N-nitroaniline in the presence of $^{15}NO_3^-$ and $^{15}NO_2^-$ is taken as evidence in support of the reaction's intramolecular nature[26].

In the case of the Fischer-Hepp Rearrangement, however, no such distinctions between the two proposed mechanisms can be made. In the presence of ^{15}N labelled sodium nitrite, N-methyl-N-nitrosoaniline has been shown to undergo label exchange at a rate far in excess of the rate of rearrangement[27]. This incorporation of the ^{15}N label is a consequence of the fast reversible denitrosation reaction and the observation casts no light upon the mechanism of rearrangement.

In the present case it would appear that a definite choice between the two mechanisms may be made only on the basis of a thorough kinetic analysis.

For the sake of convenience the schemes are reproduced below:-

Intermolecular Scheme
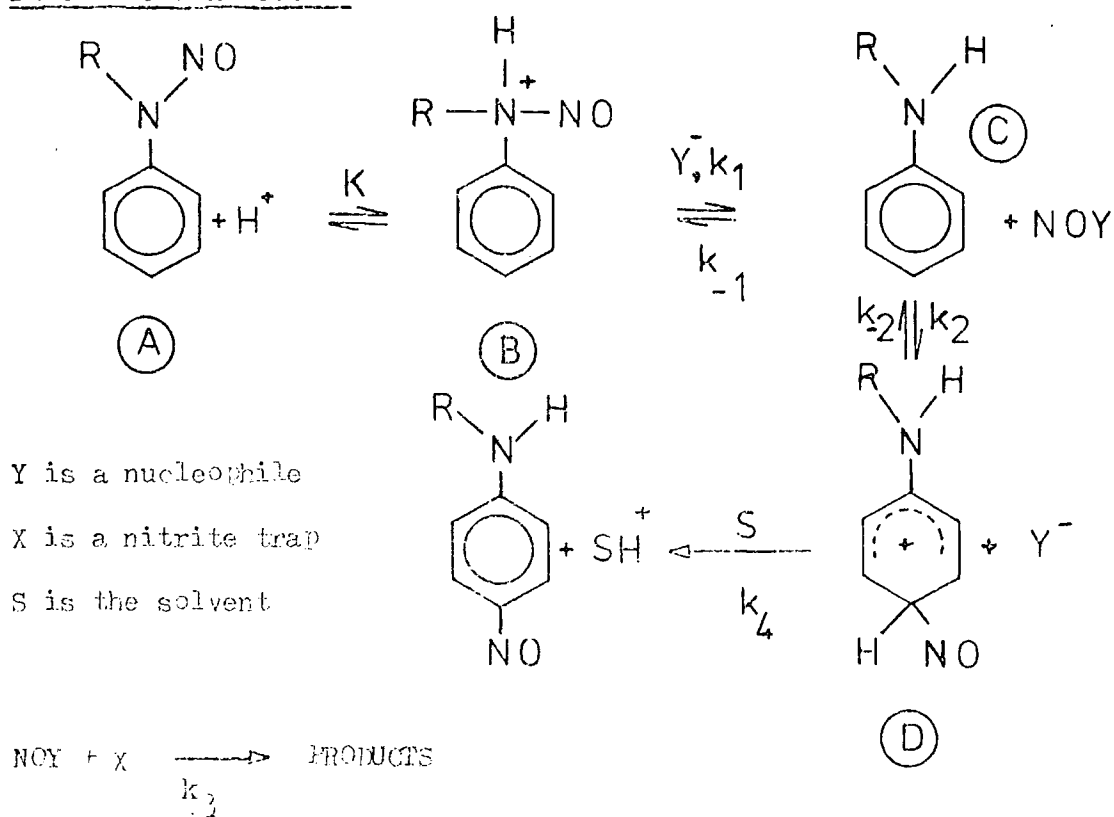
Y is a nucleophile

X is a nitrite trap

S is the solvent

$$NOY + X \xrightarrow{k_3} PRODUCTS$$

Intramolecular Scheme

Y is a nucleophile

X is a nitrite trap

S is the solvent

NOY + X $\xrightarrow{k_3}$ Products
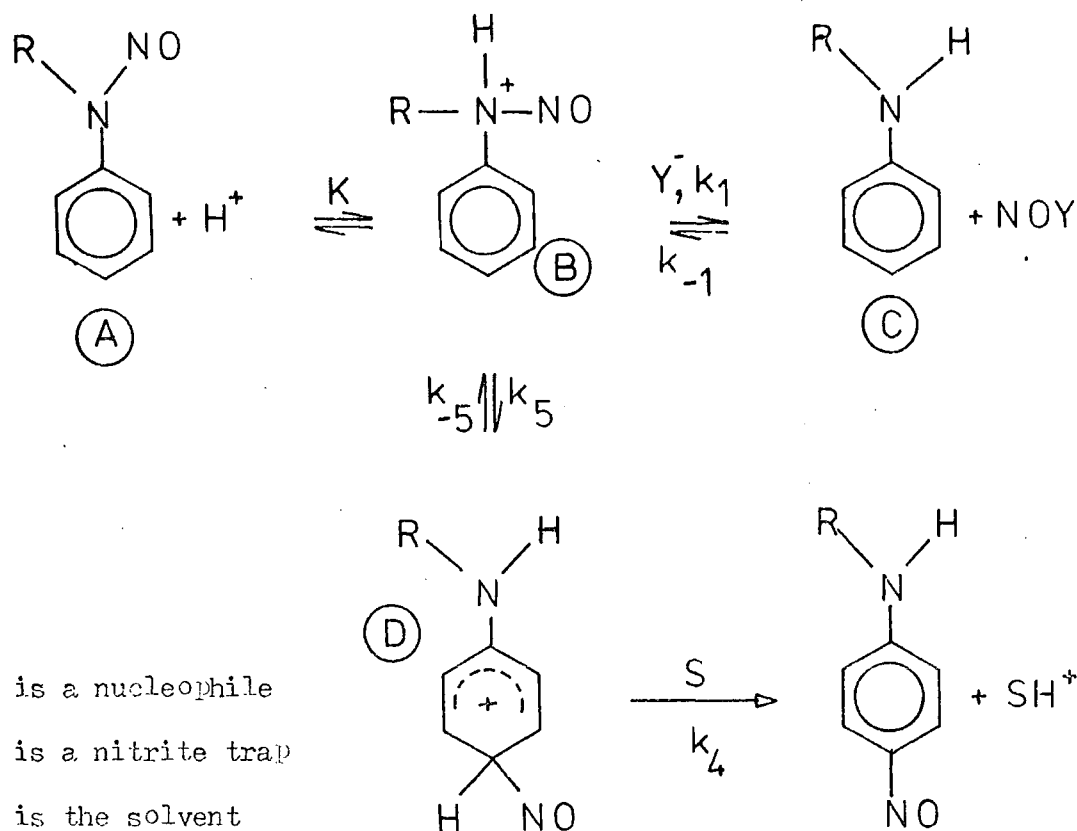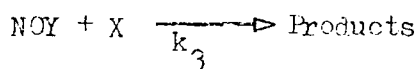
Observation of the similarity which exists between the spectra of N-methyl-N-nitrosoaniline in acidic and neutral aqueous solutions respectively have lead to the conclusion that the extent of protonation is small. As the results of experiments carried out in D_2O rule out the existence of a primary kinetic isotope effect it may also be assumed that the protonation is fast. If the intermediates, D and NOY are assumed to exist only in low concentration we may derive expressions for the rate of reaction via an application of the Steady State Principle[28].

INTERMOLECULAR:-

Let   Rate $= k_o[A] = k_1[B][Y] - k_{-1}[C][NOY]$

where k_o is the observed first-order rate constant.

Application of the Steady State Principle to the reactive

intermediates, D and NOY, results in the following expressions,

$$[D] = \frac{k_2[C][NOY]}{k_{-2}[Y] + k_4}$$

$$[NOY] = \frac{k_1[B][Y]}{k_{-1}[C] + k_3[X] + k_2'[C]}$$

where $k_2' = k_2 - \left[\frac{k_{-2}\, k_2\, [Y]}{k_{-2}[Y] + k_4}\right]$

Substitutions for $[D]$ and $[NOY]$ in the rate equation give,

$$\text{Rate} = k_o[A] = \frac{k_1[B][Y]\,(k_3[X] + k_2'[C])}{k_3[X] + (k_{-1} + k_2')[C]}$$

On the assumption that the nitrosamine acts as a Hammett base we may

write, $[B] = Kh_o[A]$, whence

$$\text{Rate} = k_o[A] = \frac{k_1 Kh_o[A][Y]\,(k_3[X] + k_2'[C])}{k_3[X] + (k_{-1} + k_2')[C]}$$

Thus,

$$k_o = \frac{k_1 Kh_o[Y]\,(k_3[X] + k_2'[C])}{k_3[X] + (k_{-1} + k_2')[C]}$$

INTRAMOLECULAR:-

Since the protonation of the nitrosamine, A, is assumed to be fast

and reversible the rate of its disappearance may be described in terms of

the disappearance of the protonated form, B.

Whence,

$$\text{Rate} = k_o[A] = k_1[B][Y] - k_{-1}[C][NOY] + k_5[B] - k_{-5}[D]$$

Application of the Steady State Principle to the two reactive intermediates, D and NOY, results in the following expressions.

$$[D] = \frac{k_5[B]}{(k_{-5} + k_4)}$$

$$[NOY] = \frac{k_1[B][Y]}{k_{-1}[C] + k_3[X]}$$

Substituting for $[D]$ and $[NOY]$ in the rate equation now gives,

$$\text{Rate} = k_o[A] = k_1[B][Y] - \frac{k_{-1}[C]k_1[B][Y]}{k_{-1}[C] + k_3[X]} + k_5[B] - \frac{k_{-5}k_5[B]}{k_{-5} + k_4}$$

Simplification followed by the substitution of $[B] = Kh_o[A]$ yields,

$$\text{Rate} = k_o[A] = \frac{k_1[Y]k_3[X]Kh_o[A]}{k_{-1}[C] + k_3[X]} + \left[\frac{k_4 k_5}{k_{-5} + k_4}\right] Kh_o[A]$$

Whence,

$$k_o = \frac{k_1[Y]k_3[X]Kh_o}{k_{-1}[C] + k_3[X]} + \left[\frac{k_4 k_5}{k_{-5} + k_4}\right] Kh_o$$

Whilst the rate expressions, derived via the intermolecular and intramolecular schemes respectively, may be seen to have certain features in common, such as a first-order dependence on h_o under all conditions, a comparison of their behaviour under certain limiting conditions does yield sufficient evidence to enable a choice between the two mechanisms to be made.

Reaction at high concentrations of X

As the concentration of the nitrite trap, X, is increased $k_3[X]$ may be expected to become greater than both $k_{-1}[C]$ and $k_2'[C]$. Under such

conditions we may apply limiting forms of each of the two rate expressions.

$$k_o = k_1[Y]Kh_o \qquad \text{INTERMOLECULAR}$$

$$k_o = \frac{k_4 k_5 Kh_o}{k_4 + k_{-5}} + k_1[Y]Kh_o \qquad \text{INTRAMOLECULAR}$$

Both mechanisms thus predict k_o to be independent of both the nature and concentration of X provided that X is present in solution in quantities large enough to maintain the limiting conditions. The predicted behaviour is observed experimentally. The results of Williams et al.[11] which were obtained for the reaction of N-methyl-N-nitrosoaniline in 3.05 M HCl (aq.) are given as illustration.

Added Nitrite Trap	$[X]/M$	$10^4 k_o/s^{-1}$
HN_3	1×10^{-3}	16.6
HN_3	3×10^{-3}	17.3
HN_3	5×10^{-3}	16.4
NH_2SO_3H	5×10^{-3}	16.9

Experimental evidence may also be provided to demonstrate the existence of catalysis by H^+ and halide ions[11]. Such behaviour is to be expected on the basis of both the intermolecular and the intramolecular mechanisms.

$[HN_3]/M$	Added $[Br^-]/M$	Added $[Cl^-]/M$	$[HCl]/M$	$10^4 k_o/s^{-1}$
5×10^{-3}	0	0	3.05	16.4
5×10^{-3}	0	3.57	3.05	23.0
5×10^{-3}	0.52	0	3.05	176
5×10^{-3}	0	0	4.47	32.3

The two rate expressions predict different trends for the % rearrangement product:% denitrosation product ratio as $[X]$ is increased and their differing behaviour in this respect permits a direct test of mechanism to be applied. The intramolecular expression may be seen to be composed of two parts. The first part pertains to the rate of the rearrangement reaction and the second to the rate of denitrosation. As the rearrangement: denitrosation product ratio is proportional to the relative rates of the two reactions it is clear that the intramolecular mechanism predicts the yield of the rearrangement product to be a constant at any one acidity and concentration of Y and to be independent of both the nature and concentration of X at high $[X]$. As a consequence of the direct competition for NOY between added X and C required by the intermolecular mechanism the limiting form of the relevant rate expression lacks the term relating to rearrangement. The intermolecular scheme thus predicts the yield of rearrangement product to fall to zero at high concentrations of X.

The experimental results[11] for the reaction of N-methyl-N-nitrosoaniline in 2.75 M H_2SO_4 (aq.) are given below.

Added nitrite trap	$[X]$/M	% rearrangement product
HN_3	6.53×10^{-4}	21
HN_3	16.3×10^{-4}	21
NH_2SO_3H	3.1×10^{-3}	21
$CO(NH_2)_2$	1.0×10^{-1}	21
NH_2OH	1.56×10^{-3}	20

At high values of $[X]$ the percentage yield of rearrangement product at any one acidity and nucleophile concentration is seen to have a constant finite value which is independent of both the nature and concentration of X.

These results are consistent only with an intramolecular mechanism.

Reaction at high concentrations of C

As we move towards high added concentrations of the denitrosation product, C, we approach a limiting condition under which

$$k_{-1}[C] >> k_3[X].$$

The intramolecular rate expression is reduced to a form given by,

$$k_o = \frac{k_4 k_5 Kh_o}{k_4 + k_{-5}} \qquad \text{INTRAMOLECULAR}$$

The limiting form of the intramolecular rate expression at high $[C]$ thus consists merely of a term previously ascribed to the rearrangement reaction. In physical terms we therefore envisage a suppression of the denitrosation reaction by the added excess concentrations of the denitrosation product. Loss of NOY to the solvent becomes negligible and the rate of N-nitrosation is greatly increased. Under these conditions the rearrangement reaction may be expected to compete favourably with the denitrosation route and high yields of the rearrangement product will be obtained. On the basis of the intramolecular mechanism k_o is expected to attain a non-zero limiting value which will be independent not only of $[X]$ and $[C]$ but also of both the nature and concentration of Y.

The full form of the intermolecular rate expression may be rewritten as below.

$$k_o = k_1[Y]Kh_o \left[1 - \frac{k_{-1}[C]}{k_3[X] + (k_{-1} + k_2')[C]} \right] \qquad \text{INTERMOLECULAR}$$

The rate of C-nitrosation, represented by $k_2[C]$ is likely to be several orders of magnitude less than $k_{-1}[C]$ the rate of N-nitrosation. Since k_2' exceeds k_2 by definition (see above) it transpires that the term enclosed by the large brackets is characterised as being of a low value. The intermolecular mechanism thus predicts that values of k_o

close to zero will be obtained in the presence of an excess of added denitrosation product. Furthermore the form of the intermolecular expression demands that k_o should never become independent of $[Y]$ under these conditions.

Williams et al.[11] have studied the reaction of N-methyl-N-nitrosoaniline in the presence of 5.90 M HCl (aq.) using various concentrations of added N-methylaniline, C. The results of the study are given below.

$10^3 [\text{N-methylaniline}]$ added/M	$10^4 k_o/s^{-1}$	% Rearrangement
0	5.07	28
0.44	3.74	54
0.94	3.61	60
3.08	2.80	76
3.96	2.78	78
5.71	2.84	80

The observed first-order rate constant, k_o, is seen to decrease towards a limiting value of approximately 2.8×10^{-4} s^{-1} at high concentrations of added N-methylaniline. This behaviour is consistent only with the intramolecular mechanism. Note also that the yield of rearrangement product tends towards 100% at high $[C]$ as is to be expected on the basis of an intramolecular mechanism.

The absence of halide ion catalysis at high $[C]$ has also been demonstrated by Williams et al.[11] for the reaction of N-methyl-N-nitrosoaniline in aqueous acid. The data are given over.

10^3 [N-methylaniline] added/M	[Halide Ion] added/M	$10^4 k_o/s^{-1}$
4	0	1.75
4	0.24 (Cl$^-$)	1.79
4	0.10 (Br$^-$)	1.77

Thus k_o is found to be independent of both the nature and concentration of the nucleophile, this observation being consistent only with an intramolecular mechanism.

The kinetic evidence thus leads us towards a firm acceptance of the intramolecular mechanism. It is perhaps pertinent to point out however that all of the kinetic data used in the analysis refers specifically to the reaction of N-methyl-N-nitrosoaniline in an aqueous medium. This restriction perhaps demands the introduction of a degree of qualification into our acceptance of the intramolecular mechanism since a direct extrapolation to the general case might be unwise. Indeed the present work notes the exact form taken by the nucleophile catalytic effect to be dependent on the choice of both the nitroso compound and the solvent.

The general case is discussed in a later section.

1.5 Other Mechanistic Studies

The intermolecular and intramolecular schemes discussed above by no means represent the only attempts to rationalise the experimental observations. Three alternative mechanisms are reviewed below in summary.

Baliga[15] has examined the reaction of N-nitrosodiphenylamine in methanolic HCl and reports that chloride catalysis of the rearrangement is not observed. The reaction is reported to be first-order in both the nitrosamine and HCl. The following reaction scheme has been tentatively proposed.

$$Ph_2NNO + HCl \xrightarrow{\text{slow}} \begin{bmatrix} Ph_2NN\text{--------}O \\ \vdots \qquad\qquad \vdots \\ H\text{--------}Cl \end{bmatrix}$$

$$\downarrow$$

$$Ph_2NH + NOCl$$

$$Ph_2NH + NOCl \xrightarrow{\text{fast}} p\text{-}NOC_6H_4NHPh + HCl$$
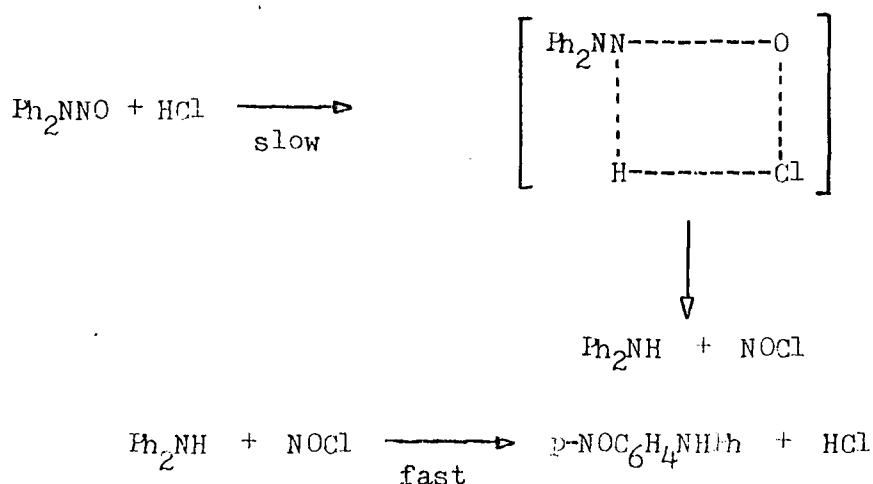
The first stage of the reaction is thus envisaged as yielding the secondary amine and NOCl via the slow formation of a cyclic four-centre transition state. Fast reaction between the secondary amine thus formed and the free nitrosating agent then leads to formation of the rearrangement product. On the basis of results obtained in aqueous solvents using N-methyl-N-nitrosoaniline Williams[11,29] has offered criticism on a number of aspects of the cyclic mechanism.

(a) Reaction via the kinetically free nitrosating species, NOY, has been ruled out by the observation of significant yields of rearrangement product in the presence of large excess concentrations of added nitrite traps.

(b) The observed solvent isotope effects obtained in aqueous solution point towards a fast initial protonation.

(c) The observed ring deuterium isotope effects indicate the final proton loss to be the rate determining step in the rearrangement reaction.

(d) The cyclic mechanism cannot account for the observed acid catalysis at any one concentration of chloride ion.

(e) Even in the absence of chloride ion quantitative yields of the rearrangement product are obtained in the presence of excess added concentrations of the denitrosation product.

It must be pointed out that since Williams' results were obtained
for reactions in aqueous solutions the applicability of his criticisms
of Baliga's proposal must be in question. In particular recent work by
Johal et al.[30] on the reaction of N-nitrosodiphenylamine in ethanolic
HCl does point to the existence of a slow initial protonation in that
particular case.

Russian workers[16,17] have recently re-examined the rearrangement of
N-nitrosodiphenylamine in methanolic HCl on the basis of the
intermolecular mechanism depicted below.

$$Ph_2\overset{+}{N}HNO \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Ph_2NH + N\overset{+}{O} \xrightarrow{k_3} p\text{-}NOC_6H_4NHPh$$

(A)   (B)   (C)
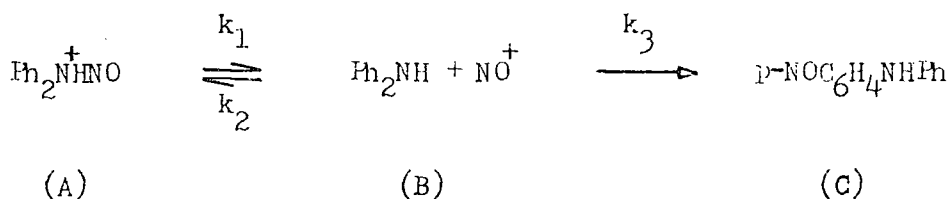
On the assumption that $N\overset{+}{O}$ is a reactive intermediate application
of the steady state theorem results in the formulation of a rate
expression for the reaction.

$$Rate = k_o[A] = \frac{k_1 k_3 [A]}{k_2 + k_3}$$

where k_o is the observed first-order rate constant.

A value of 9.167×10^{-4} s^{-1} was reported for k_o. By observation of
analogous reaction schemes in which the possibility of rearrangement was
excluded it was hoped that the first stage of the reaction could be
studied in isolation. Using results obtained from experiments on a series
of di-para-substituted N-nitrosodiphenylamines values of K_d the
equilibrium constant for the denitrosation reaction were found for each
substrate. A Hammett correlation[31] of log K_d vs σ , the relevant
substituent constant, thus yields, by interpolation, a K_d value of
2.1×10^{-3} for the reaction of N-nitrosodiphenylamine itself.

Similarly it may be seen that the study of a reaction system involving

the nitrosation of N-substituted diphenylamines might reasonably be expected to yield data concerning the second stage of the reaction scheme, the C-nitrosation reaction. Values of k_3 were obtained for the nitrosation of a series of N-substituted diphenylamines and a value of k_3 for the reaction of diphenylamine itself was obtained by interpolation of a plot of log k_3 vs σ_R.

Since

$$k_o = \frac{k_1 k_3}{k_2 + k_3}$$

and

$$K_d = \frac{k_1}{k_2}$$

substitution of the known values of k_o, k_3 and K_d into these equations yields

$$k_1 = 5 \times 10^{-3} \text{ s}^{-1}$$
$$k_2 = 2.45 \text{ l mol}^{-1} \text{ s}^{-1}$$
$$k_3 = 5.3 \times 10^{-1} \text{ l mol}^{-1} \text{ s}^{-1}$$

for the reaction involving N-nitrosodiphenylamine itself. On the basis of the individual rate constants the rate determining step is seen to be that of denitrosation. Values of k_1, k_2 and k_3 were then used in an attempt to predict the form of the observed kinetic curve.

Solving the system of differential equations defined by

$$\frac{d[A]}{dt} = -k_1[A] + k_2[B]^2$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]^2 - k_3[B]^2$$

$$\frac{d[C]}{dt} = k_3[B]^2$$

using analogue electronic techniques leads to the generation of a curve of $[C]$ vs time which closely resembled that obtained by experiment. The similarity between the theoretical and experimental curves has been

taken as confirming the applicability of the intermolecular mechanism.

Several aspects of the Russian approach merit criticism. In the first instance it must be pointed out that until data concerning the analysis of the intramolecular scheme by an analogous method is made available no meaningful comparison of the two mechanisms may be made. Secondly, it has become clear in recent years[32] that the interpolation of Hammett type correlations which rely on a small basis set can be the source of significant degrees of error. In the present case the accuracy of the interpolated results must be in question as only four experimental points were employed in each correlation.

In addition the Russian inclusion of the nitrosonium ion, NO^+, as the free nitrosating agent would appear to be unjustified. In aqueous sulphuric acid[33] nitrosation via the agency of the free nitrosonium ion is thought to occur only at acidities in excess of 9 M H_2SO_4. Under conditions such as these $-H_o \sim 4.5 \longrightarrow 5.0$ and the water activity is sufficiently low as to preclude solvation of the nitrosonium ion. The same may not be said for the 4 M methanolic solution of HCl used by the Russian workers. As 4 M HCl has been reported[34,35] to yield a $-H_o$ value of approximately 1.4 in both aqueous and ethanolic solvents it might be expected that solvation of the nitrosonium ion to yield the nitrous acidium ion $H_2NO_2^+$ would occur. There exists also the possibility of nitrosyl chloride formation. NOCl may be detected spectrophotometrically in a 4 M aqueous solution of HCl which contains added sodium nitrite and it has been calculated[36] that under such conditions free nitrous acid would be converted almost quantitatively to give the nitrosyl halide. A similar situation might be expected to exist regarding a 4 M solution of HCl in methanol.

If denitrosation is proposed as being the rate determining step in

the rearrangement reaction then catalysis by chloride ion is to be expected. This catalysis of the rearrangement reaction is not observed[15].

Finally it must be said that the Russian approach takes no account of the influence of substituents upon the acid/base properties of the substrate. As it has already been shown that the relative reactivities of various ring-substituted N-methyl-N-nitrosoanilines are a function of the acidity of the medium the omission may be a serious one. In short the Russian scheme appears unacceptable.

The existence may be postulated of a reaction scheme involving direct transfer of an NO group from the protonated form of a nitrosoamine ArNRHNO to the para-position of the corresponding secondary amine.
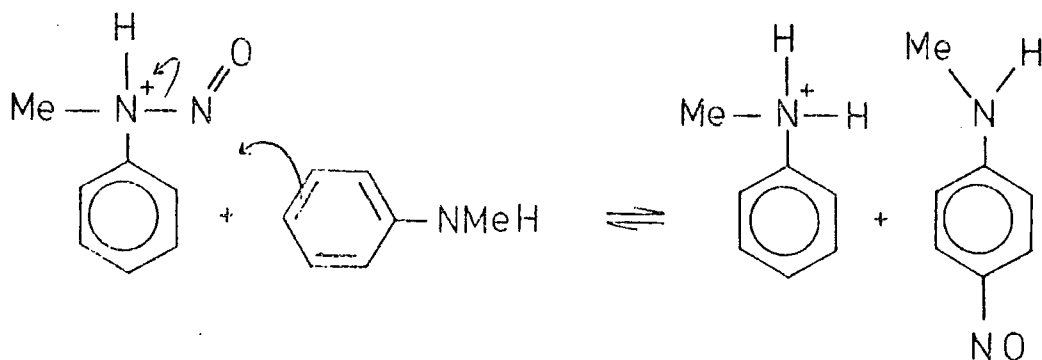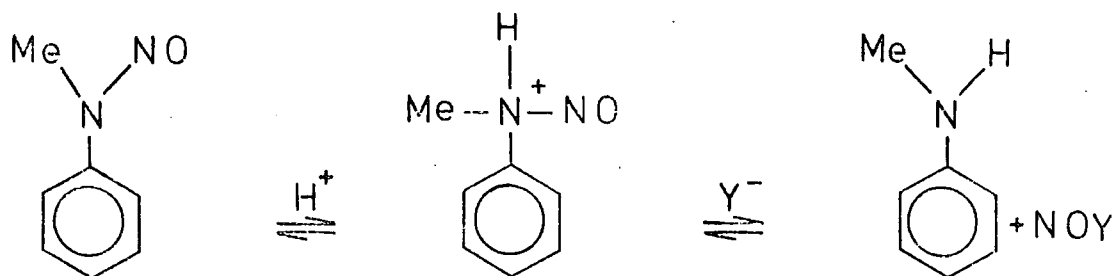
The SE2 reaction mechanism thus described would account for a number of the experimental observations including the lack of halide catalysis in the rearrangement reaction. The operation of such a scheme however appears unlikely on two counts[11].

(i) Our rejection of the intermolecular scheme on the evidence of the kinetic analysis, (Section 1.4), points to the general inability of the free nitrosating agents such as nitrosyl halide or the nitrous acidium ion to bring about the required C-nitrosation at the para-carbon site of N-methylaniline. Since the protonated nitrosamine is very probably a much weaker electrophile than either NOCl or $H_2NO_2^+$ it seems unlikely that it will be capable of such a reaction.

(ii) The reaction of N-nitroso-N-methylaniline in aqueous acidic solutions has been shown to be zero order in X, the nitrite trap, at high$[X]$. This observation rules out the possibility of a direct reaction involving the transfer of NO^+ from the protonated form of the nitrosoamine to the trap species such as urea and hydrozoic acid. Direct transfer of NO^+ to the much weaker nucleophilic para-carbon site of N-methylaniline is therefore unlikely.

1.6 The Mechanism of the Rearrangement Reaction

In preceeding sections we have discussed a series of mechanistic schemes which are said to represent the reactions of N-nitrosamines in acidic solutions. It is now pertinent to consider one of these schemes in some greater detail. On balance the intramolecular scheme of Williams would seem to be the appropriate choice although the discussion has cast some doubts upon its applicability in describing reactions in alcoholic solvents. The present work demonstrates that the scheme may be extended to account for the behaviour of a wide range of N-nitroso compounds in acidic solution and it is felt that the scheme's potential as having

general applicability justifies its selection at the expense of the
alternatives. The scheme is depicted below for convenience.

$$NOY + X \xrightarrow[k_3]{} \text{Products}$$
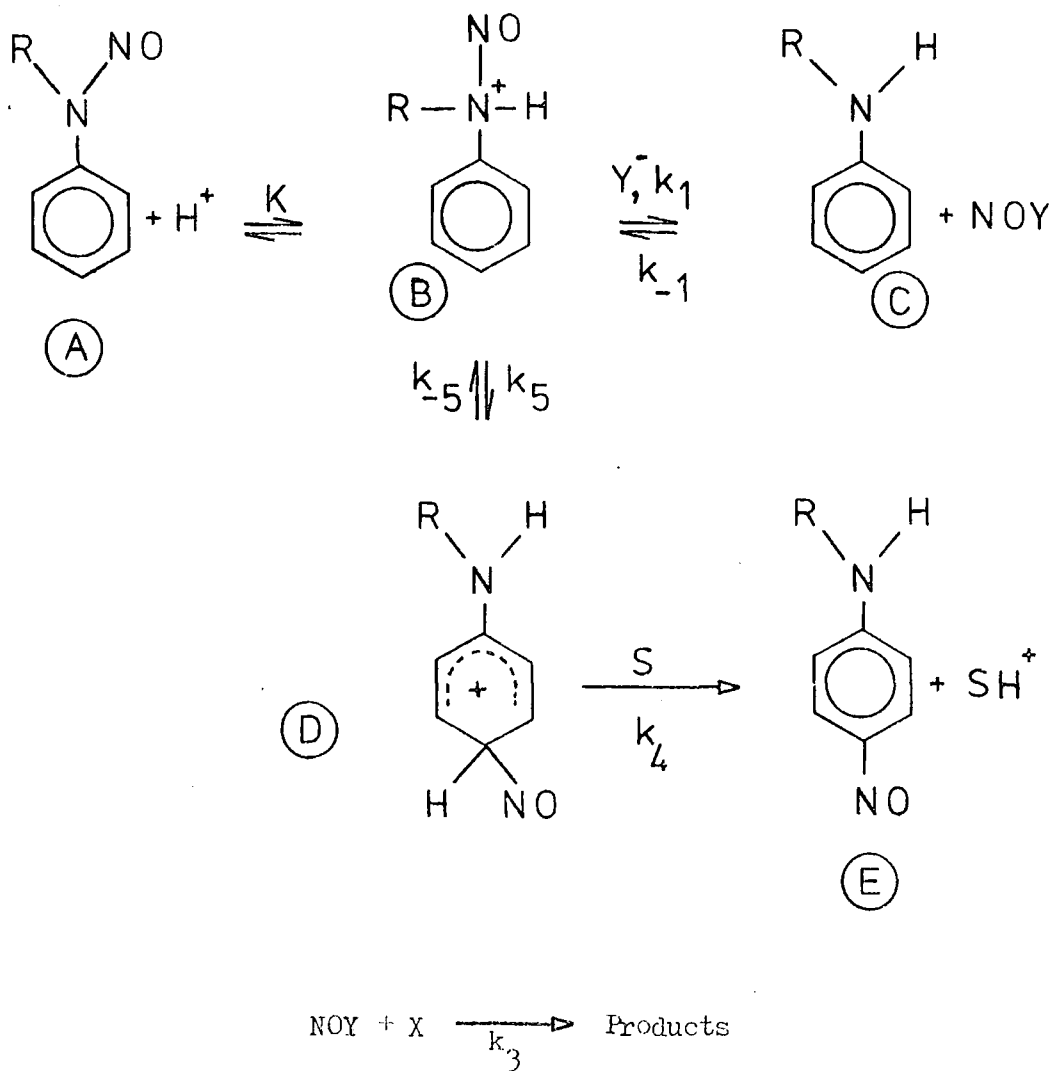
X is a nitrite trap   S is the solvent Y^- is a nucleophile

The denitrosation reaction is retarded by the omission of the nitrite
trap, X. If the denitrosation pathway is further suppressed by the
addition of an excess of the denitrosation product (C) almost quantitative
yields may be obtained from the rearrangement reaction. In such
circumstances the limiting condition, $k_{-1}[C] >> k_3[X]$ obtains and the
reduced form of the intramolecular rate expression may be represented as
follows.

$$k_o = \frac{k_4 k_5 K h_o}{k_4 + k_{-5}}$$

k_o is now independent of the concentration of C and of the nature and concentration of X and Y. Thus for the reaction of N-methyl-N-nitrosoaniline in 5.90 M aqueous HCl solutions consecutive increases in the concentration of added N-methylaniline result[19] in a reduction of the value of k_o towards a limiting value of $k_o = 2.8 \times 10^{-4} s^{-1}$. A simultaneous increase in the observed yield of the rearrangement product is reported with the percentage yield of 28% obtained in the absence of added N-methylaniline being increased to an 80% yield by the addition of a 5.71×10^3 M concentration of the same reagent. The remaining 20% may well be accounted for in terms of the generation of a yellow side product of uncertain composition which is especially prominent at the higher acidities. Similarly high yields of the rearrangement product are obtained using aqueous solutions of sulphuric acid under the same conditions.

Williams et al.[18,37] have studied the acidity dependence of the rearrangement reaction. In the range $[HCl] = 2$ M to $[HCl] = 8$ M a plot of $\log k_o$ vs $-H_o$ gives a linear correlation with a slope of 1.2. This result indicates the absence of catalysis by nucleophilic species such as chloride ion and implies that the reaction proceeds via the mono-protonated form of the nitrosamine as has been proposed. The rate profile against $-H_o$ is observed to reach a maximum at $[HCl] = 10$ M corresponding to full protonation of the N-nitroso substrate. At higher acidities $\log k_o$ decreases linearly with increasing $-H_o$, the observed trend being interpreted in terms of a rise in proton activity which leads to a retardation of the proton loss from the intermediate σ-complex, (D), in the final stage of the rearrangement reaction.

In general the introduction of electron-withdrawing substituents into the meta-position of the benzene ring in N-methyl-N-nitrosoaniline results in a reduced rate of rearrangement. Conversely meta-positioned electron-donating groups are observed to activate the substrate towards

rearrangement. In the extreme case noted in an earlier section (Section 1.3) reaction of the meta-NO_2 substituted compound yields the denitrosation product exclusively even in the absence of a nitrite trap. Conversely the meta-OMe substituted compound yields the rearrangement product exclusively even in the absence of added concentrations of the denitrosation product.

The rearrangement reaction is thus viewed in overall terms as an intramolecular, electrophilic nitrosation at a carbon site.

Isotopic substitution experiments[18] have shown the rate of rearrangement of 2,4,6-trideutero-N-methyl-N-nitrosoaniline to be slower than that recorded for the non-deuterated compound by a factor of 1.7. It would thus appear that the final proton loss to the solvent is, at least in part, rate-determining. An increase in the added concentration of the denitrosation product leads to a corresponding increase in the k_H/k_D ratio; the final value of 2.4 being characteristic of the magnitude of the primary isotope effect normally reported for the nitrosation of an aromatic substrate[38]. Challis et al. have attributed the observed result to be due, in the general case, to a rapid regeneration of reactants. In the present case k_{-5} is by implication significantly larger than k_4.

The effects of increasing degrees of N-alkyl substitution in a series of N-nitrosoanilines have been explained by Biggs and Williams[37] in terms of the effect upon the basicity of the substrate.

Although the kinetic data has led to our adoption of the intramolecular model for the rearrangement reaction and has in addition yielded a small amount of information concerning the operation of the final proton loss from the Wheland Intermediate (D) as the rate determining step little in the way of further information may be gleaned from this source. Consideration of the distances involved would seem to indicate that the transfer of the nitroso group between the amino nitrogen and the para-, ring carbon site

to yield the σ -complex, (D), must occur via the agency of an additional intermediate not considered in the above scheme. This "additional intermediate" is generally accepted as taking the form of a π -complex[19] such as that depicted below.
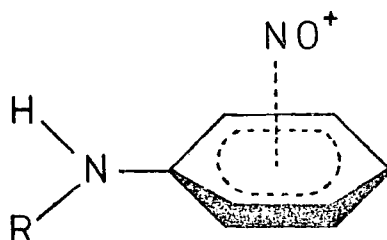
Although the π -complex species have never been positively identified in the present context a certain amount of evidence is available to support their proposed existence. The deep red colourations observed when aromatic compounds such as benzene or toluene come into contact with acidic solutions of sodium nitrite have been explained by Dimitrov and Frateev[39] in terms of an initial π -complex formation and a subsequent $\pi \longrightarrow \sigma$ transition. Their proposals are supported by the recorded visible spectra and by observations concerning the destruction of the proposed π -complex by donor solvents. Similarly Allan et al.[40] have proposed the involvement of π -bonded complexes in the production of red-brown colourations during the reaction of $NO^+ HSO_4^-$ with aromatics such as benzene and phenol. Evidence here is based upon an examination of the collected U.V., visible and N.M.R. spectral data. Further evidence for the existence of π -bonded NO^+ complexes may be obtained from observations of the extensive gas phase chemistry of NO^+. Thus NO^+ is seen[41] to react with alkanes in the gas phase to produce a number of novel species via a hydride transfer reaction. As the transition state chemistry of a species

in aqueous solution often parallels its behaviour in the gas phase the observed results may well be indicative of the importance of including the Lewis acidity of NO^+ in any consideration of its transition state chemistry. On this basis the postulation of a π -bonded NO^+ complex as a transition state species in the Fischer-Hepp rearrangement would not seem unreasonable.

It should however be remembered that under the mildly acidic conditions generally employed in the Fischer-Hepp rearrangement solvent ac tivity is fairly high and destruction of the proposed π -complex by donor solvents such as methanol and water might well be expected. Involvement of a π -bonded transition state species is thus seen to demand that the activation energy of its formation be of such a low value as to allow the complete transference of the NO^+ group across the π -cloud to the para-carbon ring site before collision with a solvent molecule can occur. Although tentative the proposal is at least compatible with experimental observations since the rate determining step for the rearrangement of N-nitrosoanilines in aqueous solution is found to be the final proton loss to the solvent.

One aspect of the rearrangement which has been the cause of some concern is its ability to yield the p-nitroso isomer exclusively. Allan[42] has attempted to explain this facet of the reaction in terms of a direct transfer of NO^+ from the amino nitrogen to the p-carbon site in an immediate environment provided by the bent-ring intermediate described below.
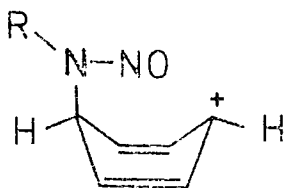
The close proximity of the nitroso group and the para-carbon site might thus be expected to favour the formation of the para-nitroso isomer. This postulate is compatible with the observed ring deuterium isotope effect if proton loss is assumed to occur simultaneously with the transfer of the NO^+ group. The scheme attracts criticism on a number of points. The primary criticism must centre around the existence of a possible alternative configuration for the proposed intermediate such as that depicted below.
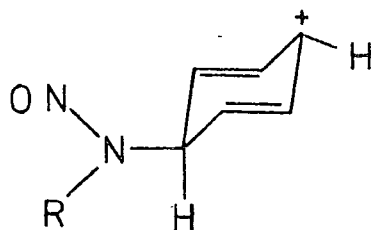
This alternative configuration minimises the steric interactions and may therefore be expected to be of lower energy than that proposed by Allan. The molecule thus spends a greater proportion of its time in the chair form where stereochemically speaking transference of NO^+ to the p-carbon site is the last thing we would expect. One could also argue that the transfer of a nitrosonium ion carrying a partial positive charge to a positively charged carbon site is at best an unlikely event. It must also be said that the availability of evidence to support the postulated ring protonation is extremely limited. A study of the acid catalysed detritiation of a number of aromatic substrates has lead to the calculation of a pK_{BH}^+ value of -15.3 for the ring protonation of anisole[43]. On the assumption that the pK_{BH}^+ value for the reaction of the N-nitrosoanilines is of a similar magnitude, the extent of protonation is calculably negligible. Indeed Olah[44] has reported only N-protonation of aniline and

its derivatives in super acid media. Finally we note that the solvent isotope effect k_{D_2O}/k_{H_2O} of around 3, which is observed for the reaction of N-methyl-N-nitrosoaniline, indicates the presence of a rapid initial protonation. The rates of proton transfers to unsaturated carbon sites, such as that required by the present postulate, are generally low. The scheme proposed by Allan would not seem to be particularly appropriate.

Although the Fischer-Hepp reaction is a particularly well documented[12] example of a rearrangement which gives, exclusively, the para-isomer it is by no means alone in this respect. Thus the Reilly-Hickinbottom rearrangement[45] of N-n-propylaniline yields largely the para-n-propyl isomer. Challis et al.[38] have additionally noted that, in general, nitrosation of mono-substituted electron-rich aromatics by $H_2NO_2^+$ or $NOCl$ yield primarily the p-nitroso isomer. e.g.
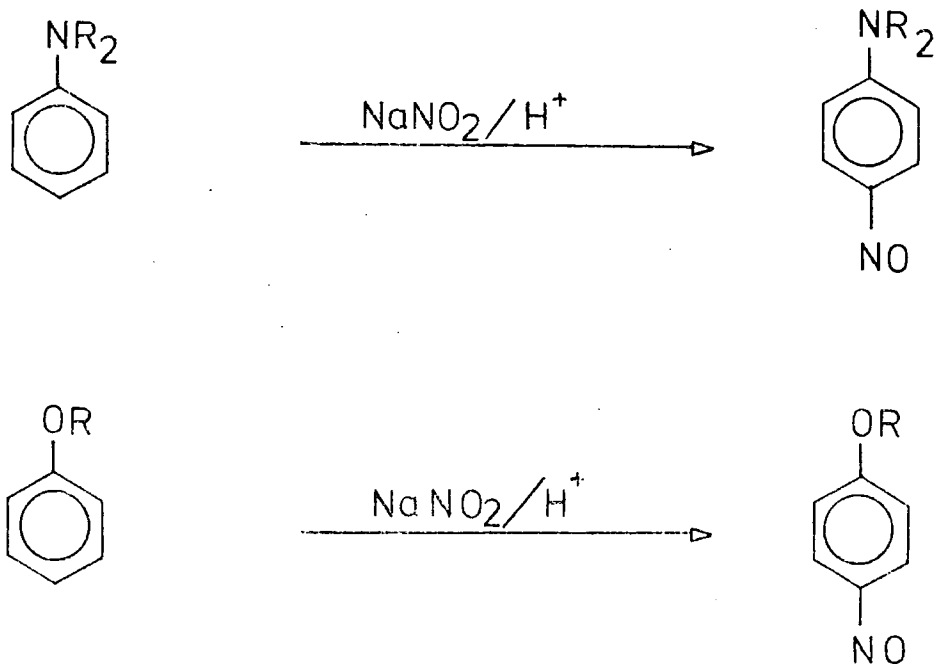
It may be, therefore, that explanation of the orientation effects operational in the Fischer-Hepp rearrangement can be gained from a

consideration of the orientation effects which operate generally in electrophilic substitution reactions. Recourse to a novel explanation peculiar to the Fischer-Hepp may be unnecessary. Working from this basis Thompson[46] has reviewed a number of aromatic substitution reactions and reports that in general reaction of an aromatic π -system with any "soft" electrophile such as Br^+ or NO^+ tends to yield the para-isomer exclusively. The result is rationalised in terms of modern generalised perturbation theories[47].

An alternative approach to the question of the reaction's specificity is via a consideration of π -system charge densities. Dewar[48] has calculated the π -cloud charge densities for azulene as follows.
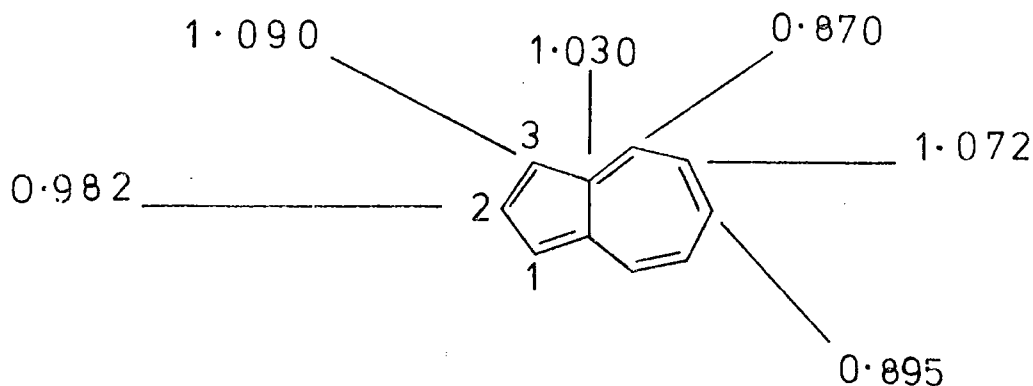
The electrophilic nitrosation of azulene might thus be expected to yield the 3-nitroso product. This result is indeed obtained. It is possible that calculations of a similar nature might indicate a high π -cloud charge density at the para-position of N-methyl-N-nitrosoaniline as compared with that at the ortho-position. It is thus probable that the orientation effects which operate in the Fischer-Hepp rearrangement are explained without recourse to novel mechanisms of limited applicability.

Mention has already been made of the yellow side-product obtained by

Williams from the reaction of N-methyl-N-nitrosoaniline in aqueous acid

solutions. Whilst this yellow product has not been the object of

positive identification analogy with observations concerning the

reaction of N-nitrosodiphenylamine in aqueous acid solution suggests the

formation of a radical cation species. Given the absence of both added

nucleophiles and nitrite trap species, the reaction of N-nitrosodiphenyl-

amine in 3 M aqueous sulphuric acid is observed by Thompson[49] to lead to

the rapid formation of a deep blue colouration with an associated visible

absorption maximum at 500nm. Addition of recognised radical scavengers

such as ascorbic acid or diphenylpicrylhydrazyl to the solution results in

rapid decolourisation. The blue coloured product would therefore appear to

exist in solution in the form of a free radical species. As the blue

colouration is also discharged by the addition of an excess of sodium

carbonate the free radical species probably takes the form of a radical

cation. This proposal is supported by the observation that the blue

species may not be extracted from aqueous solution into non-hydroxylic

solvents such as cyclohexane or diethyl ether. An e.s.r. study of the

species in aqueous solution, which was undertaken by the present author

with the co-operation of the Chemistry Department of the University of

York, has confirmed the presence of a radical species although the spectral

resolution was such as to preclude a positive identification of the species

involved.

The blue coloured species is noted to share a number of common

features with the Würster radical cation observed[50] as a product of the

reaction of diphenylamine with sodium nitrite in concentrated sulphuric

acid. In particular, both species possess U.V. absorption maxima which

lie in the range 450 ———▷ 610nm.

Furthermore Thompson has been able to isolate the product obtained

from reduction of the blue species by sodium azide. T.L.C. investigation

suggests that the blue colouration is due to a single species and on the basis of the recorded I.R. spectra the following structure has been assigned to the reduced form.
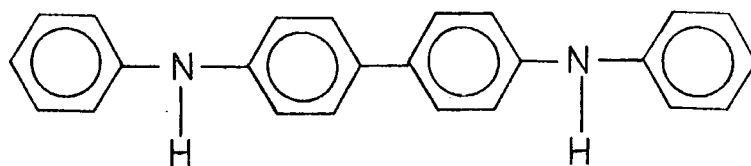
On the basis of the above information it therefore seems likely that the blue colouration evident in solution is due to the presence of a Würster radical cation as depicted below.

The following reaction scheme is proposed.

$$Ph_2NNO + H^+ \rightleftharpoons Ph_2\overset{+}{N}HNO \rightleftharpoons Ph_2\overset{+\bullet}{N}H + \bullet NO$$

$$2Ph_2\overset{+\bullet}{N}H \rightleftharpoons Ph_2\overset{+}{N}H\overset{+}{N}HPh_2$$

(Würster Radical Cation)

$$Ph_2\overset{+}{N}\overset{+}{H_2}$$

Polymer
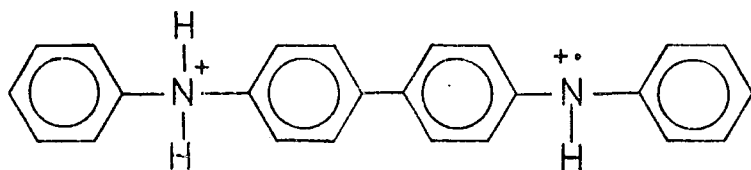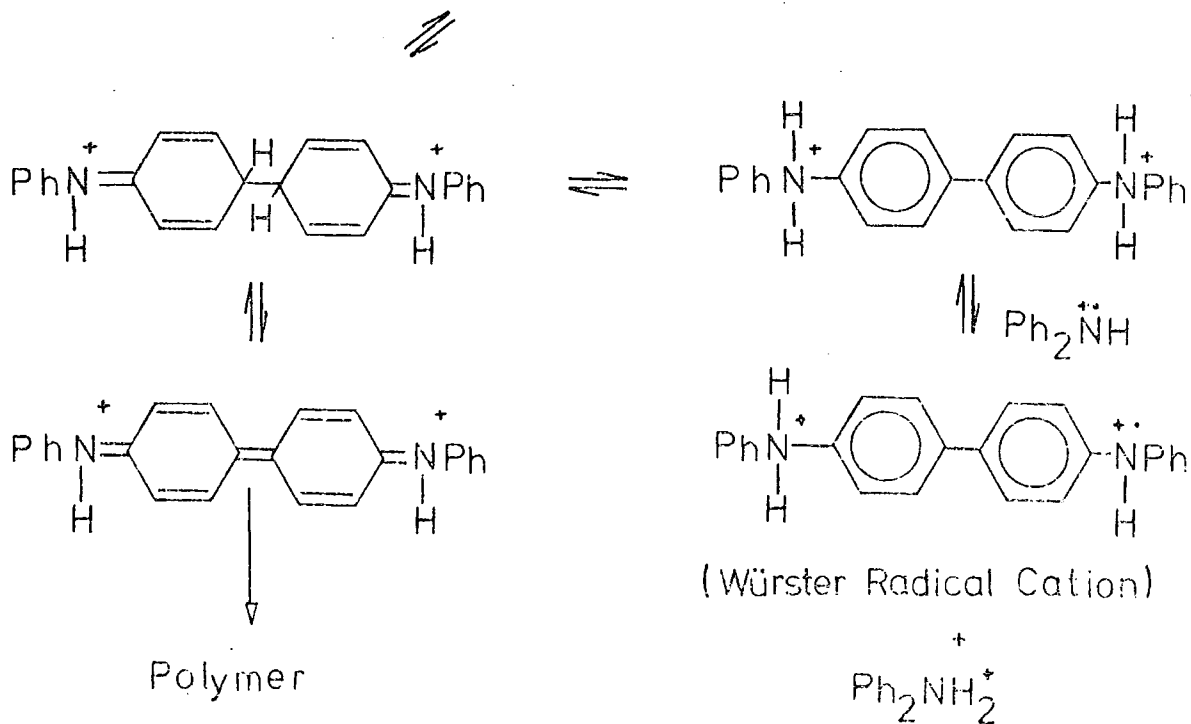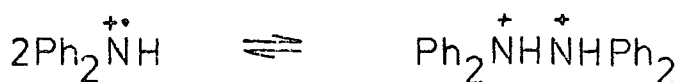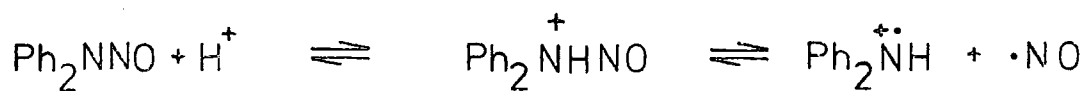
The scheme has experimental support. Formation of the diphenylamine radical cation, $Ph_2\overset{+\bullet}{N}H$, has been observed[51] during the irradiation of solutions of diphenylamine in toluene and the existence of hydrazium radical cations in acidic solutions of tetra-aryl hydrazines has been confirmed by e.s.r. studies[52,53]. The latter part of the reaction scheme is recognised as a benzidine rearrangement[54].

Although little definite information regarding the production of the yellow species from N-methyl-N-nitrosoaniline is available its nature is tentatively proposed as being similar to that of the blue species derived from N-nitrosodiphenylamine. Certainly there are common features; the yellow species is observed to be increasingly prominent at higher acidities and is destroyed by the addition of sodium azide.

Whilst it is clear that this aspect of the Fischer-Hepp reaction merits further study the following reaction scheme is proposed for the reaction of N-methyl-N-nitrosoaniline given the present availability of information.
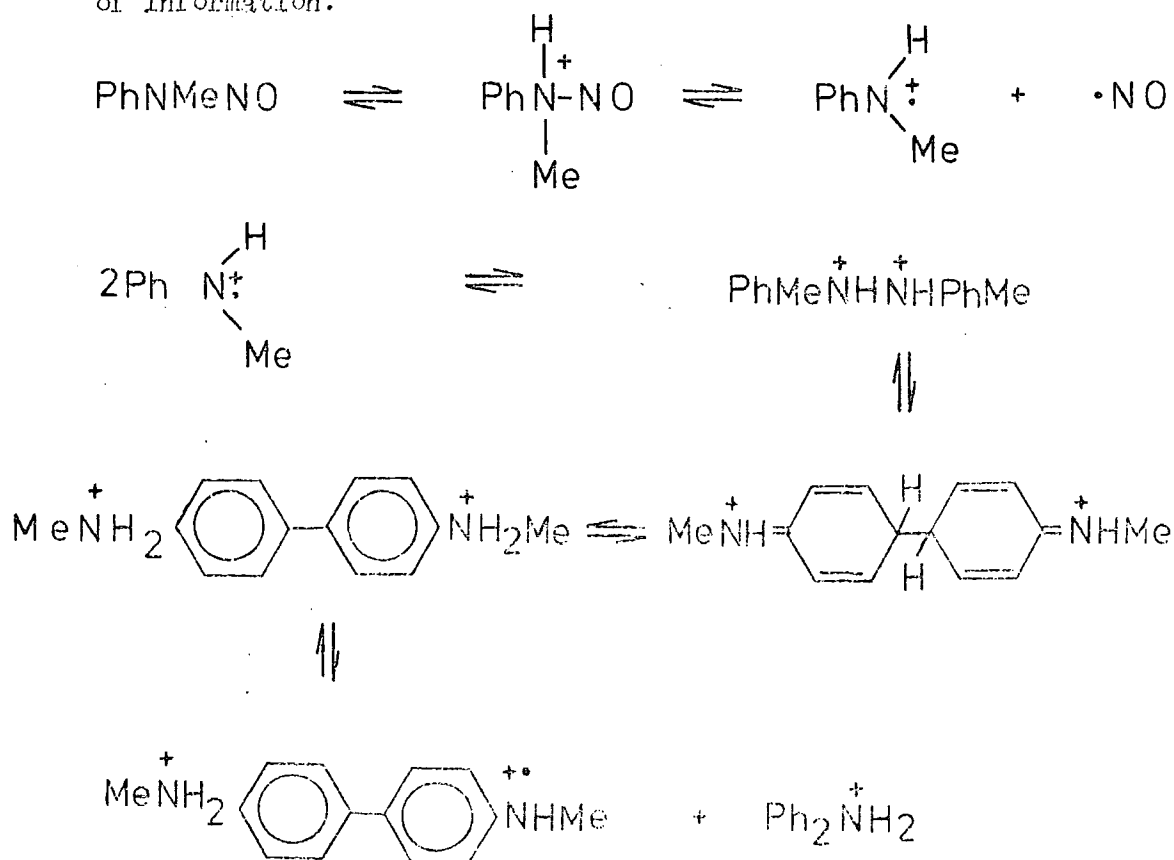
1.7   The Mechanism of the Denitrosation Reaction

It is stated in Section 1.1 of the present work that N-nitrosamines react under acidic conditions in the presence of large concentrations of an added nitrite trap such as urea to yield mainly the corresponding amine[7].   Despite its long-acknowledged existence the denitrosation reaction has, until recently, attracted attention only in the wider context of its role in the Fischer-Hepp rearrangement and it's detailed mechanism has remained a mystery.

In 1964 Russian workers[55,56,57] noted that the denitrosation of aromatic N-nitrosamines proceeded at a much higher rate in solutions containing HCl than in solutions containing H_2SO_4 at a comparable acid strength and attempted to explain their observations in terms of the operation of two parallel reaction mechanisms.   Thus nucleophilic attack by the anion HSO_4^- or Cl^- on a hydrogen bonded complex of the nitrosamine and the acid HA was purportedly accompanied by a concurrent unimolecular fission of the complex to yield the secondary amine and free NO^+.   It was further claimed that observation of the reactions of para-substituted aromatic N-nitrosamines lent support to the proposed scheme.

A later paper by the same authors[58] discussed the denitrosation in H_2SO_4 on the basis of reaction via mono- and di-protonated forms of the nitrosamine.

It would now seem that these early attempts to provide a mechanistic model for the denitrosation reaction are at best a little unrealistic. Despite claims regarding the support given by observation of the reaction of a series of aromatic N-nitrosamines it would appear that no compelling evidence exists in favour of the scheme.   The untenability of any arguement for the existence of free NO^+ in acidic solutions such as those employed above has been voiced in Section 1.5 of the present work.

On the basis of present information the reaction of the aromatic N-nitrosamines is best described in terms of Williams' intramolecular rearrangement scheme.

$$NOY + X \xrightarrow{k_3} Products$$
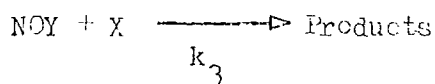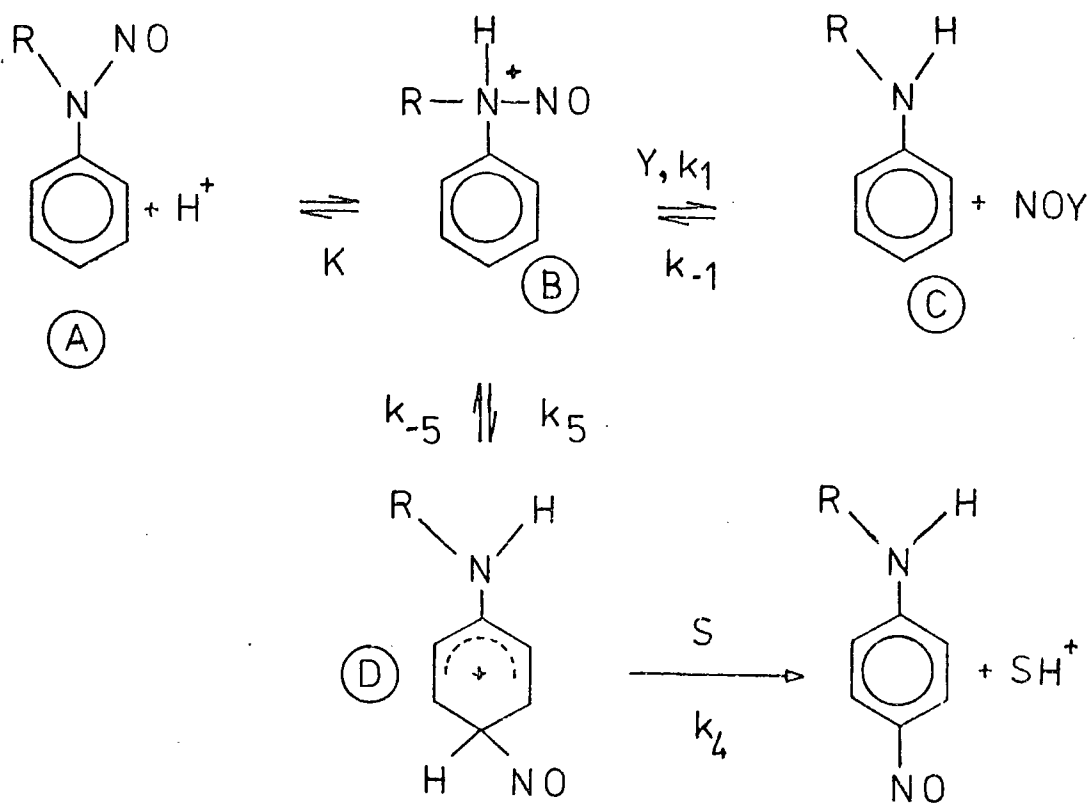
When the reaction is carried out in the presence of an excess concentration of an added nitrite trap $k_{-1}[C]$ becomes small compared with $k_3[X]$ and the full intramolecular rate expression may be simplified to yield the reduced form shown below.

$$Rate = \frac{k_4 k_5 K h_o[A]}{k_4 + k_{-5}} + k_{-1}[Y] K h_o[A]$$

In physical terms the swift irreversible removal of the free
nitrosating agent by reaction with the nitrite trap precludes the
possibility of reverse N-nitrosation of the secondary aromatic amine.
Using N-methyl-N-nitrosoaniline under such conditions in aqueous HCl
solution Williams[29] has found the rate of denitrosation to be far greater
than that of rearrangement except at very low chloride concentrations
when small yields of the rearrangement product are obtained. At
moderate chloride concentrations the system thus provides us with an
opportunity to study the mechanism of the now irreversible denitrosation
reaction without the further complication of a competing rearrangement.
Similar results are obtained using aqueous solutions of other mineral
acids in the presence of added concentrations of nucleophiles such as
chloride, bromide, iodide and thiocyanate.

A wide variety of nitrite traps have been employed to produce the
limiting conditions of $k_3[X] >> k_{-1}[Y]$ and in all cases the rate of
reaction is seen to become independent of both the nature and concentration
of the nitrite trap once the limit is reached. In the presence of an
excess concentration of the nucleophile, Y, the denitrosation reaction
becomes first-order. Investigation of the observed first-order rate
constant, k_o, as a function of the concentration of added N-methylaniline
has enabled Williams[59] to measure the relative reactivities of the various
nitrite traps towards the free nitrosating agent, NOY, and thus obtain
information regarding the efficiency of their operation as nitrite traps.
The following order of reactivities was evident.

Hydrazoic acid ~ hydrazine > sulphamic acid > hydroxylamine > urea.

Thus, at least for the reaction of N-methyl-N-nitrosoaniline in aqueous
solution, the classical nitrite trap, urea, is shown to be relatively
inefficient. Direct reaction between the nitrite trap, X, and the

protonated form of the nitrosamine is not thought to occur.

As might be expected from the form of the rate expression H^+ catalysis is observed. Analysis of the detailed form of acid catalysis in aqueous HCl solutions has shown[60] that catalysis is not exclusively by H^+ or its hydrated analogue since a plot of $\log k_0$ vs H_0 is linear with a slope of -1.62. Whilst plots of k_0 vs h_0 and $[Cl^-]$ respectively both result in curves a plot of k_0 vs $h_0[Cl^-]$ has proved linear over the acidity range studied.

Experiments have been carried out using deuterated solvents and a solvent isotope effect $k_{D_2O}:k_{H_2O}$ of ~ 3 has been observed[60] for the reaction of N-methyl-N-nitrosoaniline in aqueous solution at moderate acidities. This result rules out the possibility of a rate determining proton transfer under these conditions.

It would seem that the experimental observations are best rationalised on the basis of a reaction mechanism involving fast protonation of the nitrosamine followed by a rate-determining nucleophilic attack on the protonated form of the nitrosamine by the nucleophile Y^-.

Thus

$$PhN(Me)NO + H^+ \underset{K}{\rightleftharpoons} Ph\overset{+}{N}H(Me)NO$$

$$Ph\overset{+}{N}H(Me)NO + Y^- \xrightarrow{k_1} PhNHMe + NOY$$

$$NOY + X \xrightarrow{fast} Products$$
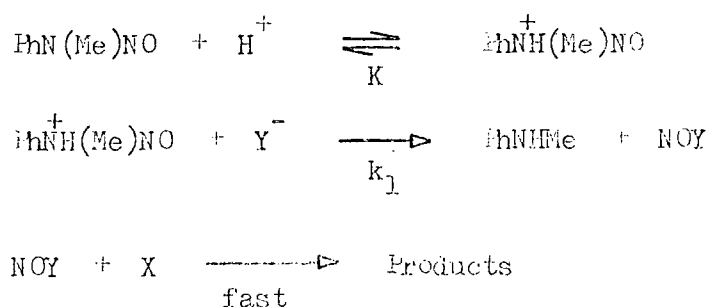
Identification of the site of nitrosamine protonation has been the subject of some study[61] in recent years and the indications are that a number of different species exist in relative concentrations which are dependent on the acidity of the reaction medium. Whilst the assumption that the reaction goes via the amino-protonated species is almost certainly an over-simplification an extension of the scheme to include other

protonated species is not expected to bring any changes to the rate expression for the reaction at moderate acidities.

Since k_o is given by,

$$k_o = \frac{k_4 k_5 K h_o}{k_4 + k_{-5}} + k_1 [Y^-] K h_o$$

and h_o is known a plot of k_o vs $[Y^-]$ might be expected to yield a value for $k_1 K$. Values of K are not obtainable by direct methods for the N-nitrosamines because of their reactions in acidic solution and $k_1 K$ must therefore suffice as a measure of nucleophile reactivity. Biggs and Williams[60] have observed the reaction of a number of nucleophiles and report that linear plots of k_o vs $[Y^-]$ were obtained for each and every nucleophile in the moderate concentration ranges employed. Values of $k_1 K$ for the reaction of a series of nucleophiles with N-methyl-N-nitrosoaniline in aqueous solution are given below.

Nucleophile	$k_1 K$
Cl^-	0.42×10^{-4}
Br^-	22×10^{-4}
CNS^-	0.22
I^-	0.63

Values of $k_1 K$ were found to be independent of both the nature and concentration of X providing the nitrite traps were present in concentrations sufficient to enforce the limiting conditions. The values of $k_1 K$ were also found to be independent of the acidity. The expected trend of nucleophile reactivity was observed. Thus,

$$I^- > CNS^- > Br^- > Cl^-$$

with relative rate constants for the denitrosation of N-methyl-N-nitroso-aniline of

15,000:5,300:54:1

The denitrosation reaction is therefore seen to be very sensitive to the nature of the nucleophile employed. This observation is in direct contrast to the results obtained for the reaction of these nucleophiles with the nitrous acidium ion, $H_2\overset{+}{N}O_2$, where the relative rates for the different nucleophiles are seen to change only by a factor of 1.5 over the complete range[62]. The anomaly is removed when it is recollected that the rates for the reaction with $H_2\overset{+}{N}O_2$ lie close to the diffusion controlled limit. Under such conditions no great discrimination between various nucleophiles is to be expected.

Application of the Swain-Scott equation[63] to the nitrosamine data yields a good straight line correlation from a plot of $\log k_1K$ against n, the nucleophilic constants of the respective nucleophiles. The value of 2.1 obtained for s, the susceptibility constant, from the slope of the plot demonstrates formally the sensitivity of the denitrosation reaction towards nucleophile reactivity.

The denitrosation reaction is also observed[60] in aqueous H_2SO_4 in the absence of added nucleophiles, albeit at a rate far below that recorded for the reaction in aqueous HCl solutions of comparable acidity. The reduced rate of denitrosation now allows more effective competition by the rearrangement reaction and the yield of the rearrangement product is correspondingly increased. For the reaction of N-methyl-N-nitrosoaniline[60] in a 2.5 M aqueous solution of H_2SO_4 the percentage yield of the rearrangement product is 20%. Since the rearrangement reaction now represents a substantial fraction of the total reaction observed values of k_o required correction to give k_d the rate constant for the denitrosation reaction, with the degree of correction to be applied being proportional to the observed yield of the rearrangement product.

Biggs and Williams[60] have investigated the denitrosation of N-methyl-N-nitrosoaniline in the absence of added nucleophiles using aqueous H_2SO_4 solutions as solvents. A plot of log k_d vs H_0 is found to be linear with a slope of -1.58 demonstrating the rate of increase of k_d with increasing acidity to be greater than that expected on the basis of a simple first-order h_0 dependence. On the assumption that the hydrogen sulphate anion, HSO_4^-, acts as a nucleophile a plot of k_d vs $h_0 + h_0[HSO_4^-]$ might be expected to be linear. In practice no such correlation is found. Furthermore addition of large concentrations of sodium sulphate has been observed to produce only small increases in k_d, the magnitudes of the increases being such as to permit their rationalisation in terms of salt effects. The most appropriate correlation of k_d vs $F(h_0)$ would appear to take the form given below.

$$k_d = \text{constant} \times h_0 + \text{constant'} \times h_0^2$$

A plot of k_d/h_0 vs h_0 thus yields a straight line. Even here the situation is not totally straight forward since the plot is composed of two straight lines of different slopes which intercept at a point corresponding to $h_0 = 200$. It may be that on reaching a sulphuric acid concentration of approximately 5M, ($h_0 = 200$), a change in the nature and extent of solvation of H_3O^+ takes place. Such a change would result in an alteration of the rate constant for attack by H_3O^+ which would be reflected by a corresponding change in constant'.

As the extent of catalysis by HSO_4^- has been shown to be insignificant we must contemplate the possibility of nucleophilic attack by species such as H_2O and H_3O^+. The reaction scheme is given below

$$PhNH(Me)NO^+ + H_2O \longrightarrow PhNHMe + H_2NO_2^+$$

$$PhNH(Me)NO^+ + H_3O^+ \longrightarrow PhNH_2Me^+ + H_2NO_2^+$$
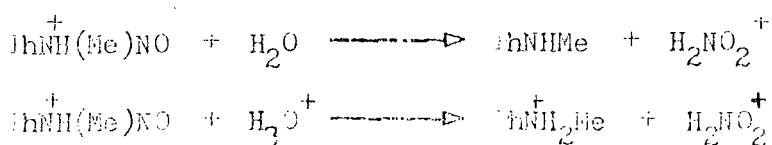
Here two concurrent reactions are proposed involving attack of H_2O and H_3O^+ respectively upon the protonated form of the nitrosamine. The latter reaction might reasonably be expected to become increasingly important at higher acidities. Whilst the proposed reaction between two positively charged species may appear a little unusual it should be remembered that an analogous reaction between the nitrous acidium ion $H_2NO_2^+$ and the protonated form of an aromatic amine has been demonstrated[15] as forming part of the mechanistic scheme describing the nitrosation of secondary aromatic amines at high acidities.

The realisation that the denitrosation reaction has an acidity dependence differing from that obeyed by the rearrangement reaction has been instrumental in explaining the observed variation of the rearrangement: denitrosation product ratio with changing acid concentration.

It is stated earlier that the observed rate constant for the denitrosation of N-methyl-N-nitrosoaniline in the presence of excess added concentrations of a nitrite trap, X, is independent of both the nature and concentration of the nitrite trap providing that the concentration is sufficient to ensure attainment of the limiting conditions of $k_3[X] >> k_{-1}[C]$. It is perhaps pertinent to point out that deviation from the zero-order dependence on $[X]$ is observed at high acidities[64]. For the reaction involving aqueous HCl solutions in the presence of excess added concentrations of various nitrite traps a plot of $\log k_o$ vs $-H_o$ shows that the zero-order dependence of k_o on $[X]$ is obeyed at all acid concentrations below HCl = 5.5 M. At higher acidities the reaction begins to lose the zero order dependence on $[X]$ the exact shape of the curve obtained being dependent on both the nature and concentration of X. As the nitrite trap species are considered to be relatively weak nucleophiles when in aqueous solution the deviation from linearity is not easily explained in terms of a direct reaction between the trap species

and the protonated form of the nitrosamine. As the acidity is increased the departure from zero-order behaviour in X occurs first for urea, then hydroxylamine followed by sulphamic acid, hydrazine and hydrazoic acid in that order. It is interesting to note that this series parallels the series constructed earlier for the increasing order of reactivity of the trap species towards the free nitrosating agent, NOY. The change of behaviour observed at high acidities is therefore suggested to be associated with the relative efficiencies of the different trap species. For the less efficient nitrite traps such as urea, hydroxylamine and sulphamic acid the observations are easily explained in terms of the extensive protonation of the trap species which is to be expected at high acidities. As the protonated forms of the trap species might reasonably be expected to be less reactive towards NOY than their unprotonated counter-parts, raising the acidity of the reaction medium will result in a lowering of the bulk reactivity of the added nitrite trap. At some stage the degree of protonation becomes such as to preclude the operation of the limiting condition, $k_3[X] >> k_{-1}[C]$ and the simple relationship between k_o and $-H_o$ no longer obtains. The reaction must now be treated in terms of the full intramolecular rate expression given below.

$$k_o = \frac{k_4 k_5 K h_o}{k_4 + k_{-5}} + \frac{k_1[Y] k_3[X] K h_o}{k_{-1}[C] + k_3[X]}$$

For the more reactive nitrite traps such as hydrazine and hydrazoic acid the reactive species in acid solutions are considered to be the protonated species, $NH_3NH_2^+$ and HN_3 respectively and further protonation is not thought to occur. In contrast to the curves obtained for the less efficient nitrite traps a plot of k_o vs $-H_o$ at high $[X]$ for hydrazine and hydrazoic acid does not exhibit a maximum in the acidity range studied. The deviation from linearity must be explained on a different basis to that employed for the less reactive nitrite traps and is

attributed to the extensive protonation of the nitrosoamine at high
acidities. Under such conditions the simple form of the rate expression,

$$k_o = k_1 [Y] Kh_o$$

must be modified to yield the expression,

$$k_o = \frac{k_1 [Y] Kh_o}{1 + Kh_o}$$

The latter expression may be simplified if protonation of the
nitrosamine is assumed to be complete so that we obtain the expression
given below.

$$k_o = k_1 [Y]$$

At very high acid concentrations k_o is therefore expected to become
independent of the acidity of the reaction medium. It is interesting to
note that measurements at very high acidities would therefore yield
a value for the individual rate constant, k_1, and thus permit a calculation
of the value of K, the equilibrium constant for the initial protonation,
which may not be obtained by direct methods. For example consider the
data of Williams.

$$[HCl] = 7.5 \ M \qquad\qquad k_o \sim 0.1 \ s^{-1}$$
$$H_o = -2.6$$

Thus if

$$k_o = k_1 [Y]$$

then

$$k_1 = 1.3 \times 10^{-2} \ l \ mol^{-1} \ s^{-1} \ for \ Cl^{-}$$

But

$$k_1 K = 0.42 \times 10^{-4}$$

Thus

$$K = 3.2 \times 10^{-3}$$
$$pK_a = -2.5$$

This value compares favourably with the value calculated by Williams

via an alternative approach.

The effects of N, meta- and para-substituents upon the rate of denitrosation of aromatic N-nitrosamines have been examined in a quantitative manner by Biggs and Williams[64]. For the reaction of a series of N-alkyl substituted N-nitrosoanilines in aqueous HCl solutions a rate sequence as depicted below was observed.

$$Pr^i >> Et > Pr^n \sim Bu^t > Me$$
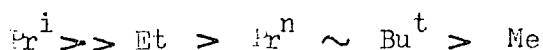
The relative rate constant ratios $k_o(Bu^t):k_o(Me)$ of 2.45, 2.39, 1.45 and 0.94 obtained in sulphuric acid solution with the nucleophiles Cl^-, H_2O, Br^- and I^- respectively lend support to the argument that the anomolous reactivity of the Bu^t compound is, at least to some extent, due to a steric hindrance of the incoming nucleophile. Whilst the general trend, excluding the Bu^t compound, is perhaps reflective of the effect of electron-donating substituents upon the equilibrium constant for the initial protonation there exists also the possibility of an effect upon k_1. The progression along the reaction coordinate is from the positively charged protonated form of the N-nitrosoamine towards, at least in the case of nucleophilic attack by negatively charged species such as the halide ions, a less positively charged transition-state. An increasing degree of N-alkyl substitution might therefore be expected to raise the activation energy for the nucleophilic attack resulting in a lowering of k_1. It is likely that both effects operate.

For substitution at the para-position of the benzene ring the complications of steric hinderance effects do not, of course, exist. The relatively small magnitude of the observed effect of substitution upon k_d has been explained in terms of the fine balance which exists, in the case of the ring substituted compounds, between the separate opposing effects upon K and k_1. For the halide catalysed reaction the rate sequence for

the denitrosation of the p-substituted N-methyl-N-nitrosoanilines is
as follows.

$$Cl > H > Me > OMe$$

The major effect in the case of the halide catalysed reaction is
thus shown to be that upon k_1.

For the uncatalysed reaction in aqueous H_2SO_4 solutions a reversed
sequence is obtained which is in line with the proposal of nucleophilic
attack by the positively charged hydroxonium ion, H_3O^+.

Further work involving the use of ring-methyl substituted
N-methyl-N-nitrosoanilines has been carried out by the same authors with
analogous results. Rate constants obtained in aqueous HCl solutions for
the denitrosation of the 2-methyl, 3-methyl, 2,6 dimethyl and 3,5 dimethyl
compounds show that in general ring-methyl substitution at the ortho and
meta positions activates the system towards the denitrosation reaction.
The results are again explained in terms of the separate effects upon
K and k_1 and it would appear that for meta and ortho-methyl substitution
the effect on K is the more dominant. The anomalous low reactivity of
the 2,6 dimethyl substituted compound appears to be due to a steric
hindrance of the solvation of the protonated form. A reluctance to form
the high energy protonated form is reflected in the low reactivity of the
substrate. This effect might also be expected to yield a low reactivity
for the rearrangement reaction and indeed this action is observed. Clearly
in the case of the denitrosation reaction there exists also the possibility
of steric hindrance of the incoming nucleophile. In aqueous H_2SO_4 the
increased magnitude of the general activation effect reflects the fact
that nucleophilic attack is now, at least in part, due to the positive
hydroxonium ion.

In the forgoing discussions nucleophilic catalysis of the denitrosation
of N-nitrosamines is described as being provided via the agency of the

simple nucleophilic species, I^-, SCN^-, Br^-, Cl^- and H_2O respectively. Whilst this does in fact represent the classical case, the list is by no means exhaustive and other possibilities do exist.

Early work by Storch[66] concerning the reactions of thiourea lead to the observation of a reaction between thiourea and nitrous acid which in the presence of moderately strong aqueous solutions of mineral acids yields a transient red colouration. This red colouration is observed to fade with time and a final product may be obtained from solution in the form of colourless crystals. The reaction was subsequently recognised as representing a nucleophilic attack by thiourea upon nitrous acid to yield the C,C'-dithiodiformamidium ion depicted below.

$$(NH_2)_2 \ C = \overset{+}{S} - \overset{+}{S} = C \ (NH_2)_2$$
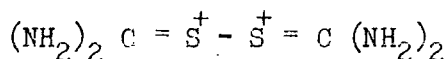
On the basis of these observations we might expect reaction between thiourea and the N-nitrosamines.

Williams[67,68] has studied the action of thiourea upon the denitrosation reaction of N-methyl-N-nitrosoaniline in aqueous acid solutions and gives the following report.

In the absence of an added nitrite trap the nitrosamine is seen to react with thiourea to give a transient yellow colouration. The same yellow colouration ($\lambda_{max} \sim 400nm$) is also reported by Stedman et al.[69] for the reaction of thiourea with nitrous acid. The apparent contradiction which exists between Stedman's results and those of Storch is explained in terms of the different reagent concentrations employed in the two studies. It is interesting to note that whilst Werner[70] reports a red colouration for the reaction of thiourea with sodium nitrite in aqueous H_2SO_4 solutions, the reaction in aqueous acetic acid solutions where on the basis of the acid catalysis described later the equilibrium concentration of the nitrosation product might be expected to be lower was reported to

yield a yellow colouration. The comment regarding the observed colouration to be a function of the product concentration would thus appear to be valid. The yellow colouration produced by the reaction of thiourea with the nitrosamine is observed to fade with time and good yields of the C,C'-dithioformamidinium cation are obtained from the equilibrium mixture. The observations are interpreted on the basis of an initial S-nitrosation to give the yellow S-nitrosothiourea intermediate which subsequently decomposes to yield the disulphide dication and nitric oxide according to the following scheme.

$$PhNMeNO + H^+ \underset{K}{\rightleftharpoons} PhN\overset{+}{H}(Me)NO$$

$$PhN\overset{+}{H}(Me)NO + S{=}C(NH_2)_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O{=}N{-}\overset{+}{S}{=}C\overset{NH_2}{\underset{NH_2}{\diagup}} + PhNHMe$$

YELLOW

$$(NH_2)_2C\overset{+}{S}\overset{+}{S}C(NH_2)_2 + 2NO$$

COLOURLESS
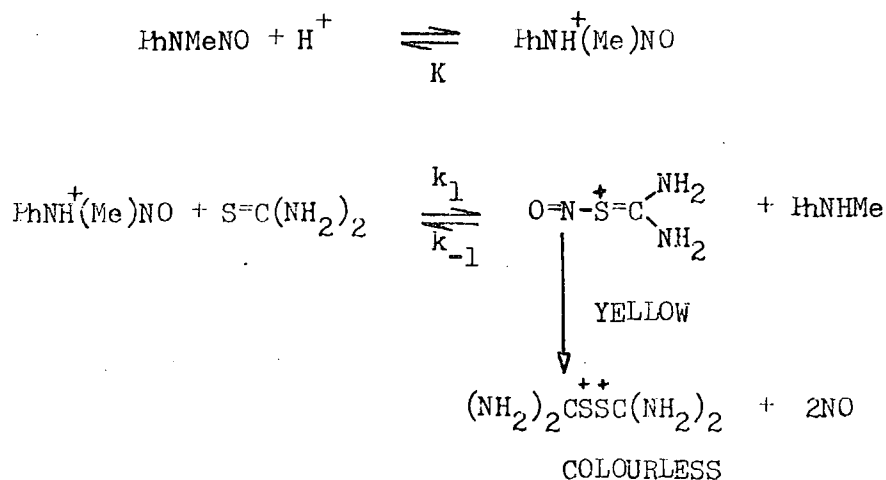
In the absence of a nitrite trap the nitrosamine is regenerated via the reaction of N-methylaniline with the nitrous acid formed from the oxidation of dissolved nitric oxide. In deoxygenated solutions the regeneration reaction is not observed. In the presence of an excess added concentration of a nitrite trap, such as hydrazine, the nitroso-thiourea adduct is rapidly removed by irreversible reaction with the nitrite trap with a consequent regeneration of the thiourea. Under these conditions the C,C'-dithiodiformamidinium ion is not obtained as a product of the reaction. As the rearrangement is not observed except at very low thiourea concentrations the reaction scheme may be described as over.

$$PhNMeNO + H^+ \underset{K}{\rightleftharpoons} Ph\overset{+}{N}H(Me)NO$$

$$Ph\overset{+}{N}H(Me)NO + SC(NH_2)_2 \longrightarrow PhNHMe + ON-\overset{+}{S}C(NH_2)_2$$

$$NO-\overset{+}{S}C(NH_2)_2 + X \longrightarrow SC(NH_2)_2 + Products$$
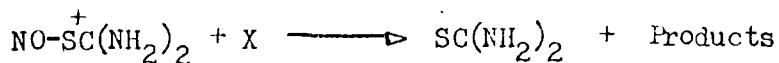
The reaction is found to be first-order in acid concentration. At moderate concentrations of thiourea the reaction is also found to be first-order in thiourea concentration. Assuming a Hammett acidity dependence for the initial protonation we may write,

$$k_o = k_1 K h_o [Y]$$

We may now calculate a value of $k_1 K$ for thiourea in a manner analogous to that employed for the more usual nucleophiles such as the halide ions. From the calculated $k_1 K$ value of 0.55 thiourea is shown to be almost as reactive as iodide ion as regards its nucleophilic attack upon the protonated form of the nitrosoamine.

Values of n, the nucleophilic constant, given by Pearson et al.[70] suggest that thiourea lies between thiocyanate ion and iodide ion in its nucleophilic reactivity. Acknowledging that we are now dealing with a nucleophilic substitution at a nitrogen site a plot of log $k_1 K$ vs n yields a particularly good correlation and serves to confirm Pearson's order of reactivities. The slope of this line is 1.40, significantly larger than the slopes generally found for conventional $S_N 2$ substitutions at a carbon centre and shows that the reactivity of the nucleophile (i.e. the bond-making process in going to the transition state) is particularly important in the present case.

A similar correlation (with a smaller slope) is found[125] for the reactions of the more reactive N-nitrosodiphenylamine with these nucleophiles.

Whilst the nitroso-thiourea adduct could, in principle, act as a nitrosating agent its action in this role is inefficient compared with that of species such as NOCl or $H_2\overset{+}{N}O_2$. Addition of N-methylaniline to the reaction system does result in a decrease in k_o, indicative of the reversability of the second step governed by k_1. A plot of $1/k_o$ vs [NMA] at constant acidity, [thiourea] and [X] leads to values of k_{-1}/k_3 for each of a number of trap species. The data for thiourea is given below together with that for bromide and thiocyanate so that comparison may be made.

Trap Species	k_{-1}/k_3		
	NOBr	$NOS\overset{+}{C}(NH_2)_2$	NOSCN
HN_3	3.2×10^{-2}	5.0×10^{-2}	4.5×10^{-2}
$NH_2\overset{+}{N}H_3$	4.8×10^{-2}	1.9×10^{-1}	1.8×10^{-1}
NH_2SO_3H	1.8	4.3	4.0
$\overset{+}{N}H_3OH$	30	85	88
$CO(NH_2)_2$	1,170		2,670

Consideration of the ranked values of k_{-1}/k_3 indicates the same trends of trap reactivity to be operative in each of the three cases. On the basis of a selectivity ⟷ reactivity correlation it would seem that whilst the two sulphur containing nitrosating agents NOSCN and $NOS\overset{+}{C}(NH_2)_2$ show similar abilities with respect to their nitrosation of the trap species, their reactivity in this aspect is substantially less than that of NOBr. It would thus seem that NOSCN and $NO-\overset{+}{S}C(NH_2)_2$ are not particularly reactive nitrosating agents.

Examples of the nitrosation of sulphur-containing nucleophiles are by no means limited to the reactions of thiourea. Recent work has demonstrated the nitrosation of thiols[71] and thioamides[72] by nitrous

acid and both the alkyl-thioureas[73] and cysteine are believed to undergo a similar S-nitrosation under the action of the same reagent. The reaction of N-alkyl-N-nitrosourethane in the presence of cysteine has been reported as yielding a "bewildering array of products"[74].

Discoveries concerning the actions of nitrosating agents upon naturally occuring sulphur containing species such as cysteine may well have a significant impact upon our consideration of the biological action of N-nitroso compounds. We shall pursue this point in some greater detail in Section 6.

The present work looks at the reaction of a series of alkyl thioureas with N-methyl-N-nitrosoaniline in aqueous acid solution. The reactions of N-methyl-N-nitrosoaniline with methionine, glutathione, cysteine and S-methylcysteine respectively are also considered.

The aspect of the denitrosation of N-nitroso compounds pertaining to catalysis by added metal ions is currently the cause of much debate. Preussman et al. have reported[75] that the degradation of N-ethyl-N-nitrosourea in aqueous acidic solution may be catalysed by the addition of any one of a series of transition metal ions. Thompson[76] has pointed out that the observed order of reactivity parallels exactly the Irving-Williams series[77] for the stability constants of complexes of the divalent first row Transition Metal ions.

$$Cu^{2+} >> Ni^{2+} > Co^{2+} \sim Mn^{2+} \sim Fe^{2+}$$
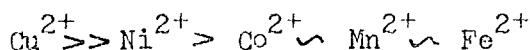
The catalytic effect has therefore been described in terms of the rate determining decomposition of a complex such as that depicted below.
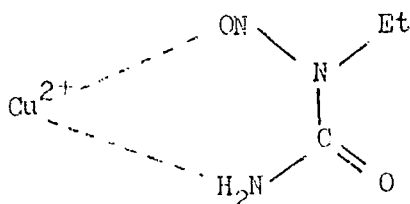
No such catalysis has been reported for the denitrosation reactions of N-methyl-N-nitrosourethane[75], N-methyl-N-nitrosoaniline[78] or N-methyl-N-nitrosotoluene-p-sulphonamide. It is assumed that these latter compounds are unable to form the chelate complex.

In the present work the action of Fe^{2+} upon the denitrosation of N-methyl-N-nitrosoaniline has been studied. From the results obtained it would seem that the "catalytic" action of the low oxidation state Transition Metal ions is, at least in part, due to their action as nitrite traps.

1.8 The N-nitrosation Reaction

It is perhaps pertinent at this stage of the present introduction to discuss a few, selected aspects of the N-nitrosation reaction which represents a reversal of the denitrosation reaction discussed above. Historically the approach has been to separate the N-nitrosation reaction for discussion and in deference a separate treatment is made here. The present work has provoked a common treatment of the nitrosation and denitrosation reactions which goes further towards providing a desirable portrayal of their interdependence. This second treatment is given in Section 2.

The nitrosation reaction has recently been reviewed in an excellent article by Ridd[62]. It is hoped that the short summary which follows will provide a background for the later discussion of the catalysis of nitrosation and denitrosation reactions in general.

The character of the products obtained from the reaction of an amino-compound with nitrous acid is to a large extent dependent on the class of amino-compound employed. Thus primary aliphatic amines react to initiate a reaction pathway which continues as follows.

$$RNH_2 \longrightarrow RNHNO \longrightarrow RNNOH \longrightarrow RN_2^+$$
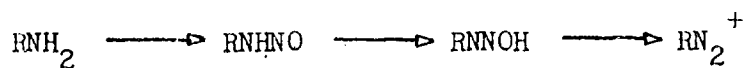
Unstable Diazonium Salt

$$RN_2^+ \longrightarrow R^+ \longrightarrow \text{Deamination Products}$$
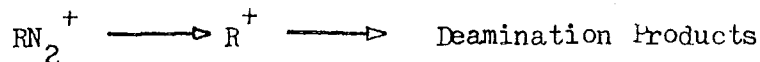
As the diazonium salts of primary aromatic amines are relatively stable reaction of a primary aromatic amine with nitrous acid yields the corresponding diazonium salt. The reaction of secondary amines, both aliphatic and aromatic yields the N-nitrosamine. Historically speaking reaction of the tertiary amines has been considered to occur only in the case of compounds such as N,N'-dimethylaniline where electrophilic attack by nitrosating agents upon the activated benzene ring has been invoked in explaining the production of the observed para-nitroso derivative. It is now clear that tertiary aliphatic amines react to give the N-nitroso derivative of a corresponding secondary amine. To some extent this reaction is paralleled by the tertiary aromatic amines especially those which possess para-substituents. In all cases the expelled group appears in the reaction products in the form of an aldehyde or ketonic derivative. The amides are observed to follow a similar pattern of reaction.

For the general case the observed reaction kinetics have been recognised as being indicative of the existence of a slow, initial N-nitrosation step which, at low acidities, is rate determining. Thus the observed kinetic complexity is due to an incursion of the several different mechanism of N-nitrosation. A number of these mechanisms possess two potentially rate-determining sub-stages. A study of the kinetics of the reaction of the amino-compounds with nitrous acid therefore yields information concerning the mechanism of the initial, rate-determining, N-nitrosation reaction. At low acidities, where $[H^+] > 0.5$ M, three

distinctly different mechanisms are porposed for the reaction in aqueous solution.

The Nitrous Anhydride Mechanism

In 1928 Taylor[79] demonstrated that the kinetic form observed for the deamination of methylamine in dilute aqueous solutions of nitrous acid could be described by the rate expression given below.

$$\text{Rate} \; = \; k \, [\text{amine}] \, [\text{HNO}_2]^2 \qquad (1)$$

The same kinetic form was observed by Taylor for the deamination of ammonia[79] and dimethylamine[80] and subsequently by Schmid[81] for the diazotisation of aniline in 0.2 M aqueous sulphuric acid. Hammett[82] suggested that the observed third-order rate expression is the consequence of a mechanism which involves nitrosation by nitrous anhydride. The following scheme of reaction was proposed.

$$\text{H}^+ + \text{HNO}_2 \; \underset{\text{fast}}{\rightleftharpoons} \; \text{H}_2\text{O}^+ - \text{NO}$$

$$\text{H}_2\text{O}^+ - \text{NO} + \text{NO}_2^- \; \underset{\text{fast}}{\rightleftharpoons} \; \text{N}_2\text{O}_3 + \text{H}_2\text{O}$$

$$\text{ArNH}_2 + \text{N}_2\text{O}_3 \; \underset{\text{slow}}{\longrightarrow} \; \text{ArNH}_2\text{NO}^+ + \text{NO}_2^-$$

$$(\text{ArNH}_2\text{NO}^+ \; \underset{\text{fast}}{\longrightarrow} \; \text{ArN}_2^+)$$
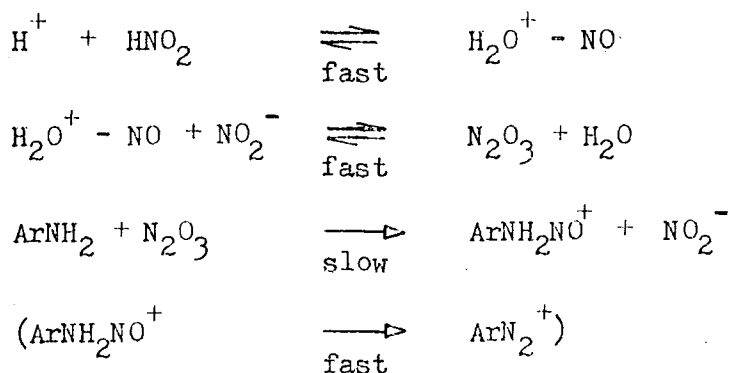
Supporting evidence for the proposed scheme is obtained from the results of experiments at very low acidities[83,84]. As the acidity of the reaction medium is reduced the rate of production of the proposed nitrosating agent, N_2O_3, might be expected to fall. At low acidities the proportion of the amine which exists in the free form is increased so that there should arise at some stage a situation in which all N_2O_3 is removed, as soon as it formed, by the amine. The rate of N-nitrosation is now governed by the rate of formation of the nitrosating agent. This rate

is independent of amine concentration. Thus the Hammett scheme implies that the rate of N-nitrosation should become independent of the amine concentration at very low acidities. With reactant concentrations of 10^{-3} M in 0.002 M perchloric acid Hughes et al. have found the rate of N-nitrosation of a series of amines to be described by the expression shown below.

$$\text{Rate} = k[HNO_2]^2 \qquad (2)$$

Thus the rate of reaction is found to be independent of the concentration of the amine. Over a limited range of amine basicities the rate of reaction is also independent of the nature of the amine. The result has been taken as supporting the proposals of Hammett. Further support for the scheme has been derived from an observation of the rate of ^{18}O exchange between nitrous acid and water in the absence of the amine[85,86].

The nitrous anhydride mechanism is now regarded as having a general applicability in describing the reaction of aniline and other primary aromatic amines of similar basicity in dilute aqueous solutions of hydrochloric, perchloric and sulphuric acid respectively. The dilution criteria are obeyed by solutions of perchloric and sulphuric acid of less than 0.5 M concentration and by solutions of hydrochloric acid of less than 0.1 M concentration. The lower limit for the latter case is due to the incursion of a halide catalysed mechanism at higher acidities. The nitrous anhydride mechanism may also be used to describe the first stage of the deamination of aliphatic amines under similar conditions. For a series of p-substituted anilines the values of k defined by equation 1 are found to exhibit a close correlation with the relative basicities of the respective substrates[62]. This trend however does not continue upon consideration of the aliphatic amines which are far less reactive than their high basicities would lead us to predict.

As the equilibrium constant for the formation of nitrous anhydride is known[87,88] the true rate constant for the reaction of the free amine with nitrous anhydride may be estimated. For aniline itself this value of k' defined by,

$$\text{Rate} = k' [PhNH_2][N_2O_3] \qquad (3)$$

is found to be of the order of $10^{-7} \text{ mol}^{-1} \text{ s}^{-1}$ l at 25°C. The reaction of nitrous anhydride with the free amines is therefore thought not to represent an example of a diffusion controlled process. This result is in marked contrast to that obtained for the reaction of the nitrosyl halide species with the free amine.

The Nitrosyl Halide Mechanism

Although the catalysis of diazotisation by hydrochloric acid had previously been reported by a number of authors[89] the exact form of the halide catalysis did not become apparent until its elucidation by Schmid in 1937[90,91]. The effect of halide catalysis is to add an extra term to the rate expression given as equation 1 to yield equation 4.

$$\text{Rate} = k[amine][HNO_2]^2 + k''[amine][H^+][HNO_2][Y^-] \qquad (4)$$

where Y^- represents the halide ion.

As the value of k" is found to be several orders of magnitude higher than that of k' we may generally re-write equation 4 as equation 5.

$$\text{Rate} = k''[amine][H^+][HNO_2][Y^-] \qquad (5)$$

This approximation may not of course apply at very low halide concentrations or at very low acidities. Hammett[82] has pointed out that the concentration terms in the equation are now equivalent to the product of the concentrations of the nitrosyl halide, NOY and the amine. On this basis he proposed the following mechanism for the halide catalysed reaction

$$HNO_2 + H^+ \underset{fast}{\rightleftharpoons} H_2O^+ - NO$$

$$H_2O^+ - NO + Y^- \underset{fast}{\rightleftharpoons} NOY + H_2O$$

$$NOY + ArNH_2 \xrightarrow{slow} Ar\overset{+}{N}H_2NO + Y^-$$

$$(Ar\overset{+}{N}H_2NO \xrightarrow{fast} ArN_2^+)$$
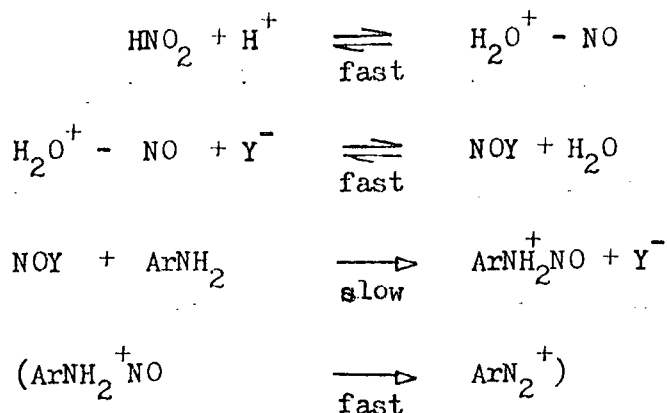
Note that, in common with the nitrous anhydride mechanism the nitrosyl halide mechanism possesses as its first stage an inorganic reaction step which does not involve the amine. Evidence in support of the proposed mechanism may therefore be collected, in a manner similar to that employed for the case of the nitrous anhydride reaction, by operating the reaction under conditions which make the production of the nitrosating agent, NOY, the rate determining step. Thus Hughes and Ridd[92] have demonstrated that reaction in the presence of a large added excess of the amine is governed by a reduced form of the rate expression as given below.

$$Rate = k''[H^+][HNO_2][Y^-]$$

The rate of the reaction is now independent of the amine concentration, the observed form of the rate expression being interpreted in terms of a rate-determining attack by the halide ion upon the nitrous acidium ion, $H_2\overset{+}{N}O_2$.

Use of rate coefficient data from Equation 4 in conjunction with the available data concerning the equilibrium constants for the formation of the nitrosyl halides has enabled Schmid and co-workers[93,94,95] to obtain true rate constants, k''', for the reaction of the molecular nitrosyl halides, NOY, with the free amine. Values of k''', defined by,

$$Rate = k'''[amine][NOY]$$

are tabulated below for the reaction of a number of substituted anilines with nitrosyl chloride at 25°C.

Amine	$10^{-9}k'''/mol^{-1} s^{-1} l$
o-chloroaniline	1.16
m-chloroaniline	1.63
p-chloroaniline	1.89
aniline	2.60
o-toluidine	2.44
m-toluidine	2.70
p-toluidine	3.00

The striking feature here is that although basicity is increased by a factor of 250 on descending the table the recorded values of k''' remain approximately constant. Furthermore the values of the rate constants lie close to the value of 10^{10} l mol^{-1} s^{-1} expected for a diffusion controlled reaction under these same conditions[87]. It would thus appear that the reaction of the free amine with NOCl is a bimolecular encounter controlled process. Support for this proposal has been derived from calculations of the activation energies using the observed dependence of k''' upon temperature. The values of 19.06 kJ mol^{-1} and 20.59 kJ mol^{-1} obtained for the reaction of aniline and p-chloroaniline respectively are noted to possess a great similarity with the apparent activation energy of $\sim$ 17 kJ mol^{-1} at 25°C calculated for the general diffusion controlled process on the basis of the variation of solvent viscosity with temperature.

To proceed further we must turn to the recent study by Crampton et al.[119] which represents an application of stopped-flow techniques to the nitrosation of a series of substituted anilines by nitrosyl halides. The

approach differs from earlier works in that it recognises the reversibility of the N-nitrosation as being an important factor particularly at high halide ion concentrations and also for aniline derivatives containing electron-withdrawing groups. Whilst this realisation does add to the complexity of interpreting substituent effects the idea of a diffusion controlled process is retained for aniline derivatives with $pK_a > 4$.

Acid Catalysed Mechanisms

For the reaction of aniline in aqueous solutions of perchloric and sulphuric acids equation 1 is seen to predict that an increase in acidity should be accompanied by an corresponding decrease in the rate of reaction due to the reduced availability of the free amine. As the perchlorate and sulphate ions are incapable of forming covalent nitrosating agents we may expect that an increase in acidity would result in an overall decrease in the rate of reaction. Whilst this is, in the first instance, true we note that the rate of reaction is subsequently increased due to the incursion of further acid catalysed mechanisms at these higher acidities.

The first of the acid catalysed mechanisms to be identified[96] was found to be described by the following rate expression.

$$\text{Rate} = k[ArNH_2][HNO_2][H^+] \qquad (6)$$

This result has been interpreted[84] in terms of the existence of a rate-determining reaction between the free amine and the nitrous acidium ion $H_2NO_2^+$. Thus:

$$HNO_2 + H^+ \underset{\text{fast}}{\rightleftharpoons} H_2NO_2^+$$

$$ArNH_2 + H_2NO_2^+ \xrightarrow{\text{slow}} ArNH_2NO^+$$
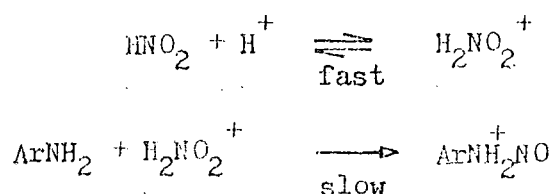
Reaction of the nitrosonium ion, NO^+, with the free amine which would have provided an alternative explanation for the observed kinetic form has been ruled out on the basis of results obtained from ^{18}O-exchange experiments. Larkworthy[97] has been able to show that the rate of reaction via the present mechanism is much less sensitive to variation in amine substrate basicity than is the rate of reaction via the nitrous anhydride mechanism and it is therefore apparent that the nitrous acidium ion is the more reactive of the two nitrosating agents concerned.

Reaction at High Acidities

Analysis of the observed kinetic behaviour of the system in terms of reaction mechanisms becomes increasingly more difficult as the acidity of the aqueous reaction medium is raised above o.5 M. In the absence of halide catalysis two main factors would appear to be operative in modifying the observed kinetic form at high acidities. The first factor, essentially a rate enhancement by medium effects, is observed as the only modifying factor in the reaction of the less basic amines in solutions of perchloric acid of up to 3M acidity. Thus the reaction of p-nitro aniline in aqueous solutions of perchloric acid , with added sodium perchlorate to maintain a constant ionic strength,is observed to follow equation 6 with the proviso that the H^+ concentration term is replaced by the more appropriate acidity function, h_o. The observed behaviour[98] of the medium effect as a function of the added concentration of the perchlorate ion and as a function of the nature of the metal ion in added concentrations of metal perchlorates indicates that we are not looking at an example of specific perchlorate ion catalysis.

For the reaction of the more basic amines at these acidities an additional modifying factor operates in the form of the incursion of a new mechanistic pathway. This new pathway is characterised by the

following expression[98].

$$\text{Rate} = k\left[\text{ArNH}_3^+\right]\left[\text{HNO}_2\right]h_0$$

Whilst the details of this mechanism are as yet uncertain it would appear that the observed kinetic form is best explained by considering the manner of the nitrosation of the protonated amine to be such as to retain the "out-going" proton in the transition state. Thus if the amine exists almost entirely in the protonated form at these acidities then a direct interaction between the appropriate nitrosating agent and the protonated form of the amine can apparently become significant.

In addition to the two factors discussed above a postulated reaction involving N-nitrosation by the nitrosonium ion may be expected to become an increasingly realistic possibility at higher acidities. As the nitrosonium ion may reasonably be expected to be the most reactive of the nitrosating agents so far discussed it is likely to be the least susceptible to variations in amine substrate basicity. The incursion of the nitrosonium ion mechanism may therefore be most easily detected in the reactions of the less basic amines. The kinetic form observed for the reaction of benzamide with nitrous acid in strong sulphuric acid solution has recently been explained in these terms[99].

In general the rate of reaction is seen to rise towards a maximum as the acidity of the reaction medium is increased. Thus for the reaction in aqueous solutions of perchloric acid the rate profile is observed to reach a maximum at $\left[\text{HClO}_4\right] = 6$ M and thereafter to decrease with increasing acidity. Interpretation of the experimental results obtained for the reaction in solutions of perchloric and sulphuric acids respectively at acidities approaching 10 M and beyond must be made on an independent basis. The rates of reaction of aniline, p-nitroaniline and p-toluidine respectively at these high acidities are reported[100,101] to follow the expression given over.

$$\text{Rate} = k[\text{ArNH}_3^+][\text{HNO}_2]\, h_o^{-2}$$

In view of the large magnitude of the reported[33] solvent isotope effect, $k_H/k_D = 10$, it is difficult to describe the system in any terms compatible with the existence of a rate-determining N-nitrosation. Raman studies have shown[102] that at these very high acidities nitrous acid exists almost entirely in the form of the free nitrosonium ion, NO^+, and it is therefore believed[62] that the reaction mechanism may be represented as follows.

$$\text{ArNH}_3^+ + NO^+ \underset{k_{-1},\ \text{fast}}{\rightleftharpoons} \text{ArNH}_2^+ NO + H^+$$

$$\text{ArNH}_2^+ NO \xrightarrow{\text{slow}} \text{ArNHNO} + H^+$$

$$\text{ArNHNO} \xrightarrow{\text{fast}} \text{ArN}_2^+ + OH^-$$
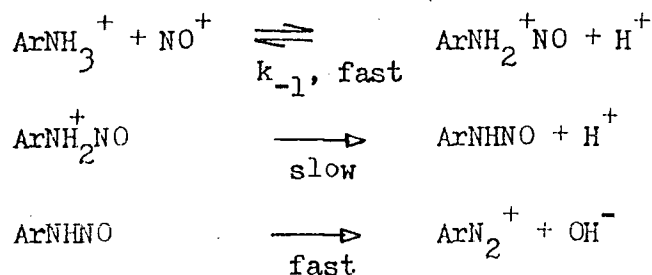
It is not difficult to understand why the second stage depicted above should become rate-determining under such conditions since the transfer of a proton from $\text{ArNH}_2^+ NO$ to a very acidic reaction medium may not be expected to occur easily. Furthermore the rate of the backward reaction, governed by k_{-1}, will be enhanced as a consequence of the high proton activity in such solutions.

At these very high acidities the reaction of the amine with nitrous acid is not characterised by a rate-determining N-nitrosation and information regarding the mechanism of the N-nitrosation can not be obtained from observations of the kinetic form of the overall reaction.

SECTION TWO

THE ACID CATALYSED REACTIONS OF N-NITROSO COMPOUNDS.

A RATIONALE OF THE EXPERIMENTAL

OBSERVATIONS.

2.1 The Denitrosation Reaction

It is perhaps pertinent to note that the preceeding discussion regarding the mechanism of the denitrosation of N-nitroso compounds in acid solutions draws rather heavily on our experience of one particular compound. Whilst the reaction of this compound, N-methyl-N-nitrosoaniline, has been the subject of a comprehensive study on the part of Williams et al.[103] it must be said that our knowledge or, more precisely, our understanding of the general case has been limited in its extent. Our difficulties have not stemmed from a shortage of data, indeed the present interest in the carcinogenic properties of the N-nitroso compounds has lead to an influx of relevant factual material. Rather we have been faced with the absence of a rationale which might encompass the apparently unrelated results reported in the literature. A consideration of the results obtained in this department serves to illustrate the nature of the problem. Thus Williams[11] has reported the denitrosation of N-methyl-N-nitrosoaniline in aqueous acid solution to be subject to catalysis by both acids and nucleophiles. On the other hand the analogous reaction is ethanol using dissolved HCl as the mineral acid is not subject to nucleophilic catalysis[30]. Meyer et al.[104] have been able to demonstrate that this absence of nucleophilic catalysis may also be observed for the reaction in water given the presence of large excess concentrations of the chosen nucleophile. Similarly, whilst Thompson[105] has shown that the denitrosation of N-nitrosodiphenylamine in aqueous acidic solution is subject to both acid catalysis and catalysis by nucleophiles the observation does not extend to the reaction in ethanol.

The absence of nucleophilic catalysis for the reaction using an

aqueous medium in the presence of large excess concentrations of a chosen nucleophile has been demonstrated both by Thompson[106] and by the present author, (Section 5.2). In contrast to the observations concerning the reaction of the two N-nitrosamines discussed above, Williams[107] has reported that the rate of denitrosation of N-methyl-N-nitrosotoluene-p-sulphonamide in aqueous acidic solution is not enhanced by the addition of nucleophilic species such as the halide ions. Similarly the reaction of N-methyl-N-nitrosourea in aqueous solution is not catalysed by nucleophilic species, (Section 3.2.5).

The key to the formation of a successful rationale lies in the realisation that the observed behaviour of each system with respect to catalysis depends on the relative rates of the two component stages of the denitrosation reaction. A generalisation of the reaction scheme proposed by Williams[11] for the reaction of N-methyl-N-nitrosoaniline in aqueous solution at moderate acidities leads to the scheme shown below.

$$\underset{B}{\overset{A}{>}}N\text{-}NO + H^+ \rightleftharpoons \underset{B}{\overset{A}{>}}\overset{+}{N}\underset{H}{\overset{NO}{<}} \xrightarrow{Y^-} \underset{B}{\overset{A}{>}}N\text{-}H + NOY$$

$$\Updownarrow$$
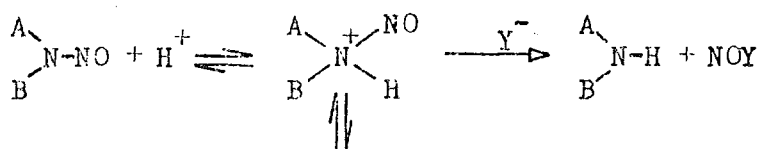
REARRANGEMENT PRODUCT (where applicable)

NOY + X $\longrightarrow$ Products

$\underset{B}{\overset{A}{>}}N\text{-}NO$ represents the generalised N-nitroso compound.

Y^- is a nucleophile

X   is a nitrite trap

Analogy with the N-methyl-N-nitrosoaniline system would suggest that if the reaction is carried out in the presence of a sufficient concentration of a suitable nitrite trap the percentage yield of any rearrangement product will be negligible. In such circumstances the scheme simplifies to the form shown below.

$$\begin{array}{c} A \\ B \end{array}\!\!\!N\text{-NO} + H^+ \quad \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \quad \begin{array}{c} A \\ B \end{array}\!\!\!N^+\!\!\!\begin{array}{c} NO \\ H \end{array} \quad \overset{Y^-}{\underset{k_1}{\longrightarrow}} \quad \begin{array}{c} A \\ B \end{array}\!\!\!N\text{-H} + NOY$$
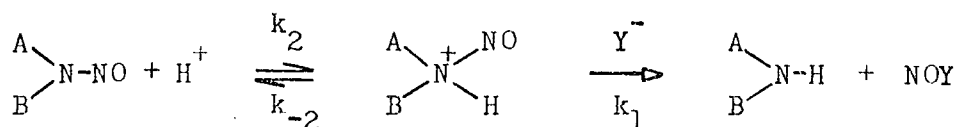
As the deprotonation of the protonated N-nitroso compound to yield the free N-nitroso species must occur via the agency of a solvent molecule the rate constant, k_{-2}, is understood to include a term corresponding to the solvent concentration, $[S]$. It is further understood that the hydrogen ion, H^+, exists in solution in the form of the appropriate solvated ion, SH^+. An expression for the rate of appearance of the product, ABNH, may be written as follows.

$$\text{Rate} = \frac{d\,[ABN\text{-H}]}{dt} = k_1\,[Y^-]\,[ABN^+H\text{-NO}]$$

Application of the steady state principle to $[ABN^+H\text{-NO}]$ yields,

$$k_2\,[ABN\text{-NO}]\,[H^+] = k_{-2}\,[ABN^+H\text{-NO}] + k_{-1}\,[ABN^+H\text{-NO}]\,[Y^-]$$

whence,

$$[ABN^+H\text{-NO}] = \frac{k_2\,[ABN\text{-NO}]\,[H^+]}{k_{-2} + k_1\,[Y^-]}$$

Substitution for $[\text{ABNH-NO}]^+$ in the rate expression yields,

$$\text{Rate} = \frac{k_2 k_1 [\text{ABN-NO}] [\text{H}^+] [\text{Y}^-]}{k_{-2} + k_1 [\text{Y}^-]} \tag{7}$$

In the presence of an excess concentration of the nucleophile we may write an expression for the rate of disappearance of the N-nitroso reactant, ABN-NO, such that,

$$\text{Rate} = k_0 [\text{ABN-NO}] \tag{8}$$

where k_0 is the observed 1st order rate constant for the disappearance.

If loss of ABN-NO is assumed to be entirely on account of the denitrosation reaction we may equate expressions 7 and 8 to give equation 9.

$$k_0 = \frac{k_1 k_2 [\text{H}^+][\text{Y}^-]}{k_{-2} + k_1 [\text{Y}^-]} \tag{9}$$

Further simplification of the present expression is possible only on the basis of assumptions concerning the relative magnitudes of k_{-2} and $k_1 [\text{Y}^-]$.

(i) if $k_{-2} \gg k_1 [\text{Y}^-]$ then equation 9 reduces to,

$$k_0 = \frac{k_1 k_2 [\text{H}^+][\text{Y}^-]}{k_{-2}} \tag{10}$$

Acid catalysis will be observed.

Catalysis by nucleophilic species, Y^-, will be observed.

(ii) if $k_1 [\text{Y}^-] \gg k_{-2}$ then equation 9 reduces to,

$$k_0 = k_2 [\text{H}^+] \tag{11}$$

Acid catalysis will be observed.

Catalysis by nucleophilic species, Y^-, will NOT be observed.

Thus the behaviour of the system with respect to added catalytic agents is governed by the relative magnitudes of k_{-2} and $k_1[Y^-]$. In physical terms we are saying that if the second stage of the denitrosation reaction is rate determining we should observe both acid catalysis and catalysis by nucleophilic species. If the first stage is rate determining we shall observe only acid catalysis. The following discussion demonstrates that with a little thought it is possible to make certain proposals concerning the relative magnitudes of k_{-2} and $k_1[Y^-]$ for a particular reaction system and thus rationalise the response of the system to each of the two types of catalytic agent described above.

The relative magnitudes of k_{-2} and $k_1[Y^-]$ for a particular reaction system are seen to be governed by a combination of three separate factors which may be described as follows.

1. Nature of the N-nitroso compound.

2. Nature of the solvent system employed.

3. Nature and concentration of the potential nucleophile, Y^-.

Consider the energy profile for the denitrosation reaction.

Extent of Reaction
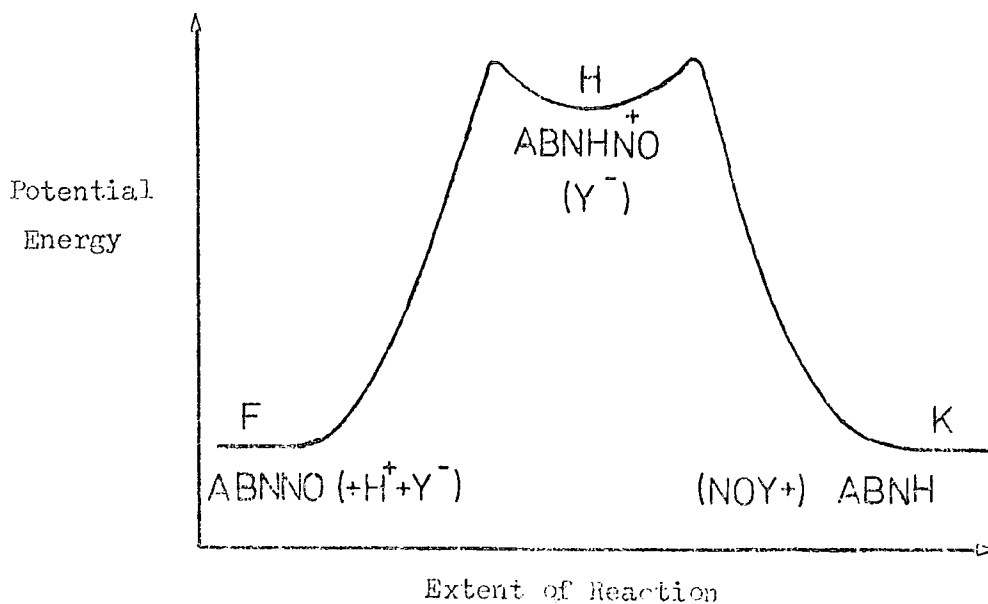
The term "potential energy" is, in this case, intended to represent all energies of the system other than those due to molecular translation. Note that the reactants and products are represented schematically as having the same potential energies: this will not generally be the case. We may of course split the energy profile to yield two separate energy profiles each describing one stage of the denitrosation reaction.

$$
\begin{array}{c}
\partial + \partial + \quad \partial - \\
A\,BN{-}NO \\
\vdots \\
H^{\,\partial +}
\end{array}
$$

G

H $\quad$ E$_2$

$$
\begin{array}{c}
+\partial + \quad \partial - \\
ABNHNO
\end{array}
$$

Potential

Energy

E$_1$

F

$$
\begin{array}{c}
\partial + \quad \partial - \\
A\,BN{-}NO \\
(+H^+)
\end{array}
$$

Extent of Reaction
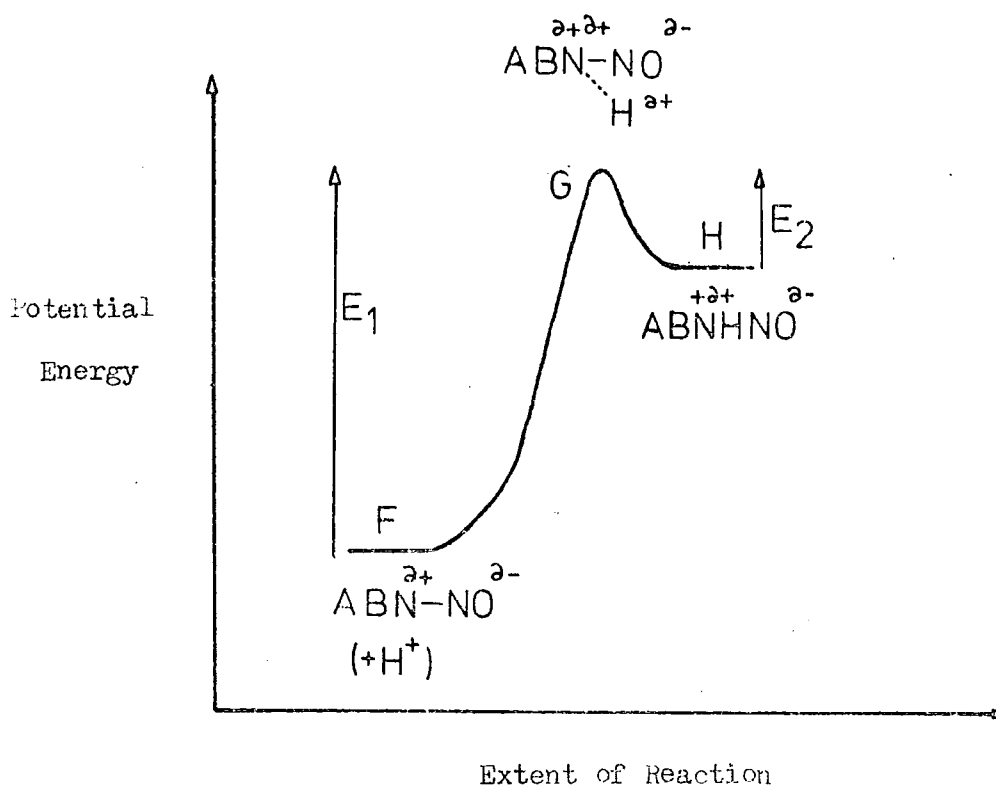
E$_1$ and E$_2$ represent the activation energies for the forward and backward reactions respectively.

Also,

Extent of Reaction
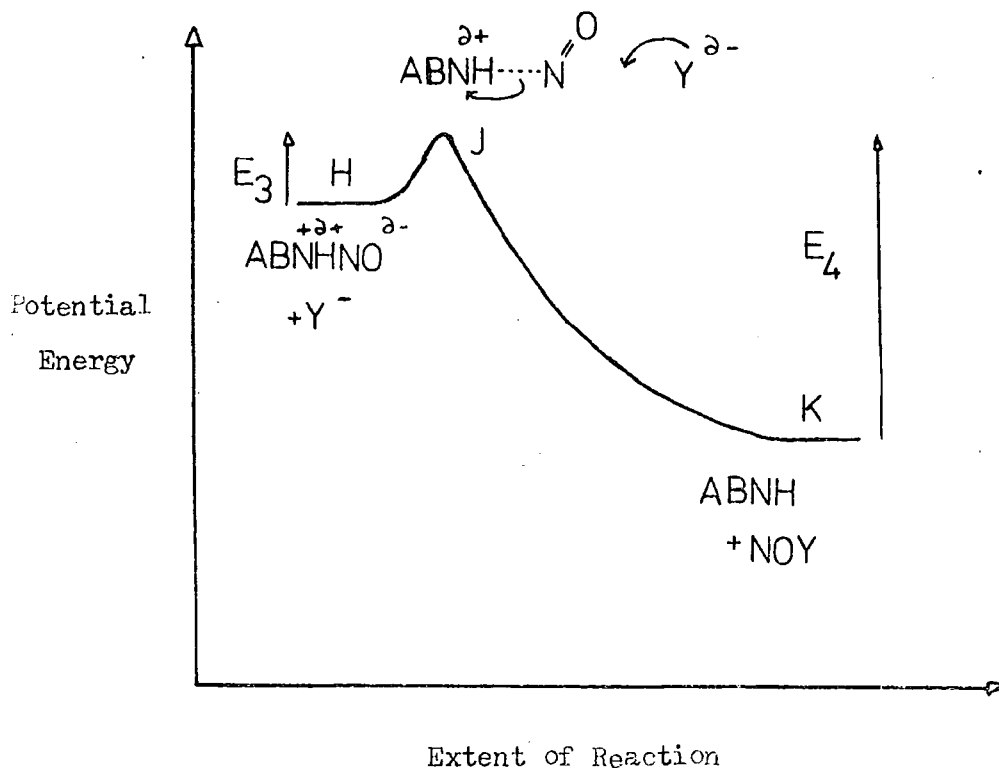
E_3 and E_4 represent the activation energies of the forward and backward reactions respectively. The depiction of the inherent polarisation of the N-nitroso function in the form shown is justified on the basis of the review by Fribush et al.[108].

We may now use these energy profiles to discuss the manner in which each of the three factors described above acts to control the relative magnitudes of k_{-2} and $k_1[Y^-]$.

1.   The Nature of the N-nitroso compound

(a)   The Effect upon k_{-2}

Take an N-nitroso compound ABNNO with substituents A and B chosen such that the net effect is one of electron donation towards the

-N-NO moiety. Referring to the first of the two separate energy profiles; a consideration of the charge distributions upon the various species leads us to note that this electron-donating effect will stabilise the product species, H, to a greater extent than it will the transition state species, G. Use of electron-donating substituents will therefore maximise the value of E_2. As $k_i \propto e^{-E_i/RT}$ the use of electron-donating substituents will lead to a low value for k_{-2}. Conversely the use of electron-withdrawing substituents will result in a high value for k_{-2}.

(b) The Effect upon $k_1[Y^-]$

Consider the energy profile for the second stage of the denitrosation reaction. If substituents A and B are chosen to be electron-donating the starting material, H, will be stabilised by their action. The transition state species, J, will be similarly stabilised but to a lesser extent. Thus the use of electron-donating substituents tends to maximise the value of E_3 and leads to a low value for k_1. For a series of experiments at constant nucleophile concentrations this effect will be reflected in low values of $k_1[Y^-]$. Conversely electron-withdrawing substituents minimise the value of E_3 and favour high values of $k_1[Y^-]$.

(c) The Effect on the $k_{-2}:k_1[Y^-]$ Ratio

As the inductive effects of a particular combination of substituents act upon the values of k_{-2} and $k_1[Y^-]$ in the same direction it is not easy to ascertain the effect of such a substituent combination upon the $k_{-2}:k_1[Y^-]$ ratio. To proceed further we need to make a decision concerning the relative magnitudes of the separate effects upon k_{-2} and $k_1[Y^-]$. Ideally this decision would be made on the basis of a

detailed knowledge of the transition state configuration but, as our information in this area is far from complete we can proceed further, in the present case, only on the basis of an assumption.

Let us assume that the effect of changes in the nature of the N-nitroso compound acts predominantly upon the value of $k_1[Y^-]$. Whilst this assumption might at first sight appear completely arbitrary it is at least consistent with the associated experimental evidence. Thus an examination of the ultraviolet spectra of N-nitroso compounds in neutral and acidic solution respectively has shown that at moderate acidities the extent of protonation is small. With reference to the energy profile for the protonation it would appear that E_2 is of a considerably lower magnitude than E_1. In other words the transition state lies much closer to the product, H, in terms of energies, than to the starting material, F. If we invoke the general principle due to Hammond[109], which states that immediately succeeding species which closely resemble each other in energy terms are also likely to have similar structures, then we note that the structure of the transition state species is likely to be closer to that of the product, H, than to that of the starting material, F. This revised view of the transition state for the first stage of the reaction leads to a consequent reduction in the magnitude of the proposed effect of differing substituent combinations upon the value of k_{-2}. The proposal of an "unsymmetrical" transition state species in which the transfer of H^+ to the N-nitroso compound is almost complete has received support from the work of Williams[107].
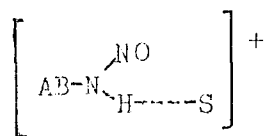
$$\left[AB-N \begin{array}{c} \diagup NO \\ \diagdown H \text{----} S \end{array} \right]^+ \qquad \text{where S represents the solvent.}$$

Working with N-methyl-N-nitrosotoluene-p-sulphonamide in aqueous

media he has reported a value of $k_H : k_D \simeq 1.5$ for the reaction in

H_2SO_4/H_2O and D_2SO_4/D_2O respectively. As the reaction is rationalised

on the basis of a rate-limiting proton transfer the observed value

of $k_H : k_D$ is seen to represent a measure of the primary kinetic isotope

effect. The observation of low values of $k_H : k_D$ for reactions in which

the kinetic isotope effect is known to be of a primary nature have

been rationalised within the confines of the Westheimer treatment[110]

in terms of the existence of an "unsymmetrical" transition state.

Thus it may be seen that our assumption concerning the relative

magnitudes of the effects of substituents upon k_{-2} and $k_1[Y^-]$

respectively is in accordance with the available experimental evidence.

It is wise to point out that such an observation may not be taken as

conclusive proof of the validity of our assumption: similar

considerations may apply for the second stage of the reaction.

Justification for the assumption must be on the basis of its allowing

us to formulate a coherent scheme which rationalises the experimental

observations. The reader may wish to satisfy himself that the opposite

assumption does not lead to a fruitful conclusion.

On the basis of our present assumption it is clear that N-nitroso

compounds having electron-donating substituents will tend to possess

relative values of k_{-2} and k_1 such that $k_{-2} >> k_1[Y^-]$. Conversely

N-nitroso compounds having electron-withdrawing substituents will tend

to possess relative values of k_{-2} and k_1 such that $k_1[Y^-] >> k_{-2}$.

2. The Nature of the Solvent

(a) The Effect upon k_{-2}

Take a particular N-nitroso compound, ABNNO. If we consider the

energy profile for the protonation stage we note that a solvent of moderate ionising ability will tend to stabilise the product species, H, to a greater extent than the transition state, G, since the charges are more localised in the former case. Thus the use of a solvent of moderate ionising ability might be expected to yield reasonably high values of E_2. These moderately high values of E_2 will be reflected in a moderate value of k_{-2}. If we were to change to a solvent of much greater ionising ability the difference in degree of stabilisation of the product, H, and the transition state species, G, would be increased and a higher value of E_2 might be expected. This increase in E_2 would be reflected in a lowered value of k_{-2}.

It is thus clear that a change to a solvent of greater ionising ability will result in a lowering of the magnitude of k_{-2}. Conversely a change to a solvent of lower ionising ability will result in an increase in the value of k_{-2}. This result is conveniently expressed by the qualitative theorem of Hughes and Ingold[111] which states that an increase in the ionising power of the reaction medium will inhibit processes which involve the destruction or diffusion of charges.

(b) The Effect on $k_1[\overset{..}{Y}]$

With reference to the energy profile for the second stage of the reaction we note that a solvent of moderate ionising ability will stabilise the starting material, H, to a greater extent than the transition state species, J, since a diffusion of charge occurs as we approach the transition state. E_3 will therefore appear fairly high and k_1 will take a reasonably low value. Changing to a solvent of greater ionising ability will reinforce this effect. Thus E_3 will be raised and k_1 lowered relative to the above case.

Changing to a solvent of greater ionising ability whilst

maintaining a constant concentration of Y^- therefore results in a lowering of the magnitude of $k_1[Y^-]$. Conversely changing to a solvent of lower ionising ability whilst maintaining Y^- constants results in an increase in the value of $k_1[Y^-]$.

(c) The effect upon the $k_{-2}:k_1[Y^-]$ Ratio

As an increase in solvent ionising power will affect k_{-2} and $k_1[Y^-]$ in the same direction we may make predictions as to the effect of such an increase on the $k_{-2}:k_1[Y^-]$ ratio only on the basis of an assumption concerning the relative magnitudes of the separate effects on k_{-2} and $k_1[Y^-]$ respectively. As the second stage of the reaction demands a much more extensive delocalisation of charges than does the first stage it would not seem unreasonable to expect the effect of solvent changes to operate mainly on k_1. Thus an increase in the ionising ability of the solvent will predispose the system towards the possession of values of k_{-2} and $k_1[Y^-]$ such that $k_2 \gg k_1[Y^-]$. Conversely a decrease in the ionising ability of the solvent will tend to predispose the system towards a condition in which $k_1[Y^-] \gg k_{-2}$.

3. The Nature and Concentration of the Potential Nucleophilic Reagent, Y^-.

At moderate concentrations of Y^- where $Y^- < \sim 2M$ we may expect media effects attributable to the presence of Y^- to be of a relatively small magnitude. Under such conditions the effect of a change in the nature or concentration of the nucleophile may be assumed to act solely upon the term, $k_1[Y^-]$. Thus the use of "poor" nucleophiles (with a correspondingly low value for k_1) at low concentrations in a particular reaction system might be expected to predispose that system towards the possession of values of k_{-2}

and $k_1[Y^-]$ such that $k_{-2} \gg k_1[Y^-]$. Conversely the use of an efficient nucleophile at high concentration might be expected to push the system towards the condition $k_1[Y^-] \gg k_{-2}$.

If we refer back to the earlier paragraphs which discussed the forms of catalysis associated with the conditions $k_{-2} \gg k_1[Y^-]$ and $k_1[Y^-] \gg k_{-2}$ respectively we note that we are in a position to make certain generalisations concerning the role of the three factors described above in determining the form of catalysis observed for a particular denitrosation reaction.

1. The Nature of the N-nitroso Compound

The presence of electron-donating substituents predisposes the system towards the condition $k_{-2} \gg k_1[Y^-]$. Acid catalysis is to be expected. Catalysis by nucleophilic species is also to be expected. The presence of electron-withdrawing substituents predisposes the system towards the condition $k_1[Y^-] \gg k_{-2}$. Acid catalysis only may. be expected.

2. The Nature of the Solvent

The presence of a solvent of relatively high ionising ability predisposes the system towards the condition $k_{-2} \gg k_1[Y^-]$. Acid catalysis may be expected. Catalysis by nucleophiles may also be expected. The presence of a solvent of relatively low ionising ability predisposes the system towards the condition $k_1[Y^-] \gg k_{-2}$. Acid catalysis only may be expected.

3. The Nature and Concentration of the Nucleophilic Species

The use of poor nucleophiles at low concentrations in a particular system predisposes that system towards the condition $k_{-2} \gg k_1[Y^-]$. Both acid catalysis and catalysis by nucleophiles is to be expected.
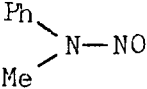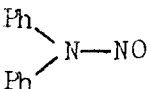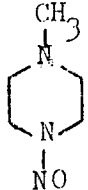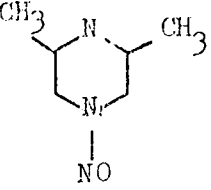
The use of good nucleophiles at high concentrations predisposes the system towards the condition $k_1[Y^-] \gg k_{-2}$. Acid catalysis only may be expected.

The exact behaviour of a particular denitrosation system towards various potentially catalytic reagents is determined by a combination of these three factors so that it is not strictly possible to predict the exact form of catalytic behaviour adopted by each system. Nevertheless the present approach would seem to go a long way towards our objective of providing a rationale for the results of the literature. The experimental observations relevant to a number of studies of the denitrosation reactions of N-nitroso compounds containing electron-withdrawing substituents are given below.

COMPOUND	SOLVENT	ACID CATALYSIS	Y^- CATALYSIS	REF.
Me RSO$_2$ N—NO (N-methyl-N-nitrosotoluene-p-sulphonamide)	H_2O EtOH	YES YES	NO NO	107 30
Bu^n HC N—NO O (N-n-butyl-N-nitrosoacetamide)	H_2O	YES	NO	112
O N — NO (N-nitroso-2-pyrolidine)	H_2O	YES	NO	113
Et NH O = C OEt (N-ethyl-N-nitrosourethane)	H_2O	YES	NO	114,115

It is apparent that in the case of N-nitroso compounds containing strongly electron-withdrawing substituents the observed form of catalytic behaviour shown by the system is determined essentially by the first factor concerning the nature of the N-nitroso compound. Thus $k_1[\bar{Y}] \gg k_{-2}$ and, whilst catalysis by acids is observed, catalysis by nucleophilic reagents is not. Within the bounds of the current experimental evidence the effects of changes in either the nature of the solvent or the nature and concentration of the nucleophile upon the catalytic behaviour of the system are not observed. In practical terms we are dealing here with compounds yielding a very high value of k_1. Under the conditions studied $k_1[Y^-]$ is high and the relative rates of the two stages of the reaction are such as to make the initial protonation rate-determining. It is not surprising, therefore, that primary deuterium isotope effects, $k_H:k_D$, of between 1.5 and 1.9 have been observed for such reactions[30,107,112,113]. As the nucleophilic catalysts act only upon the second stage of the reaction catalysis by nucleophiles is not observed the reaction being zero order in Y^- in the ranges studied. It appears that the relative magnitude of $k_1[Y^-]$ is sufficiently large as to preclude any attempt on our part to return to conditions under which the second stage might again become rate determining. Thus the use of H_2O in place of EtOH and the use of aqueous solutions in the absence of added nucleophiles effect no change in the observed catalytic behaviour.

For the denitrosation of N-nitroso compounds containing electron-donating substituents the effect of each of the three factors may be demonstrated. Consider the experimental evidence given over.

COMPOUND	CONDITIONS	ACID CATALYSIS	Y⁻ CATALYSIS	REF.
Ph Me ⟩N—NO	Dry EtOH solvent	YES	NO	30
	H_2O solvent	YES	YES(low$[Y^-]$)	11
		YES	NO (High$[Y^-]$)	104
N-methyl-N-nitroso aniline				
Ph Ph ⟩N—NO	Dry EtOH solvent	YES	NO	30
	H_2O solvent	YES	YES(low$[Y^-]$)	105
		YES	NO (High$[Y^-]$)	106
N-nitrosodiphenylamine				
4-methyl-1-nitroso-piperazine	50:50 w/v Dioxan/H_2O	YES	YES	116
3,5-dimethyl-1-nitroso-piperazine	50:50 w/v Dioxan/H_2O	YES	YES	116

Note: The results for the piperazine derivatives are derived from the observations of Singer[116] concerning the rate of trans-nitrosation of morpholine. The trans-nitrosation reaction is assumed to follow that of denitrosation and the denitrosation reaction is assumed to be rate-determining since the overall reaction is noted to be zero-order in morpholine concentration at all but the very lowest concentrations. The observed behaviour of the system towards potential catalytic agents will therefore reflect the catalytic behaviour of the denitrosation reaction.

On the basis of our structural considerations we might expect the denitrosation reaction of an N-nitroso compound containing electron-donating substituents to obey the condition $k_{-2} \gg k_1[Y^-]$. Thus catalysis by both acids and nucleophilic species is to be expected. In aqueous solution this is generally found to be the case. However, in contrast to the situation that exists for N-nitroso compounds with electron-withdrawing substituents, the balance of k_{-2} vs $k_1[Y^-]$ appears to be a fine one. Thus the use of a solvent such as ethanol, which has a lower ionising ability than water, alters conditions such that $k_1[Y^-] \gg k_{-2}$. In this instance catalysis by nucleophilic species is not observed. Similarly the use of aqueous solutions containing a high concentration of an efficient nucleophile such as thiourea or thiocyanate also serves to ensure that $k_1[Y^-] \gg k_{-2}$. The rate of reaction is not improved by any further increase in $[Y^-]$.

In physical terms we are dealing here with compounds for which the rates of the first and second stages of the reaction are of similar magnitudes. The catalytic behaviour of the system is therefore greatly influenced by changes in either the nature of the solvent or the nature and concentration of the nucleophile, Y^-. In aqueous solution, at moderate concentrations of Y^-, the observed solvent isotope effect, $k_{H_2O} : k_{D_2O}$, of around $0.3 \sim 0.5$ is indicative of a fast reversible protonation of the N-nitroso compound[18]. Stage two is rate determining and nucleophilic catalysis is observed. Whilst this situation obtains at moderate values of $[Y^-]$ subsequent increases in $[Y^-]$ may enhance the rate of the second stage to such an extent that it is no longer rate-determining, the reaction becoming zero-order in Y^-. The "leveling off" of a plot of k_0 vs $[Y^-]$ at high Y^- has been

demonstrated for a number of nucleophiles[104,106]. In ethanolic solution the greatly increased solvent isotope effect, $k_{EtOH}:k_{EtOD}$, of around 2.6∽3.8 points towards a rate-determining proton transfer[30]. Since the nucleophiles act only upon stage two of the reaction the rate of reaction is found to be zero-order in Y^-. The gradual change from conditions involving a rate-determining 2nd Stage to those involving a rate-determining 1st Stage has been demonstrated by Johal et al.[30] using aqueous ethanolic solutions. As the percentage by volume of water is increased the catalytic effect of added thiocyanate becomes increasingly important.

2.2 The N-nitrosation Reaction

In the preceeding section an approach based on the scheme of Williams[11] was used to provide a rationale for the forms of catalytic behaviour observed for a range of N-nitroso denitrosation reactions. It appears that this same approach allows a rationalisation of the forms of catalytic behaviour observed for the range of N-nitrosation reactions.

Consider the N-nitrosation of a secondary aromatic amino compound using $NaNO_2$ in the presence of added nucleophiles at low or moderate acidities. Refering to Section 1.8 we note that under these conditions the nitrous anhydride mechanism may be neglected. At these acidities we may assume that the reaction proceeds via the unprotonated form of the amino compound so that we may represent the reaction by the scheme shown over.

$$RNO_2 + Y^- + H^+$$

$$K \Updownarrow$$

$$NOY + ROH + ABNH \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} ABNHNO^+ + Y^-$$

$$K' \Updownarrow H^+ \qquad k_2 \Updownarrow k_{-2}$$

$$ABNH_2^+ \qquad ABNNO + H^+$$
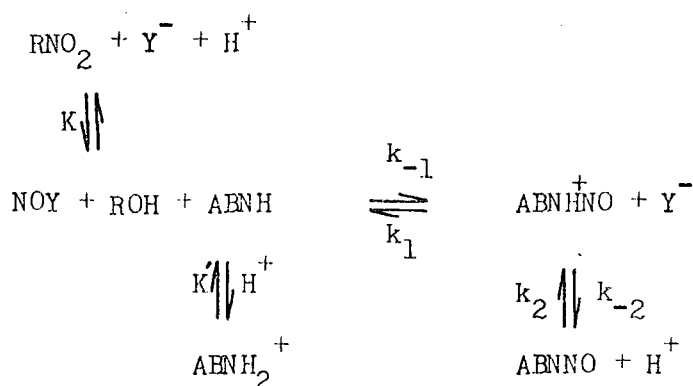
ROH   represents the solvent

ABNH   represents the generalised amino compound

Y^- represents the nucleophile

$$K = \frac{[NOY][ROH]}{[RNO_2][Y^-][H^+]}$$

As the removal of a proton from the protonated N-nitroso compound
to yield the free nitroso species must occur via the agency of a
solvent molecule the rate constant k_{-2} is understood to include a
term corresponding to the solvent concentration, $[S]$. It is further
understood that the hydrogen ion H^+ exists in solution in the form
of the appropriate solvated ion SH^+. Note that the rate constants
for the individual steps of the reaction are defined in a manner
identical to that employed for the preceeding discussion of the
denitrosation reaction. At these acidities the back-reaction k_2 will
be minimal in its extent so that the rate expression for the overall
reaction may be written as follows.

$$\text{Rate} \quad . \quad \frac{d[\text{Products}]}{dt} = k_{-2}[ABNHNO^+]$$

Application of the steady state principle to the species $ABNHNO^+$ yields,

$$[\text{ABHNO}^+_\cdot] = \frac{k_{-1}[\text{ABNH}][\text{NOY}]}{k_1[\text{Y}^-] + k_{-2}}$$

whence,

$$\text{Rate} = \frac{k_{-2}k_{-1}[\text{ABNH}][\text{NOY}]}{k_1[\text{Y}^-] + k_{-2}}$$

Substituting for $[\text{NOY}]$ in terms of the equilibrium constant, K, yields

$$\text{Rate} = \frac{k_{-1}k_{-2}[\text{ABNH}] \; K \; [\text{RNO}_2][\text{Y}^-][\text{H}^+]}{(k_{-2} + k_1[\text{Y}^-])[\text{ROH}]} \tag{12}$$

The rate expression may be simplified further by the separate consideration of two distinct cases.

(a) For compounds ABNH which is moderately acidic solution exist almost entirely in the form of the protonated species we may write,

$$[\text{ABNH}_2^+] \simeq [\text{ABNH}]_T$$

where $[\text{ABNH}]_T$ represents the total concentration of the amino compound.

Thus,

$$K' = \frac{[\text{ABNH}][\text{H}^+]}{[\text{ABNH}_2^+]}$$

$$K' \simeq \frac{[\text{ABNH}][\text{H}^+]}{[\text{ABNH}]_T}$$

Substitution for $[\text{ABNH}]$ in equation 12 now yields

$$\text{Rate} = \frac{k_{-1}k_{-2} \; K \; [\text{RNO}_2] \; [\text{Y}^-] \; K' \; [\text{ABNH}]_T}{(k_1[\text{Y}^-] + k_{-2}) \; [\text{ROH}]}$$

Defining k_o, the observed second order rate constant for the reaction, such that,

$$\text{Rate} = k_o \left[RNO_2\right] \left[ABNH\right]_T$$

we have

$$k_o = \frac{k_{-1}k_{-2} \ K \ K' \ \left[Y^-\right]}{(k_1\left[Y^-\right] + k_{-2})\left[ROH\right]}$$

(i) if $k_{-2} \gg k_1\left[Y^-\right]$ the above expression reduces to

$$k_o = \frac{k_{-1} \ K \ K' \ \left[Y^-\right]}{k_{-2} \ \left[ROH\right]} \tag{13}$$

Acid catalysis will not be observed.

Catalysis by the nucleophile, Y^-, will be observed.

(ii) if $k_1\left[Y^-\right] \gg k_{-2}$ then

$$k_o = \frac{k_{-1}k_{-2} \ K \ K'}{k_1 \ \left[ROH\right]} \tag{14}$$

Acid catalysis will not be observed.

Catalysis by the nucleophile, Y^-, will not be observed.

(b) for compounds ABNH which exist in moderately acidic solution almost entirely in the form of the non-protonated species we have,

$$\left[ABNH\right] \simeq \left[ABNH\right]_T$$

Substitution for $\left[ABNH\right]$ in equation 12 in this instance yields,

$$\text{Rate} = \frac{k_{-1}k_{-2} \ K \ \left[RNO_2\right]\left[Y^-\right]\left[H^+\right]\left[ABNH\right]_T}{(k_1\left[Y^-\right] + k_{-2}) \ \left[ROH\right]}$$

Thus,

$$k_o = \frac{k_{-1}k_{-2} K [Y^-][H^+]}{(k_1[Y^-] + k_{-2})[ROH]}$$

(i) if $k_{-2} \gg k_1[Y^-]$ then this expression reduces to

$$k_o = \frac{k_{-1} K [Y^-][H^+]}{[ROH]} \tag{15}$$

Acid catalysis will be observed.

Catalysis by nucleophilic species will also be observed.

(ii) if $k_1[Y^-] \gg k_{-2}$ then,

$$k_o = \frac{k_{-1}k_{-2} K [H^+]}{k_1 [ROH]} \tag{16}$$

Acid catalysis will be observed

Nucleophilic catalysis will not be observed.

Note that here we have a parallel with the denitrosation reaction in that the exact form of catalytic behaviour shown by a particular system is dependent on the relative magnitudes of k_{-2} and $k_1[Y^-]$. The three factors which control the catalytic behaviour of the denitrosation systems are thus expected to be operative here, namely,

(a) The nature of the secondary amino compound.

(b) The nature of the solvent.

(b) The nature and concentration of the nucleophile, Y^-.

The rationale developed to explain the catalytic behaviour of the denitrosation reaction may therefore be applied to the N-nitrosation reaction of secondary aromatic amino compounds. As the kinetics of the diazotisation of primary aromatic amino compounds is characterised[62] by a rate-determining N-nitrosation the rationale may also be applied to the diazotisation.
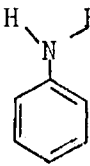
Consider the data given below which describes the N-nitrosation, at moderate acidities, of a series of amino compounds carrying electron-withdrawing substituents.

COMPOUND	SOLVENT	ACID CATALYSIS	Y^- CATALYSIS	REF.
$O=C\overset{NH_2}{\underset{NHCH_2CO_2H}{}}$ Hydantoic Acid	H_2O	?	NO	117
$\overset{H}{\underset{Et}{}}N-CO_2Et$ Ethyl-N-ethyl carbamate	H_2O	?	NO	117
$O=C\overset{Me\!\!\smallsetminus NH}{\underset{NH_2}{}}$ N-methylurea	H_2O	?	NO	118

Under the reaction conditions employed we might expect amino compounds carrying electron-withdrawing substituents to exist mainly in the form of the unprotonated species. Furthermore our experience concerning the analogous N-nitroso compounds has shown us that, on the basis of structural considerations, we may expect the condition $k_1[Y^-] \gg k_{-2}$ to hold. Referring to equation 16 we note that acid catalysis is to be expected whilst catalysis by nucleophilic species is not. We can not comment upon the prediction concerning acid catalysis but the rationale does adequately describe the behaviour of the system with respect to nucleophilic catalysis. The effects of changes in the nature of the solvent and in the nature of concentration of the nucleophile have not been investigated.

In practical terms we have a reaction in which the stage involving loss of a proton from the protonated form of the N-nitroso compound is rate-determining.

The data for the analogous reaction of amino compounds with electron-donating substituents are a little more abundant. Consider the examples given below.

COMPOUND	CONDITIONS	ACID CATALYSIS	Y^- CATALYSIS	REF.
Me–N–H / Me (Dimethylamine)	H_2O solvent, low$[Y^-]$	?	YES	117
$CH_3NHCH_2CO_2H$ (Sarcosine)	H_2O solvent, low$[Y^-]$	?	YES	117
Aniline	H_2O solvent, low$[Y^-]$	NO	YES	119
	H_2O solvent, high$[Y^-]$	NO	NO	119
	MeOH solvent, low$[Y^-]$	NO	YES	120
	MeOH solvent, high$[Y^-]$	NO	NO	120
p-methoxyaniline	H_2O solvent, low$[Y^-]$	NO	YES	119
p-methylaniline	H_2O solvent, low$[Y^-]$	NO	YES	119
p-nitroaniline	H_2O solvent, low$[Y^-]$	NO	YES	119
	H_2O solvent, high$[Y^-]$	NO	NO	119
m-nitroaniline	H_2O solvent, low$[Y^-]$	NO	YES	119
	H_2O solvent, high$[Y^-]$	NO	NO	119
p-chloroaniline	H_2O solvent, low$[Y^-]$	NO	YES	119
	H_2O solvent, high$[Y^-]$	NO	NO	119

Under the conditions employed we might expect amino compounds with electron-donating substituents to exist mainly in the form of the

protonated species. As the possession of electron-donating

substituents will predispose the system towards the condition

$k_{-2} >> k_1[Y^-]$ we might expect the reaction at low nucleophile

concentrations in solvents of high ionising ability to be governed by

equation 13. Acid catalysis is not to be expected. For the reaction

in water at low nucleophile concentrations such behaviour is indeed

observed. A change of solvent to one of lower ionising ability or an

increase in concentration of the nucleophile might be expected to

induce the condition $k_1[Y^-] >> k_{-2}$. The reaction is now governed by

equation 14 and neither acid catalysis nor nucleophilic catalysis is to

be expected. Whilst it is not possible on the basis of present

evidence to demonstrate the effect of a change in solvent the effect of

increasing nucleophile concentration is clearly shown. Presumeably in

the cases studied the $k_{-2} >> k_1[Y^-]$ inequality lies too far over to the

left to be reversed by solvent effects.

In practical terms the reaction of amino compounds with electron-

donating substituents is seen to proceed in aqueous solution at low

acidities via a rate-determining stage involving attack by NOY upon the

free amine. Addition of nucleophilic species serves to increase the

rate of this stage and therefore that of the overall reaction and

nucleophilic catalysis is observed. As $[Y^-]$ is increased this early

stage eventually becomes so fast as to make the loss of a proton from

the protonated form of the nitrosamine rate-determining. Thus plots of

k_0 vs $[Y^-]$ whilst linear in their lower reaches curve away towards a

limiting value of k_0 as $[Y^-]$ becomes high.

It should be noted that our rationale applies only in conditions

such that attack by the nitrosating species NOY (or H_2NO_2 where $Y^- = H_2O$)

is upon the free form of the amino compound. Thus the observation of

Kalatzis et al.[121] concerning the presence of acid catalysis in the reaction of N-methyaniline is explained in terms of the incursion of a mechanism involving an attack on the protonated form of the amine.

Thus we have been able to rationalise the forms of catalytic behaviour exhibited by a number of N-nitrosation and denitrosation reactions in terms of the effect of 3 factors upon the ratio k_{-2} vs $k_1[Y^-]$. The three factors are as follows,

1. The nature of the amino/nitroso compound.

2. The nature of the solvent.

3. The nature and concentration of the nucleophile.

Viewed in this light the experimental observations concerning a wide variety of denitrosation and N-nitrosation reactions are seen to be consistent with the reaction scheme devised by Williams[11] for the reaction of the N-methyl-N-nitrosoaniline/N-methylaniline system in aqueous solution. In the main the postulation of unique reaction mechanisms may be avoided.

SECTION THREE

THE PRESENT WORK

THE EFFECT OF THE NATURE OF THE
AMINO/NITROSO COMPOUND

THE REACTIONS OF N-METHYL-N-NITROSOUREA AND
N-METHYLUREA IN ACIDIC AQUEOUS SOLUTIONS

3.1 An Introduction

Whilst the preceeding section has demonstrated that the kinetic behaviour of a number of N-nitrosation/denitrosation systems may be adequately explained in terms of a common reaction scheme and the effects of three modifying factors it is perhaps valid to criticise a general acceptance of such an approach on the basis of a lack of relevant experimental data. With this possible criticism in mind it was decided to extend the spectrum of evidence available in support of the present approach. As the preceeding section was able to demonstrate the joint applicability of the present approach to both the denitrosation and the N-nitrosation reaction the subject system was chosen in such a manner as to facilitate the study of both of these reactions. Thus the N-methyl-N-nitrosourea/N-methylurea system was deemed suitable for study.

3.2 The Denitrosation of N-methyl-N-nitrosourea

3.2.1 The Literature

The results of a study related to the rate of "decomposition" of N-methyl-N-nitrosourea (MNU) are given in a paper by McCalla et al.[122] Using aqueous solutions which contained HCl at various concentrations they have been able to show that exposure of the nitroso compound to high concentrations of hydrogen ion results in its "rapid destruction". Under the conditions employed the rate of disappearance of MNU is found to follow first-order kinetics such that,

$$\text{Rate} = k_o[\text{MNU}]$$

The observation of methylamine·HCl amongst the degradation products has been explained in terms of a two-stage reaction scheme in which a

fast preliminary denitrosation of MNU is followed by a slow hydrolysis
of the methylurea product to yield ammonia, methylamine and carbon
dioxide. The effect of varying degrees of illumination is also discussed.
Upon a thorough examination of the paper it becomes clear that the
authors maintain a staunch biochemical viewpoint and indeed the aim has
been that of providing a qualitative explanation for the differing
degrees of biological activity demonstrated by a series of N-nitrosoamides.
In such circumstances the authors omission of a comprehensive kinetic
study is perhaps understandable. In particular it should be noted that,
as the mineral acid employed in the study was aqueous HCl, it is not
clear from the information given as to whether the rapid destruction of
MNU observed in the presence of high concentrations of HCl is indicative
of acid catalysis or nucleophilic catalysis or indeed both. It seems
to me that a rough extrapolation of McCalla's data might proceed as
follows.

Let us assume that reaction is limited to the denitrosation and
hydrolysis reactions discussed above. As the rate of the hydrolysis
reaction is deemed to be very slow compared with that of the denitrosation
reaction we must assume that the observed rate of disappearance of the
nitroso compound represents the rate of the denitrosation reaction. The
rate of reaction at zero HCl concentration may be expected to be very
low and if this rate is assumed to be negligible compared with those
obtained by McCalla at higher acidities we may make an addition to the
data given.

$[HCl]/M$	k_o/s^{-1}
0	0
0.0025	8.833×10^{-6}
0.01	2.833×10^{-5}

Now k_o may be proportional to either $[H^+]$ or $[Cl^-]$ but not both as a plot of k_o vs $[HCl]$ is linear with a correlation coefficient of 0.998. Our experience in Section 2.1 would lead us to suppose that we are seeing here a case in which acid catalysis acts in isolation. Whilst the procedure is, at worst, a gross approximation it does demonstrate McCalla's results to be at least indicative of the occurence of a denitrosation reaction in which only acid catalysis is operative. The system was subjected to a more rigorous kinetic analysis at the hands of the present author.

3.2.2 The Extent of the Deamination Reaction

Let us consider the scheme of the present reaction to represent a specific example of the general denitrosation scheme discussed in Section 2.1. Berry et al.[112] and Challis et al.[113] have noted that both N-n-butyl-N-nitrosoacetamide and N-nitroso-2-pyrrolidone yield substantial amounts of the deamination products and we can not at this stage discount the possibility that a parallel deamination may accompany the present reaction. An acknowledgement of this possibility leads to the following reaction scheme.

$$
\begin{array}{ccc}
\underset{O=C}{\overset{Me}{\diagdown}}\!\!\!\underset{\diagdown NH_2}{\overset{N-NO}{\diagup}} + \; H^+ & \rightleftharpoons & \text{Deamination products}
\end{array}
$$

$$k_2 \Updownarrow k_{-2}$$

$$
\underset{O=C}{\overset{Me-\overset{+}{N}\,\overset{H}{-}NO}{\diagdown}}\!\!\!\underset{\diagdown NH_2}{} \quad \underset{k_{-1}}{\overset{Y^-,k_1}{\rightleftharpoons}} \quad \underset{O=C}{\overset{Me}{\diagdown}}\!\!\!\underset{\diagdown NH_2}{\overset{N-H}{\diagup}} + \; NOY
$$

$$NOY + X \xrightarrow[\text{fast}]{} \text{Products}$$
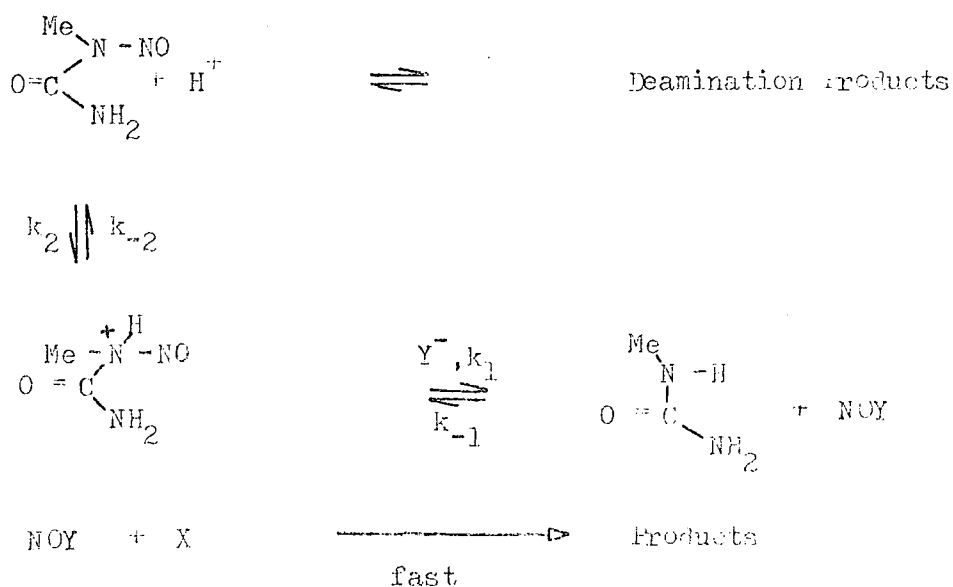
where Y^- represents a nucleophile and X represents a nitrite trap.

The representation of the protonated nitrosoamide is acknowledged as being nominal. The most likely site of protonation in an N-nitroso compound would seem to be the nitroso oxygen atom so that it is conceivable that the N-protonated form arises via an internal rearrangement. Such an argument does not materially affect the kinetic analysis and in the absence of evidence to the contrary the present representation is retained as an outline mechanism. The proposal of such a scheme is supported by the observation of a peak at 200nm in the U.V. spectrum of the reaction mixture at equilibrium. A comparison with the U.V. spectrum of an authentic sample has shown this peak to be due to N-methylurea. With the addition of a further stage involving decomposition of the methylurea the scheme becomes compatible with the conclusions of McCalla et al.[122].

If we are to consider the kinetic behaviour of such a system it is advantageous for us to carry out the reaction the presence of a high concentration of an added nitrite trap, X, so that the reverse step governed by k_{-1} may be neglected. The choice of p-chloroaniline as the nitrite trap leads us to a method of assessing the importance of the deamination pathway.

A number of kinetics runs were carried out under the conditions detailed below. Aliquots removed at various times, t, were neutralised in accordance with the details given in Section 8.2.2.

The diazo compound produced by the reaction of the free nitrosating agent, NOY, with the nitrite trap, was then coupled with an excess concentration of 2-napthol-3,6-disulphonic acid. Measurements of the magnitude of the absorption peak at 500nm, due to the resultant diazo dye, were performed for each sample. The results are given over.

$[H_2SO_4]$ = 2.155 M $[KBr]$ = 2.504 x 10^{-2} M

$[NaNO_2]_o$ = 5.078 x 10^{-4} M $[\text{p-chloroaniline}] \simeq$ 2 x 10^{-2} M

Volume of 4M NaOH aq. used = 4.70ml

t/s	abs (500)
0	0.465
780	0.474
1920	0.470
2280	0.467

Mean value of

abs (500) = 0.469 A

$[H_2SO_4]$ = 0.539 M $[KBr]$ = 2.504 x 10^{-2} M

$[MNU]_o$ = 5.526 x 10^{-4} M $[\text{p-chloroaniline}] \simeq$ 2 x 10^{-2} M

Volume of 1M NaOH aq. used = 2.99ml

t/s	abs (500)
0	0.016
570	0.190
1350	0.343
2850	0.503
4710	0.577
7230	0.607
12,990	0.620

abs $(500)(\infty)$

= 0.620 A (graphical)

$[H_2SO_4]$ = 1.078 M $[KBr]$ = 2.504 x 10^{-2} M

$[MNU]_o$ = 5.010 x 10^{-4} M $[\text{p-chloroaniline}] \simeq$ 2 x 10^{-2} M

Volume of 1M NaOH aq. used = 7.14ml

t/s	abs (500)
0	0.027
558	0.220
1366	0.313
1792	0.340
2452	0.343
4142	0.362
4230	0.362

abs $(500)(\infty)$

= 0.370 A (graphical)

$[H_2SO_4] = 1.616$ M

$[MNU]_o = 4.944 \times 10^{-4}$ M

Volume of 2M NaOH aq. used = 6.32ml

$[KBr] = 2.504 \times 10^{-2}$ M

$[p\text{-chloroaniline}] \simeq 2 \times 10^{-2}$ M

t/s	abs (500)
0	0.059
140	0.171
465	0.305
1350	0.384
2067	0.411
2800	0.387
3598	0.397

abs (500) (∞)

= 0.391 A (graphical)

$[H_2SO_4] = 2.155$ M

$[MNU]_o = 4.948 \times 10^{-4}$ M

Volume of 4M NaOH aq. used = 4.70ml

$[KBr] = 2.504 \times 10^{-2}$ M

$[p\text{-chloroaniline}] \simeq 2 \times 10^{-2}$ M

t/s	abs (500)
0	0.102
132	0.269
302	0.362
568	0.440
1122	0.451
1797	0.442
2422	0.446

abs (500)(∞)

= 0.448 A (graphical)

$[H_2SO_4]$ = 2.694 M $\qquad$ $[KBr]$ = 2.504 x 10^{-2} M

$[MNU]_o$ = 5.076 x 10^{-4} M $\qquad$ $[p\text{-chloroaniline}] \simeq$ 2 x 10^{-2} M

Volume of 4M NaOH aq. used = 5.96ml

t/s	abs (500)
0	0.085
124	0.245
245	0.322
377	0.358
605	0.382
915	0.398
1214	0.397

abs (500)(∞)

= 0.398 A (graphical)

The data for the reaction mixture involving $NaNO_2$ are assumed to represent a quantitative conversion of $NaNO_2$ into the diazo dye so that an extinction coefficient, ε (500), of value 19,710, may be calculated for the diazo dye. Plots of abs (500) vs t for each of the five remaining runs allow us to find abs (500)(∞), the infinity value of abs (500), by extrapolation. Thus values for the percentage yield of the diazo-dye from the MNU starting material are obtained. As the diazo dye is formed via the agency of the free nitrosating agent, which is formed on the denitrosation pathway only, this percentage yield represents a measure of the percentage reaction via the denitrosation pathway. The results are given below.

$[H_2SO_4]$/M	% Reaction via Denitrosation Pathway
0.539	100%
1.078	97%
1.616	102%
2.155	98%
2.694	96%
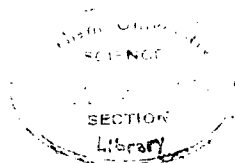
Reaction via the denitrosation pathway is quantitative there being no effective competition from a parallel deamination pathway.

The results may also be used to give approximate values for the observed first-order rate constant, k_o, defined by,

$$\text{Rate} = k_o[\text{MNU}]$$

If we discount data which refer to the final 10% of a reaction we obtain the following figures.

$[H_2SO_4]/M$	$10^3 k_o/s^{-1}$
0.539	0.56
1.078	1.31
1.616	2.90
2.155	4.59
2.694	5.78

Values of k_o obtained in this manner, often from only a few data points, are noted to agree acceptably with the more accurate values of Section 3.2.4. A detailed account of the dependence of k_o upon the appropriate acidity function is given in that same section.

As the deamination pathway has been demonstrated as being of no significance it may be removed from the proposed reaction scheme to yield the scheme depicted over.

SLOW DECOMPOSITION $\longleftarrow\!\!\sim\!\!\sim\!\!\sim$

$NOY + X \xrightarrow[\text{fast}]{} Products$
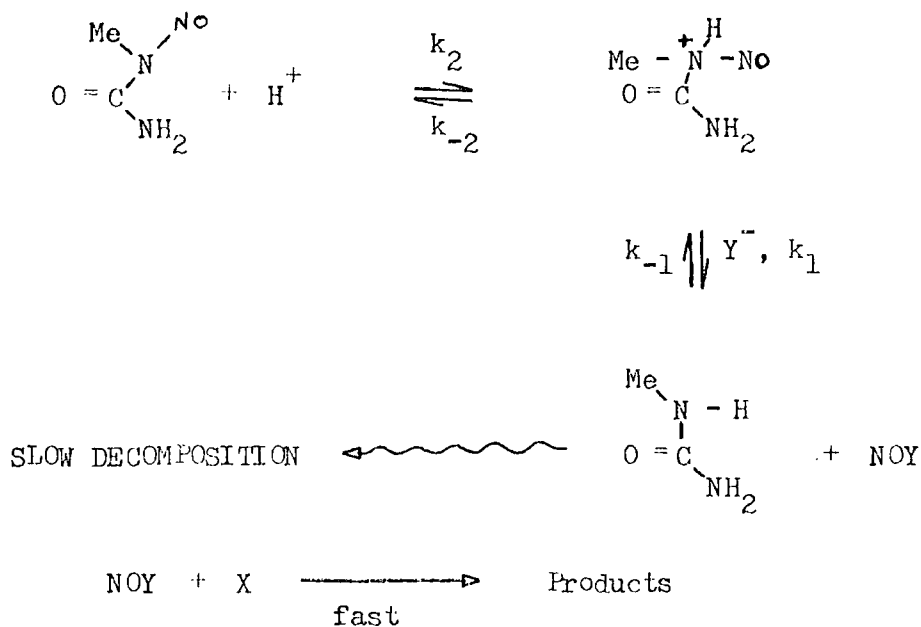
If we assume that the denitrosation reaction is kinetically independent of the slow decomposition we are left with the general scheme of Section 2.1.

For the remainder of the kinetic studies involving MNU hydrazine sulphate was used in place of p-chloroaniline as the nitrite trap. The reasons for this substitution are two-fold. Hydrazine sulphate apart from being a more efficient nitrite trap than p-chloroaniline, has the added advantage of being optically clear at 245nm. Adoption of hydrazine sulphate as the nitrite trap thus allows us to monitor the disappearance of MNU directly by following the decrease in its U.V. absorption peak at 245nm.

3.2.3 The Variation of k_o with [Hydrazine Sulphate]

With reference to the reaction scheme depicted above we note that k_o may be expected to rise with increasing concentration of the nitrite trap as a consequence of the increasing degree of suppression being applied to the back-reaction governed by k_{-1}. In the presence of a

sufficient concentration of the nitrite trap the reverse reaction may
be completely suppressed so that k_o attains a limiting value. Values
of k_o obtained for the reaction of MNU in aqueous acid solution in the
presence of various concentrations of hydrazine sulphate are given
below. In the absence of added nucleophiles the nucleophilic reagent
is believed to be H_2O.

$[H_2SO_4] = 2.148$ M	Nucleophile is H_2O
$[NH_2NH_3^+ \ HSO_4^-]$ /M	$10^3 k_o/s^{-1}$
0	8.622
2.50×10^{-5}	8.339
5.00×10^{-5}	7.673
7.50×10^{-5}	6.875
1.25×10^{-4}	4.662
2.50×10^{-4}	4.690
3.75×10^{-4}	4.439
5.00×10^{-4}	4.503
7.50×10^{-4}	4.597
1.25×10^{-3}	4.680
1.75×10^{-3}	4.729
2.50×10^{-3}	4.560
5.00×10^{-3}	4.591

The data are presented as a plot of k_o vs $[NH_2NH_3^+ \ HSO_4^-]$ in
figure one. The high values of k_o recorded at low concentrations of
hydrazine sulphate are not thought to be significant. At these low
concentrations the reverse reaction governed by k_{-1} is able to make a
considerable contribution to the kinetic behaviour of the system and

deviations from simple first-order behaviour are to be expected. As the values of k_o in question are derived from an application of the first-order guggenheim method[123] their large magnitude is assumed to arise from the application of a simple first-order kinetic method to a reaction which under the conditions stated does not show simple first-order kinetic behaviour. It should be noted that the individual plots of $-\ln(\text{abs } (\lambda)(t) - \text{abs } (\lambda)(t + \Delta))$ vs t used in the calculation of these "rogue" values of k_o appeared linear to the eye and returned values for r, the correlation coefficient, of greater than 0.999. The ability of the first-order guggenheim method to produce linear plots from data which do not depict a first-order kinetic behaviour is noted elsewhere[123]. Values of k_o for $[NH_2NH_3^+HSO_4^-] \geqslant 3.75 \times 10^{-4}$ M were calculated by application of the computer program RKISNA to data which reflected good first-order kinetic behaviour. It is clear that if we discount the early points we are left with a depiction of the expected trend which involves an increase in k_o towards a limiting value as $[NH_2NH_3^+HSO_4^-]$ is increased. In the present case where $[MNU]_o = 2.748 \times 10^{-4}$ M the plot is noted to be flat within the limits of the experimental error for all values of $[NH_2NH_3^+HSO_4^-]$ such that $[NH_2NH_3^+HSO_4^-] \geqslant 2.75 \times 10^{-4}$ M. A hydrazine sulphate concentration of greater than 2.75×10^{-4} M is therefore assumed to suppress the reverse reaction completely. In general the reaction is found to be zero-order in hydrazine sulphate for all values of $[NH_2NH_3^+HSO_4^-]$ such that $[NH_2NH_3^+HSO_4^-] > [MNU]_o$.

Figure 1

k_o vs $[NH_2NH_3^+ HSO_4^-]$
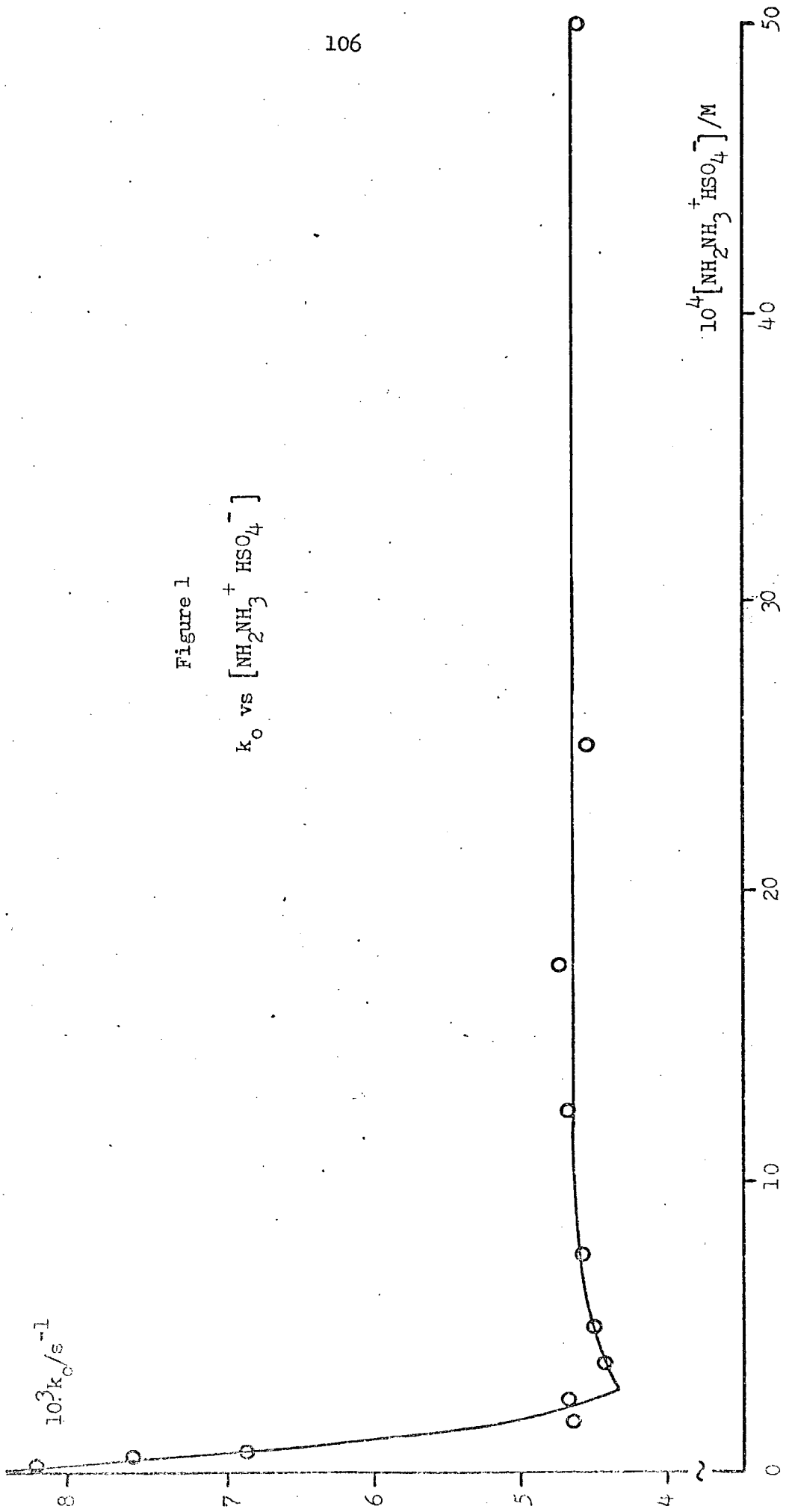
3.2.4 The Variation of k_o with acidity

If we carry out our experiments in the presence of a concentration of hydrazine sulphate larger than the initial concentration of MNU then we ensure the complete suppression of the reverse reaction governed by k_{-1}. Under such circumstances we may apply the generalised kinetic analysis of Section 2.1. From our earlier discussion concerning the effect of a nitroso compound's structure upon its kinetic behaviour we would expect the equation for the rate of denitrosation of MNU to take the form dictated by equation 11.

Thus

$$\text{Rate} = k_2 h_x [\text{MNU}]$$

where h_x represents the appropriate acidity function. It is clear that acid catalysis is to be expected. The results of a series of kinetic runs at various acidities are given below. Under the conditions shown, where $[H^+] \gg [\text{MNU}]$, the reaction is expected to follow first-order kinetics such that,

$$\text{Rate} = k_o [\text{MNU}]$$

Values of k_o were obtained by application of the computer program RKISNA to data which reflected good first-order behaviour.

$[H_2SO_4]/M$	$10^3 k_o/s^{-1}$
0.539	0.56
1.072	1.40
1.613	2.91
2.148	4.64
2.694	7.49

Clearly the reaction is acid catalysed. It should not surprise us to find that the variation of k_o is non-linear with changing $[H^+]$; clearly at these acidities some alternative acidity function must be

sought. Figure 2 depicts the variation of $\log k_0$ against $\log h_A$ and $\log h_0$. The plot of $\log k_0$ vs $\log h_A$ yields a slope of 0.93 together with a correlation coefficient of 0.999 and shows the H_A acidity function to be the more appropriate in the present case. That this result should be different from that obtained for the reaction of N-methyl-N-nitrosoaniline[11] is to be expected. The H_A acidity function is derived from measurements involving amide indicators[124] and is predictably the more appropriate here. The value of 0.93 observed for the slope of the graph is taken as being consistent with the theoretical value of unity expected for reaction via the singly protonated nitrosoamide. A similar correlation vs H_A is observed for the reaction of N-butyl-N-nitroso-acetamide in perchloric acid[112].

Under the present conditions the denitrosation of MNU appears to be governed by the rate law,

$$\text{Rate} = k_2 h_A [\text{MNU}]$$

the general behaviour of the reaction being consistent with the reaction scheme of Section 3.2.2.

3.2.5 The Variation of k_0 with [Nucleophile]

A series of kinetic runs were carried out in the presence of various concentrations of added nucleophiles so that the absence of the nucleophile concentration from the rate law of Section 3.2.4 might be confirmed. The details are given over.

Figure 2

$[H_2SO_4] = 2.148$ M $[N_2H_5^+ HSO_4^-] = 1 \times 10^{-3}$ M
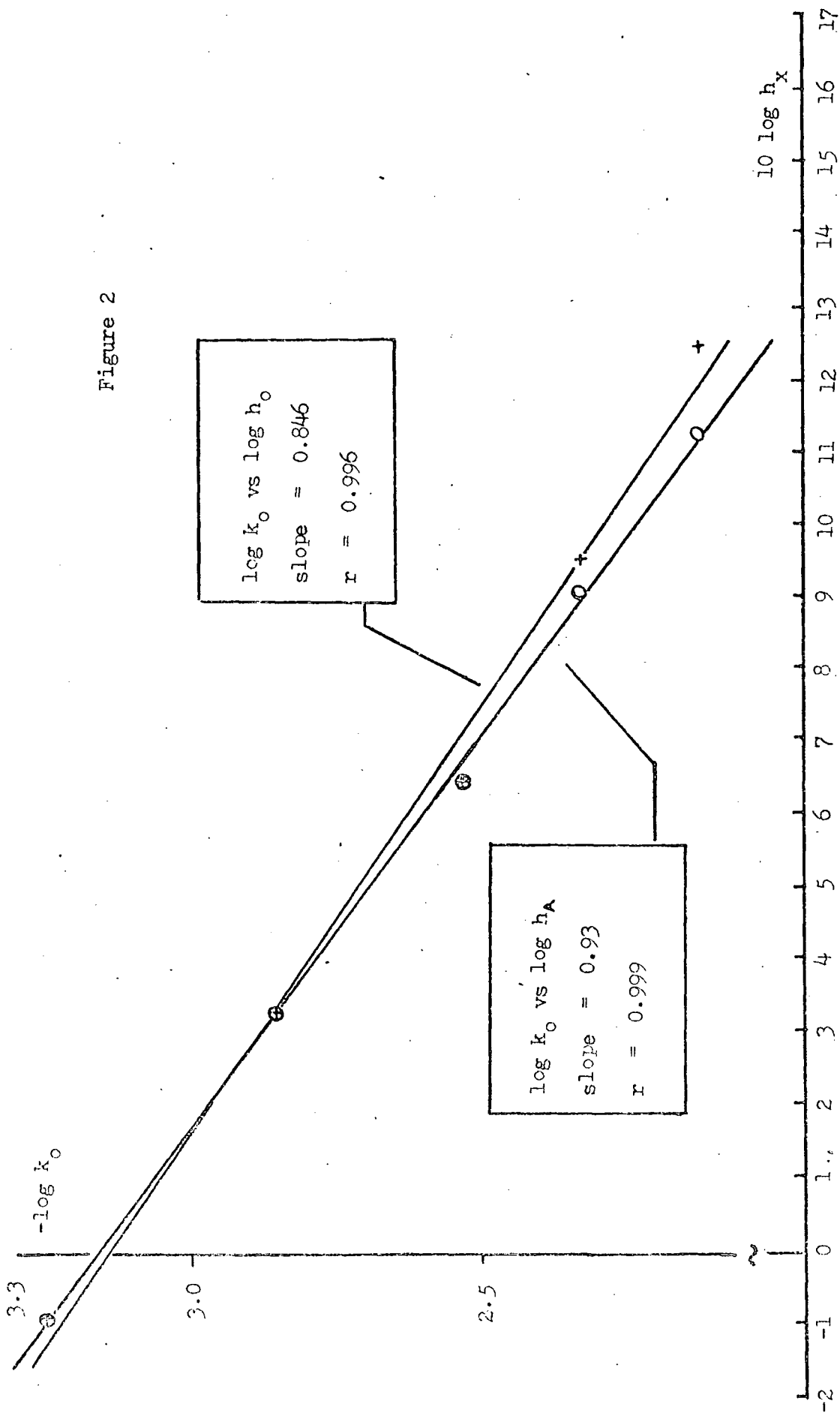
Added Nucleophile	$10^3 \, k_o/s^{-1}$
0	4.64
0.1 M KBr	4.73
1.0 M KBr	4.88
0.02 M KSCN	4.70
0.04 M KSCN	4.90
0.002 M $SC(NH_2)_2$	4.64
0.004 M $SC(NH_2)_2$	4.83

The small changes in k_o depicted by the table are, in many cases, within the experimental error of $\pm$ 5%. If these changes are "real" it seems likely that their cause lies in a general salt effect rather than in a specific nucleophile/nitrosoamide interaction. Certainly we do not observe the large kinetic effects brought about by similar concentrations of these same nucleophiles in the denitrosations of N-methyl-N-nitrosoaniline[60] and N-nitrosodiphenylamine[125]. As a comparison consider the hundred fold increase in k_o for the N-methyl-N-nitrosoaniline reaction which is brought about by the addition of a 0.001 M concentration of thiocyanate ion. It is apparent that nucleophilic catalysis by such ions is not operative for the denitrosation of MNU. This is in agreement with the proposed rate law, the observation being rationalised in terms of the arguments of Section 2.1. The electron-withdrawing character of the N-nitroso substituents causes the initial protonation to become rate determining so that the sole action of added nucleophiles upon the overall rate of reaction is of the form of minor salt effects.

3.2.6 The Variation of k_o with $[MU]$ (added).

The overall reversability of the reaction was investigated briefly by noting the variation of k_o as a function of added MU. The results of this investigation are given below.

$$[H_2SO_4] = 2.148 \text{ M} \qquad [N_2H_5^+ \ HSO_4^-] = 1 \times 10^{-3} \text{ M}$$

$[MU]$ added/M	$10^3 k_o/s^{-1}$
0.002	4.45
0.010	4.35
0.020	4.17
0.101	2.93
0.201	2.26
0.302	1.67

As k_o is appreciably decreased by the addition of MU it is clear that the reverse N-nitrosation stage governed by k_{-1} is of some significance. At high concentrations of MU the rate law of Section 3.2.4 has no application since its derivation requires that the rate of the reverse reaction be negligible. A full rate expression may be derived via the application of steady state treatments to the two intermediates,

$$NH_2CONH(Me)NO \text{ and NOY whence,}$$

$$k_o = \frac{k_{-2}h k_1[Y^-] k_3 [N_2H_5^+]}{(k_{-2} + k_1[Y^-])k_3[N_2H_5^+] + k_{-1}k_{-2}[MU]}$$

The expression has been tested at least in part by plotting k_o^{-1} against $[MU]$. The linear relationship is presented as Figure 3 with a positive slope and intercept.
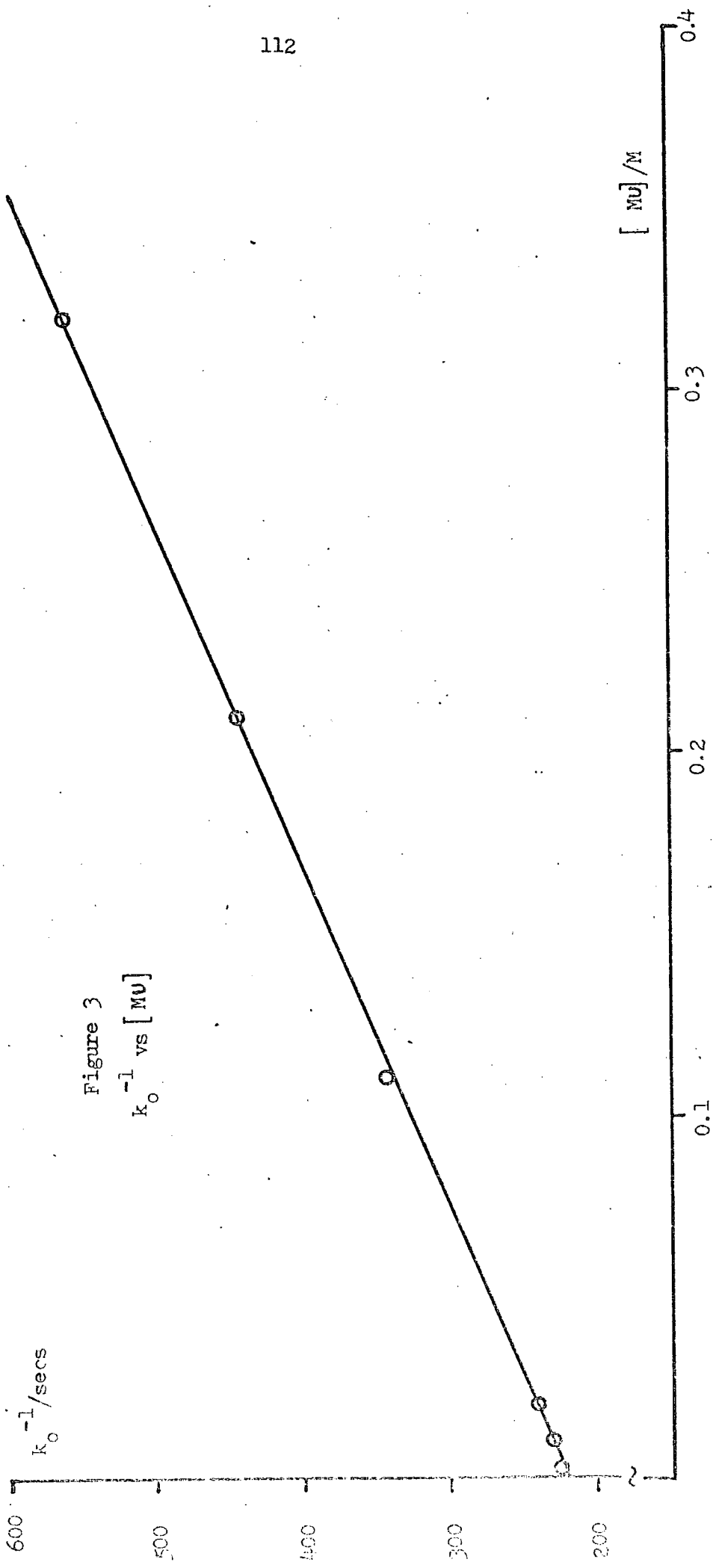
Figure 3

k_o^{-1} vs $[M\upsilon]$

3.3 The N-nitrosation of N-methylurea

3.3.1 The Literature

It is well known that in aqueous solution the nitrosation reactions of amines (diazotisation for primary amines) are generally strongly catalysed by nucleophilic species such as halide ions and thiocyanate ion[62]. This catalytic effect is thought to arise from a rapid equilibrium formation of the corresponding nitrosyl halide, etc., which acts as a more powerful nucleophile than does nitrous acid itself. For some of the more reactive amines (eg. some aniline derivatives) the rate constants approach those values expected for a diffusion-controlled process[119]. Whilst there is one report which concludes that this state of affairs exists also in the reaction of amides[126,127] there are a number which report that nucleophilic catalysis of the amide N-nitrosation reaction is not observed. Thus Berry and Challis report[112] the absence of halide catalysis from the reaction of acetamide; Stedman[128] reached a similar conclusion. The absence of halide catalysis has also been reported for the reactions of hydantoic acid and ethyl-N-ethyl carbamate[117]. In the face of the dilemma additional data would seem to be necessary. Whilst the N-nitrosation of N-methylurea (MU) had been studied to some extent by Yamamoto et al.[118] with the conclusion that nucleophilic catalysis was absent, there are areas of their approach which lend themselves to criticism; in particular their data are based on measurements of the initial rate of reaction. The system was subjected to a more rigorous kinetic analysis at the hands of the present author.

3.3.2 The Extent of the Side-Reactions

We might suppose that the N-nitrosation of MU would proceed along the lines of the reaction scheme shown below.

$$H_2O + NOY + NH_2CONHMe \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} NH_2CO\overset{+}{N}H(NO)Me + Y^-$$

$$\big\Updownarrow K \qquad\qquad\qquad k_{-2} \big\Updownarrow k_2$$

$$HNO_2 + Y^- + H^+ \qquad\qquad NH_2CON(NO)Me + H^+$$
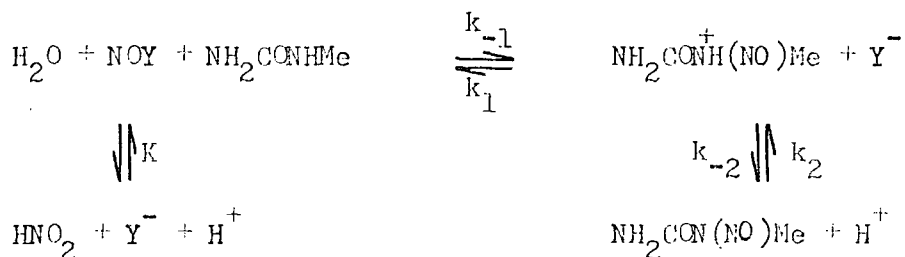
The yield of MNU was established by comparing the U.V. absorption at 265nm, obtained after ten half-lives with that obtained from a standard solution of MNU. Values for the percentage yield of between 96% and 100% indicate that the N-nitrosation reaction is quantitative. Clearly the deamination pathway is not operative here. From the results in Section 3.2.4 it is further clear that the reverse reaction governed by k_2 is of little significance at these low acidities so that the rate equation for the disappearance of MU takes the form shown below. (Section 2.2).

$$\text{Rate} = \frac{k_{-1}k_{-2}K\,[HNO_2]\,[Y^-][H^+][MU]_T}{(k_1\,[Y^-] + k_{-2})\,[H_2O]}$$

However, since our earlier experience of amines with electron-withdrawing groups lead us to expect that $k_1[Y^-] \ggg k_{-2}$, the expression simplifies to the form of eqn. 16.

$$\text{Rate} = \frac{k_{-1}k_{-2}K[H^+]\,[HNO_2]\,[MU]}{k_1\,[H_2O]}$$

Given an excess concentration of both H^+ and MU the reaction is expected to follow first-order kinetics. This behaviour is duly observed. It should be noted that these observations preclude the

operation of the "N_2O_3" nitrosation mechanism.

3.3.3 The Variation of k_o with $[MU]_o$

A series of kinetic runs were carried out at various initial concentrations of MU so that the order of the reaction with respect to $[MU]$ might be determined. Under the conditions employed good first-order behaviour was observed in all cases. The values of k_o, defined such that

$$\text{Rate} = \frac{d[MNU]}{dt} = \frac{-d[MU]}{dt} = k_o[HNO_2]$$

are given below.

$[H_2SO_4] = 0.020$ M	$[NaNO_2] = 6.026 \times 10^{-4}$ M
$10^2\,[MU]_o/M$	$10^2 k_o/s^{-1}$
1.20	1.21
2.40	2.38
3.59	3.58
4.78	4.61

From the plot of k_o vs $[MU]_o$, presented as Figure 4, it is clear that the reaction is first order in $[MU]$ as is predicted by equation 16.

3.3.4 The Variation of k_o with Acidity

A series of N-nitrosations were carried out in the presence of various concentrations of aqueous sulphuric acid. Values of k_o defined as above, were obtained from good first-order data and are tabulated over.

Figure 4

k_o vs $[MU]_o$

$10^2[MU]_o/M$

$10^2k_o/s^{-1}$
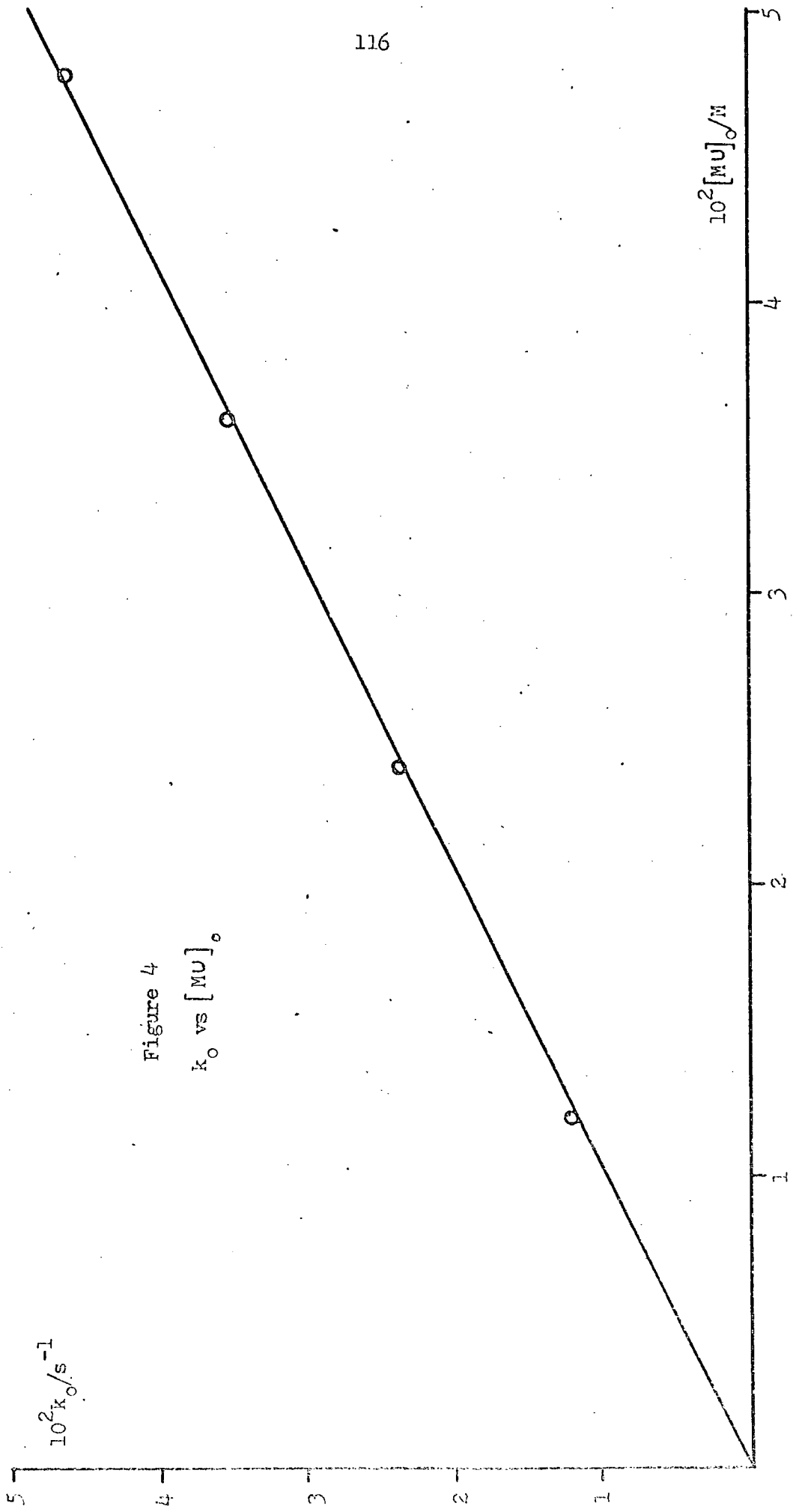
$$[MU]_o = 1.198 \times 10^{-4} \, M \qquad [NaNO_2] = 6.026 \times 10^{-4} \, M$$

$10^3[H_2SO_4]/M$	$10^2 k_o/s^{-1}$
1.00	0.50
2.00	1.23
3.00	1.84
4.00	2.44

The data are presented graphically in Figure 5 as a plot of k_o vs $[H^+]$. At these acidities the reaction appears to be first-order in $[H^+]$ which is to be expected if the reaction involves the attack of the nitrous acidium ion, the nitrosyl halide or the nitrosonium ion upon the unprotonated form of the amide. At these acidities the latter possibility appears remote as nitrosation via the free nitrosonium ion is thought to occur only at acidities in excess of 9M H_2SO_4[33]. Thus the rate law of eqn. 16 is established. It is pertinent to note that the same rate law has recently been established by Mirvish in a study (by initial rate measurements) concerned with the possible involvement of nitrosoureas in human gastric cancer[129].

3.3.5 The Variation of k_o with [Nucleophile]

A series of kinetic runs were carried out in the presence of various concentrations of added nucleophiles so the absence of the nucleophile concentration term from the rate law might be confirmed. The values of k_o are tabulated over.

Figure 5

k_o vs $[H^+]$

$10^2 [H^+]$
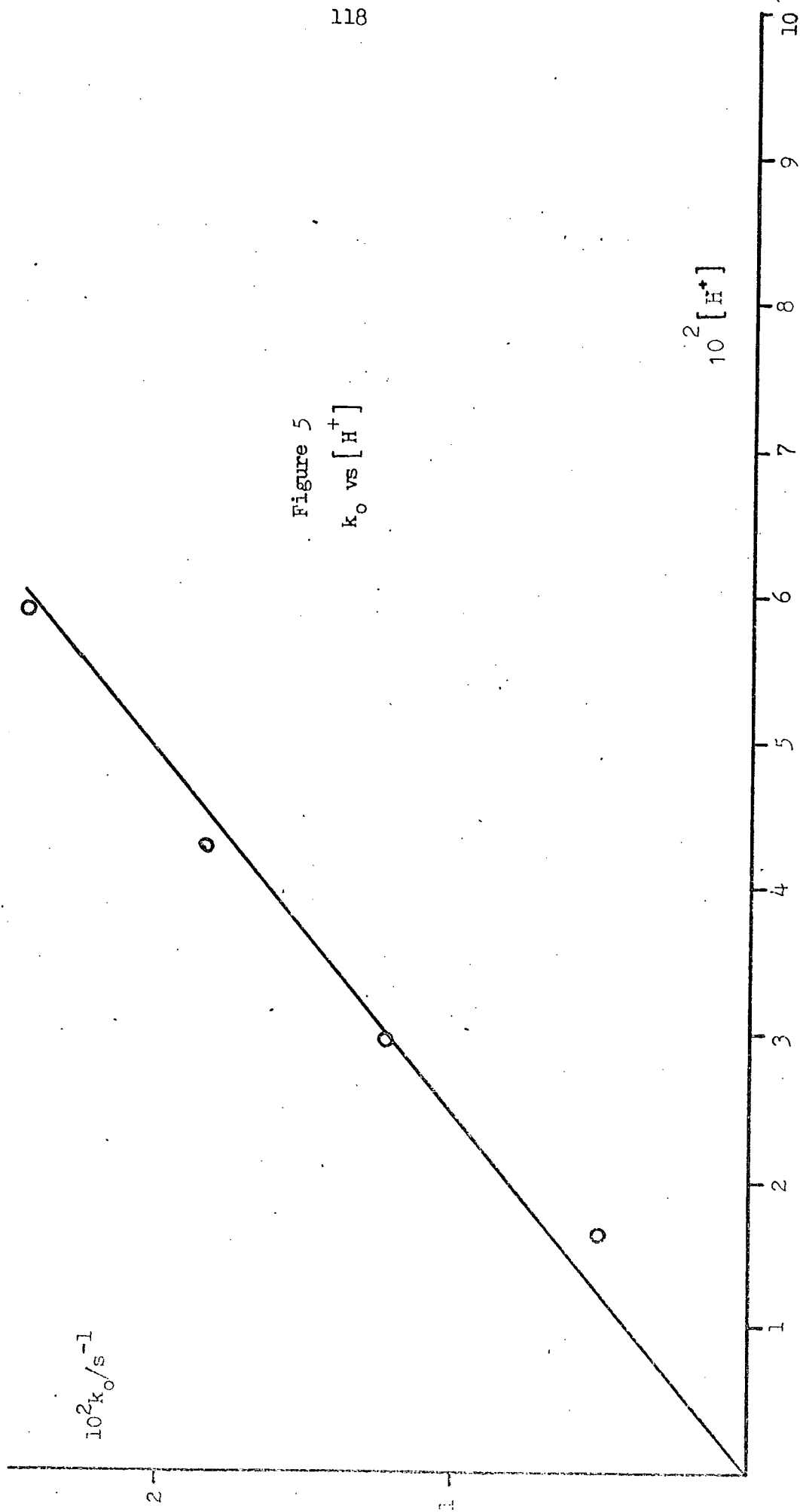
$10^2 k_o/s^{-1}$

$[H_2SO_4]$ = 0.020 M $\qquad$ $[NaNO_2]$ = 6.026 x 10^{-4} M

$[MU]$ = 1.198 x 10^{-2} M

Added Salt		$10^2 k_o/s^{-1}$
0		1.21
0.1 M	KBr	1.14
0.2 M	KBr	1.28
0.3 M	KBr	1.30
0.1 M	KSCN	1.13
0.2 M	KSCN	1.10
0.3 M	KSCN	1.11
0.4 M	KSCN	0.98

In contrast to the amine reaction the N-nitrosation of MU is virtually unaffected by the addition of quite substantial concentrations of nucleophiles. If we assume that the small increase in k_o observed with the addition of KBr is due to a salt effect then this effect might also be expected to be operative in the case of the thiocyanate. It should be remembered however that the protonation of the thiocyanate ion acts to lower the effective acidity of the solution. This latter affect would seem to be stronger than the salt effect, which it opposes, since a small reduction in k_o is noted with the addition of KSCN. Our observation concerning the absence of nucleophilic catalysis is in line with the results of Yamamoto et al.[118] and Mirvish[129] and should not surprise us greatly. Referring to Section 2.2 we note that the amides electron-withdrawing N-substituents are expected to cause a situation to arise in which the proton transfer to the solvent becomes the rate determining step. The nucleophile plays no part in this stage and may not therefore affect the overall rate of the reaction.

However this is not a full explanation of the observed facts.
Consider the expression for k_o

$$k_o = \frac{k_{-1}k_{-2}K[H^+][MU]}{k_1[H_2O]}$$

Whilst it is true that the nucleophile concentration term is missing from
this expression we should not expect the same value of k_o for all
nucleophiles since the values of k_{-1}, k_1 and K will differ from
nucleophile to nucleophile. It should be remembered that if $k_1[Y^-]$ is
large, as we are indeed saying, then the path-way for reaction via the
nitrosyl halide must be reduced in rate considerably. Such circumstances
as these will permit reaction via the nitrous acid species,
(NO^+ or $H_2NO_2^+$) to compete favourably. It seems clear, bearing in mind
the low acidites employed, that the reaction of MU proceeds via the
attack of $H_2NO_2^+$ upon the unprotonated form of the amide.

Thus

$$H_2^+NO_2 + NH_2CONHMe \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} NH_2C\overset{+}{O}NH(NO)Me + H_2O$$

$$\Big\Updownarrow K \qquad\qquad\qquad\qquad \Big\downarrow k_{-2}$$

$$H^+ + HNO_2 \qquad\qquad\qquad NH_2CON(NO)Me + H^+$$
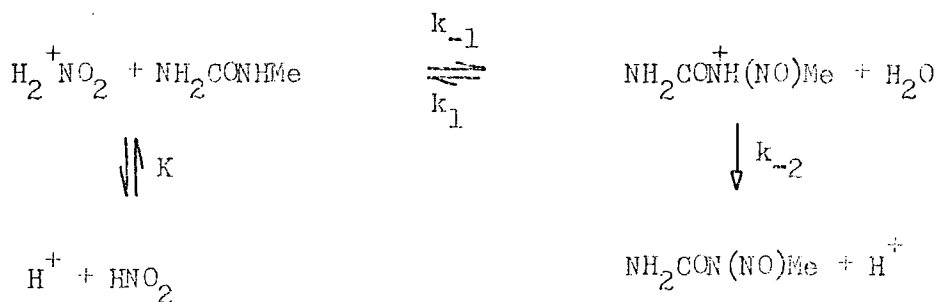
It may well be that the final proton transfer between the amide and
the solvent is not the simple one-stage process depicted above. The
most likely site of protonation in the N-nitroso compound is the nitroso
oxygen atom so that some kind of $O \cdots N$ transition may be involved.
In the absence of detailed evidence the above outline is retained.

3.4 Conclusions

It is clear that nucleophilic catalysis is not observed for either

the N-nitrosation of N-methylurea or for the reverse denitrosation

reaction. Furthermore a consultation of the results of other authors,

(Sections 2.1 and 2.2), leads us to believe that this is the normal state

of affairs for amino/nitroso compounds which possess electron-withdrawing

·substituents. Referring to these earlier sections we note that the effect

of these electron-withdrawing substituents is to make the proton-

transfer stage governed by k_{-2} rate-determining with the production of

the relevent limiting forms of the rate expressions.

This proposal of a rate-determining proton-transfer is not

unsupported. Thus both Challis et al. and Williams report that the

kinetic isotope effects $k_{H_2O}:k_{D_2O}$ observed for these systems lie in

the range 1.5 to 1.9.

SECTION FOUR

THE EFFECT OF THE NATURE OF

THE SOLVENT

THE REACTIONS OF A SERIES OF

p-SUBSTITUTED N-METHYL-N-NITROSOANILINES

IN ACIDIC ETHANOLIC SOLUTIONS

4.1 An Introduction

In the preceeding sections we have considered the effect of changes in the amino/nitroso compound structure upon the kinetic behaviour of a series of N-nitrosation/denitrosation reactions. Mention has also been made of the effect of the nature of the solvent and in this section we will consider this aspect in a little more detail.

In the case of N-nitroso systems possessing electron-withdrawing substituents the over-riding dictate over the kinetic behaviour appears to be the nature of the N-nitroso compound and it has not been possible to demonstrate the effect of solvent changes in this sphere. To find examples of this effect we must confine ourselves to species such as the N-nitrosamines. Consider the denitrosation of N-methyl-N-nitrosoaniline. Williams[11] has noted that the reaction in aqueous H_2SO_4 is characterised by the operation of both H^+ and nucleophilic catalysis. The kinetic isotope effect $(k_{D_2O}:k_{H_2O})$ of between 2.0 and 2.9 confirms that we have a fast initial protonation which is followed by the rate-determining attack of the nucleophile upon the protonated form of the N-nitrosamine. In moving to a solvent of lower ionising power we might expect from our discussion of Section 2.1 that we would predispose the system towards the condition $k_1[Y^-] \gg k_{-2}$. If this effect is large enough we may expect to reach a position in which the initial protonation becomes the rate-determining step. Johal et al.[30] have examined the reaction of N-methyl-N-nitrosoaniline, (NMNA), in ethanolic HCl solutions and report that the addition of a 4.6×10^{-3} M concentration of sodium bromide has no effect upon the rate of reaction. In contrast the addition of this same concentration of sodium bromide brings about a five-fold increase in the rate of the reaction in aqueous solution. Similarly the addition of substantial concentrations of sodium thiocyanate effects

no increase in the rate of the ethanolic reaction. Clearly the nucleophilic species plays no part in determining the rate of the reaction in ethanol. This suggests that either (a) the loss of NO^+ is now an unaided unimolecular process or (b) the rate determining stage is now the protonation. It is difficult to imagine a reason as to why explanation (a) should occur and so we prefer the second possibility, a conclusion which is supported by the observed kinetic isotope effect $(k_{EtOH}:k_{EtOD})$ of 3.8. Clearly the effect of the change to a solvent of low ionising power has been to make the initial protonation the rate-determining stage.

A similar situation exists for the denitrosation of N-nitrosodiphenyl-amine, (NDA)[30]. Thus whilst Thompson[105] has reported that the denitrosation in H_2O is subject to nucleophilic catalysis the reaction in EtOH is not[30]. In this present work the study is extended to include the effect of solvent change upon a series of p-substituted N-methyl-N-nitrosoanilines.

4.2 The Denitrosation Reactions of a Series of p-substituted N-methyl-N-nitrosoanilines

A study has been made, by Biggs et al.[64], of the reactions of a series of p-substituted NMNA's in aqueous acid solution. In each case nucleophilic catalysis was found to be operative. It is thus clear that they were dealing with a series of denitrosation reactions for which the nucleophilic attack is the rate-determining stage; a result which we might have predicted on structural grounds. Values of k_o, the observed first order rate constant for the disappearance of the nitrosamine are given below for the bromide catalysed reaction in the presence of a 2×10^{-3} M concentration of the nitrite trap sulphamic acid.

$$[H_2SO_4] = 2.15 \text{ M} \qquad\qquad [Br^-] = 0.2 \text{ M}$$

substituent	$10^4 k_o / s^{-1}$
H	39
p-Me	23.7
p-OMe	13.7
p-Cl	63.7
p-NO$_2$	50.8

Similar, although not identical, results are obtained for the chloride catalysed reaction. Let us consider the range of reactivities which whilst small does demonstrate that electron-donating p-substituents serve to reduce the reactivity of NMNA. Whilst the effect is easily observed it is less easily rationalised. Consider the reaction scheme shown below.

$$p\text{-X NMNA} + H^+ \quad \overset{K}{\rightleftharpoons} \quad p\text{-X NMNA } H^+$$

$$k_{-1} \big\Updownarrow k_1 , Y^-$$

$$p\text{-X N-methylaniline} + NOY$$
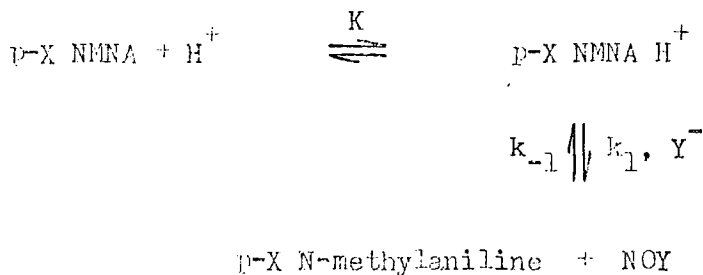
$$NOY + \text{nitrite trap} \longrightarrow \text{Products}$$

Following the example of Section 1.4 and realising that the extent of the rearrangement reaction is negligible under these conditions we may write,

$$k_o = k_1 [Y^-] K h_o$$

Whilst it is clear that the nature of the p-substituent must affect the value of K the nature of this variation is not known for the N-methyl-N-nitrosoanilines and we can only assume that the variation

in pK_a parallels that of the anilines. Values of pK_a for the anilines are given below.

substituent	pK_a
p-Me	5.10
p-OMe	5.34
p-Cl	3.98
p-NO$_2$	1.11

As pK_a is increased by electron-donating substituents the insertion of such substituents into the p-position of the nitrosamine should result in an increase in k_o. Such an increase is not observed and we conclude that the effect of substitution acts also upon k_1. Note that we now have an example of the effect of substituent upon the leaving group in a nucleophilic substitution at a nitrogen centre.

Viz.
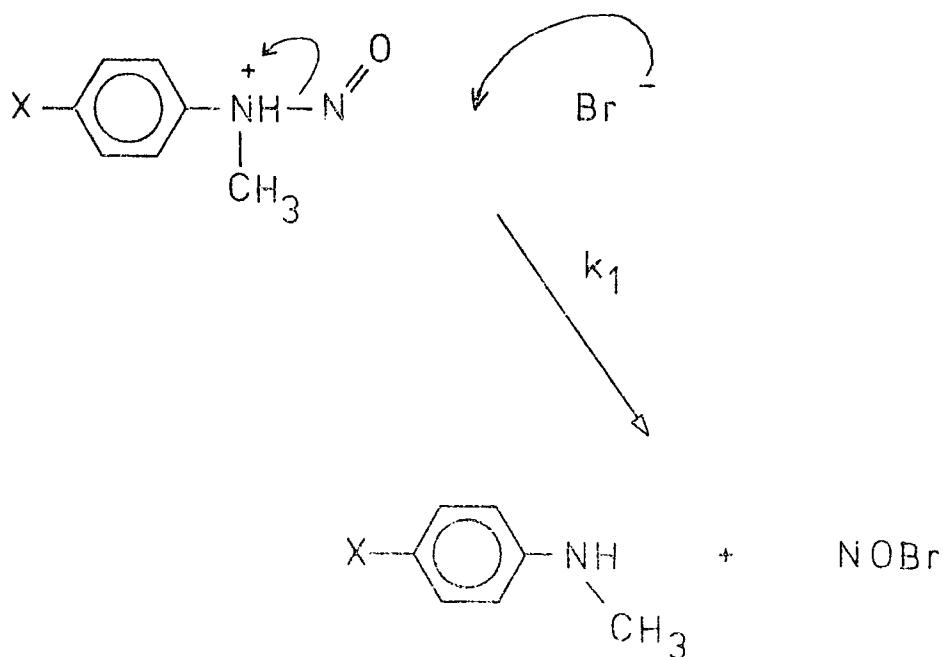
In such circumstances we would expect the insertion of an electron-donating p-substituent to decrease the value of k_1. In the case of the bromide catalysed reaction the effect upon k_1 would appear to outweigh the effect upon K so that electron-donating substituents produce a decrease in k_0. As this decrease represents the difference of the two opposing substituent effects it is understandably of a small magnitude.

Now let us go on to consider the corresponding reaction in an ethanol solvent which proceeds as follows:

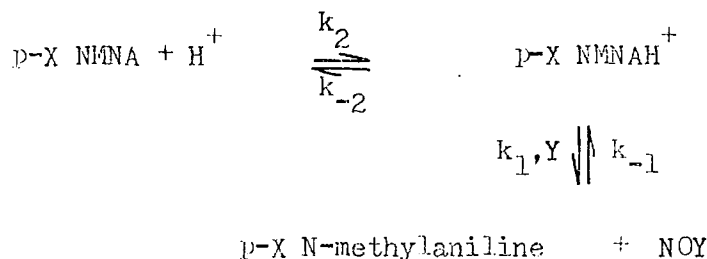$$\text{p-X NMNA} + \text{H}^+ \quad \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \quad \text{p-X NMNAH}^+$$

$$k_1, Y \updownarrow k_{-1}$$

$$\text{p-X N-methylaniline} \quad + \quad \text{NOY}$$

$$\text{NOY} + \text{nitrite trap} \longrightarrow \text{Products}$$

From our earlier discussion we are aware that a change of solvent to ethanol may suffice to make the initial protonation rate-determining so that

$$k_0 = k_2 h_x$$

In such circumstances the effect of the p-substituents upon k_1 will not be transmitted to k_0. If our earlier consideration of substituent effects is correct then the effect upon k_0 should take the form of the unopposed effect upon K or in the present case upon the forward component of K, k_2. With the removal of the opposing effect on k_1 the variation in k_0 might be expected to be of a moderately large magnitude.

Values of k_0 obtained for the reactions of a series of p-substituted NMNA's in ethanolic HCl in the presence of ascorbic acid as a nitrite

trap are given below.

p-H NMNA

$[HCl]/M$	$10^3 k_o/s^{-1}$
0.217	3.839
0.434	9.231
0.651	15.14
0.868	22.24

p-NO$_2$ NMNA

$[HCl]/M$	$10^3 k_o/s^{-1}$
0.216	1.200
0.447	2.950
0.678	4.840
0.909	5.546

p-Cl NMNA

$[HCl]/M$	$10^3 k_o/s^{-1}$
0.213	3.097
0.444	7.444
0.675	11.67
0.906	16.62

p-Me NMNA

$[HCl]/M$	$10^3 k_o/s^{-1}$
0.213	2.656
0.408	6.994
0.606	12.65
0.820	17.26

p-OMe NMNA

$[HCl]/M$	$10^3 \, k_o/s^{-1}$
0.194	2.808
0.414	7.246
0.614	11.19
0.826	17.33

A consideration of the expression for k_o given above demonstrates that a plot of k_o vs h_x, an appropriate acidity function, should lead to a straight line of slope k_2. We expect this value of k_2 to be increased by the presence of electron-donating substituents. Further we expect this effect to be transmitted, unopposed, to k_o.

Plots of k_o vs h_o using the h_o values of Braude[130] are given below. A comparison of these plots with the accompanying plots of k_o vs $h_o[Cl^-]$ does serve to illustrate the absence of nucleophilic catalysis. Clearly our change of solvent to ethanol has had the expected effect of making the initial, protonation, stage rate-determining. However on investigating the results in a little more depth it becomes clear that our foregoing rationale does not reflect the whole truth. In the first instance comment must be made concerning the apparent departure from linearity depicted by a number of the k_o vs h_o plots; clearly the Braude acidity function does not represent the acidity dependence of the reaction with any great accuracy. As no more an appropriate function has been found we must persevere. With the accepted curvature of the k_o vs h_o plots it is agreed that I lay myself open to criticism in presenting values of k_2 derived from them via a least-squares treatment. Nevertheless this calculation has been made since I believe that the results illustrate a factor which has lain in the shadows for

Figure 6

k_o vs h_o and $h_o[Cl^-]$
for NMNA

Figure 7

k_o vs h_o and $h_o[Cl^-]$

for p-NO_2 NMNA

Figure 8

$10^3 k_o / s^{-1}$

$h_o [Cl^-]$

h_o

k_o vs h_o and $h_o [Cl^-]$

for p-Cl NMNA

h_o or $h_o [Cl^-]$
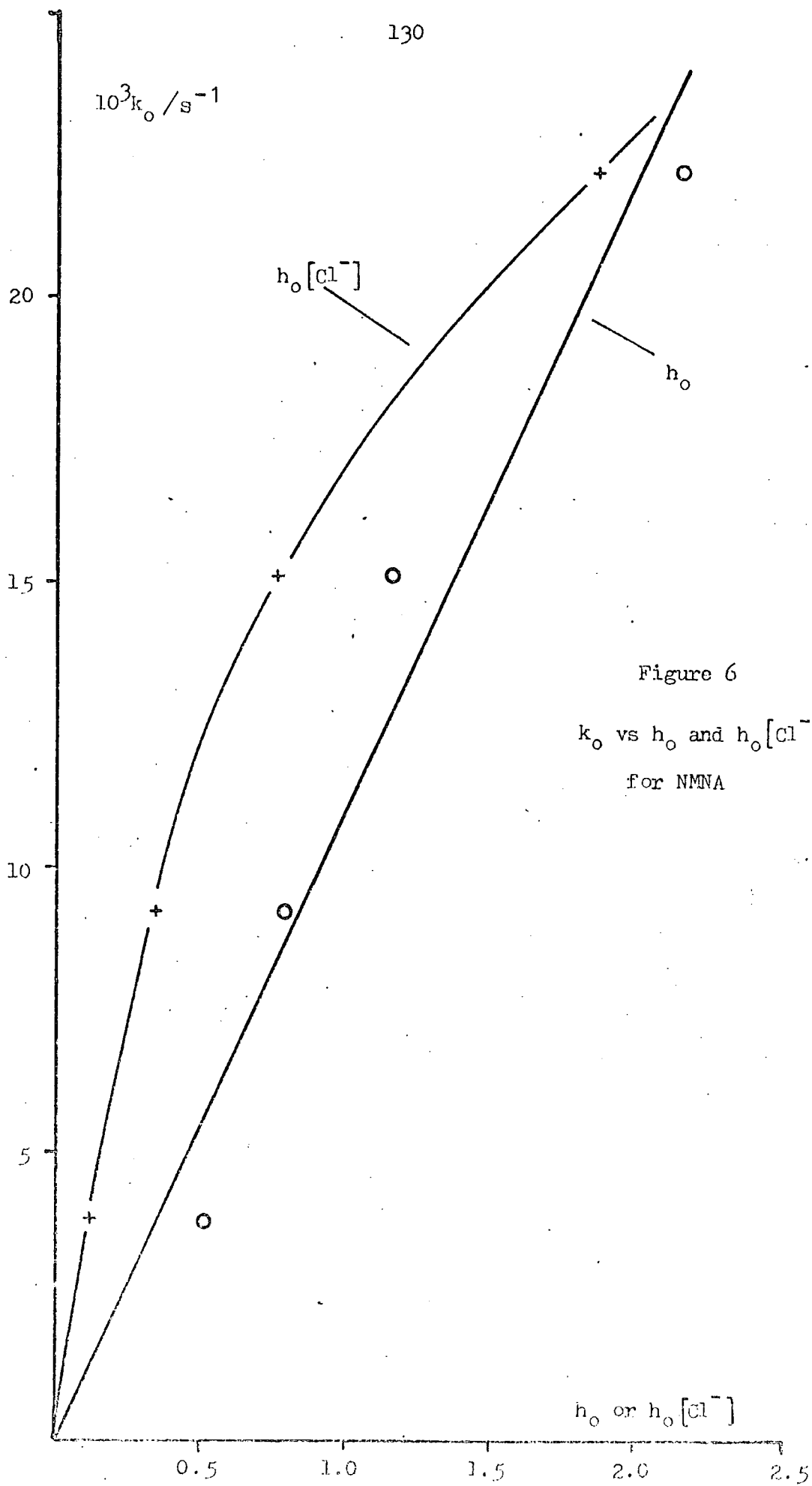
Figure 9

k_o vs h_o and $h_o[Cl^-]$

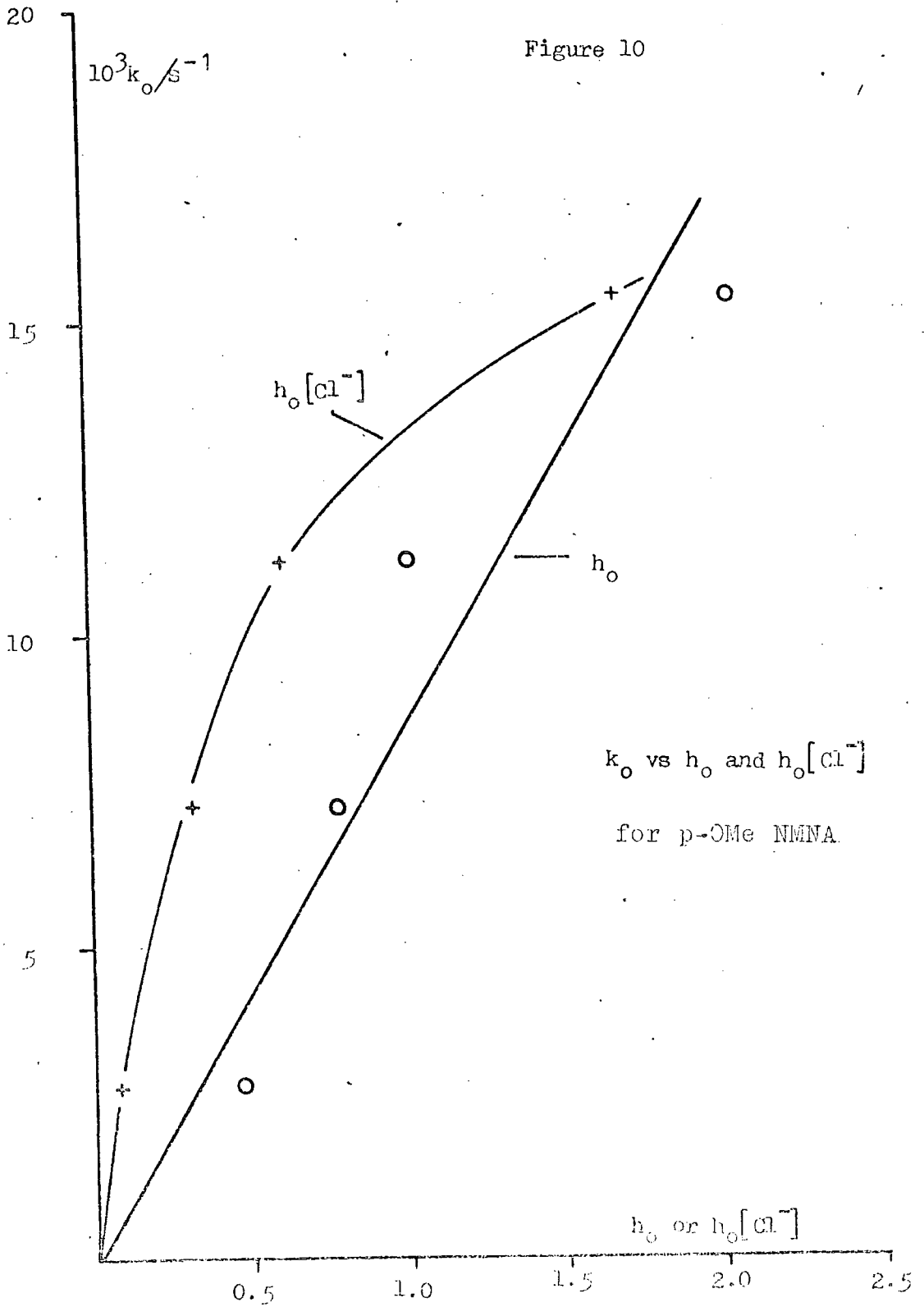for p-Me NMNA

Figure 10

k_o vs h_o and $h_o[Cl^-]$

for p-OMe NMNA
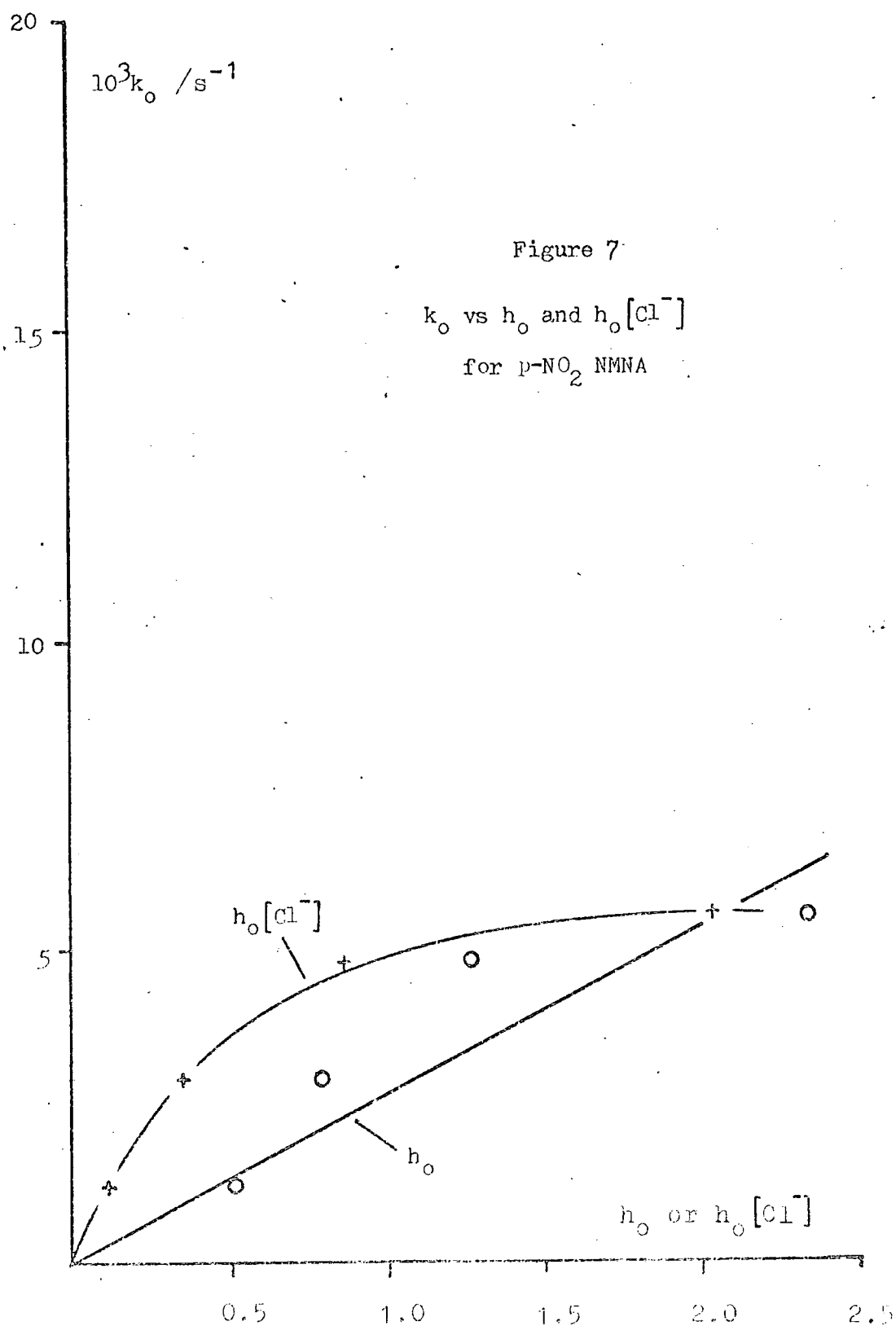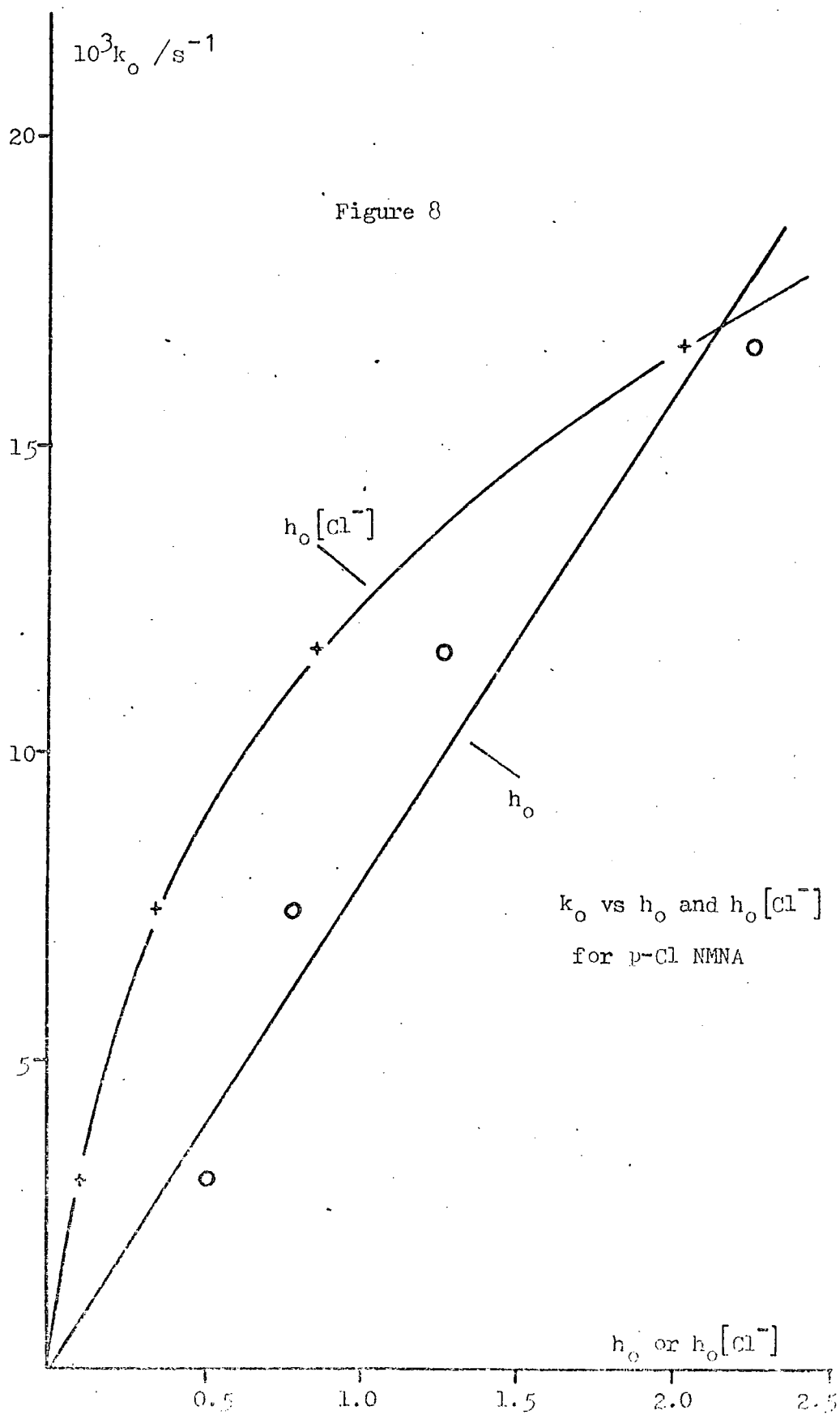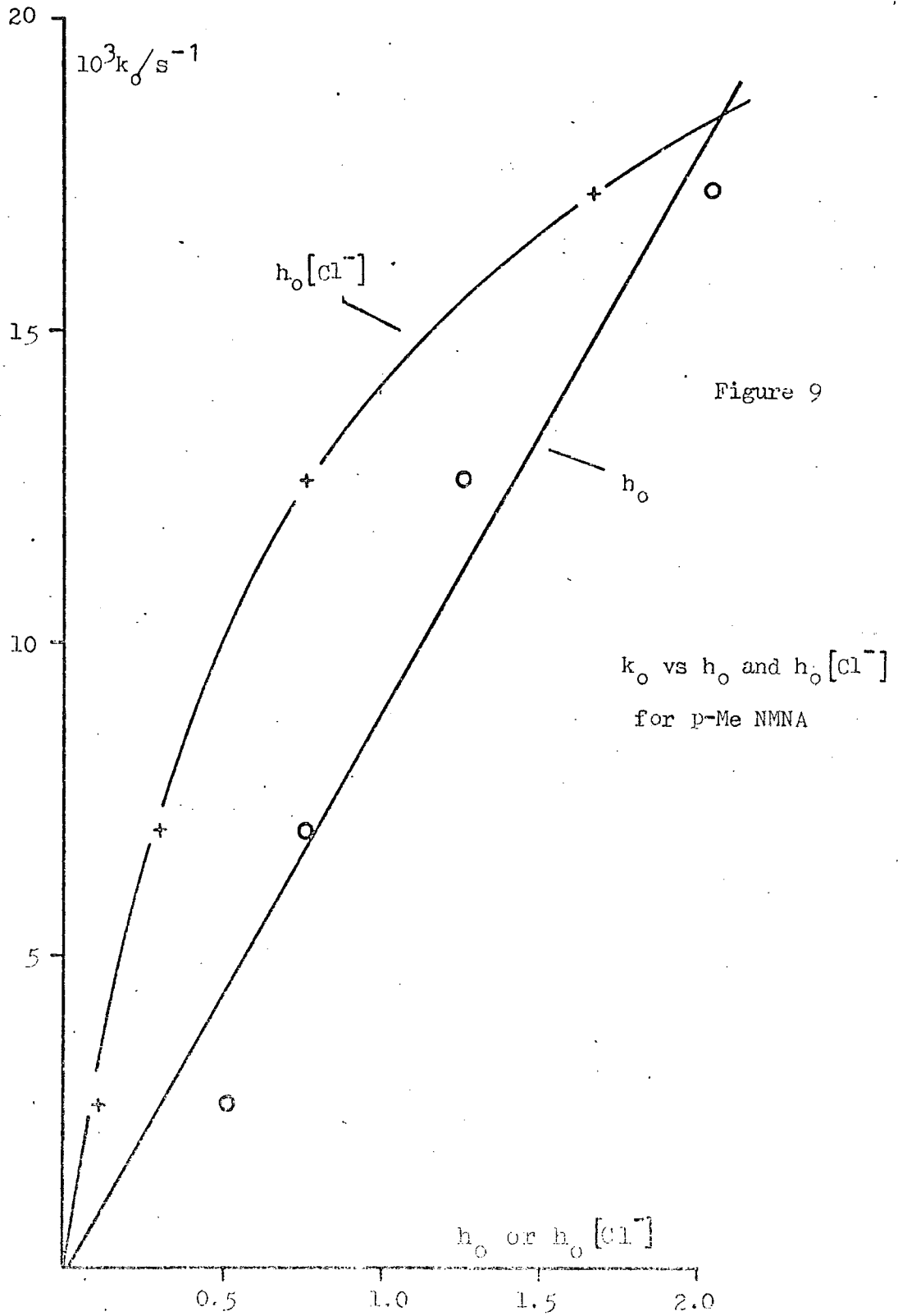
Figure 11

$3 + \log k_o$ vs $-H_o + 1$

p-H = □
p-NO$_2$ = △
p-Me = ▽
p-Cl = ○
p-OMe = ×
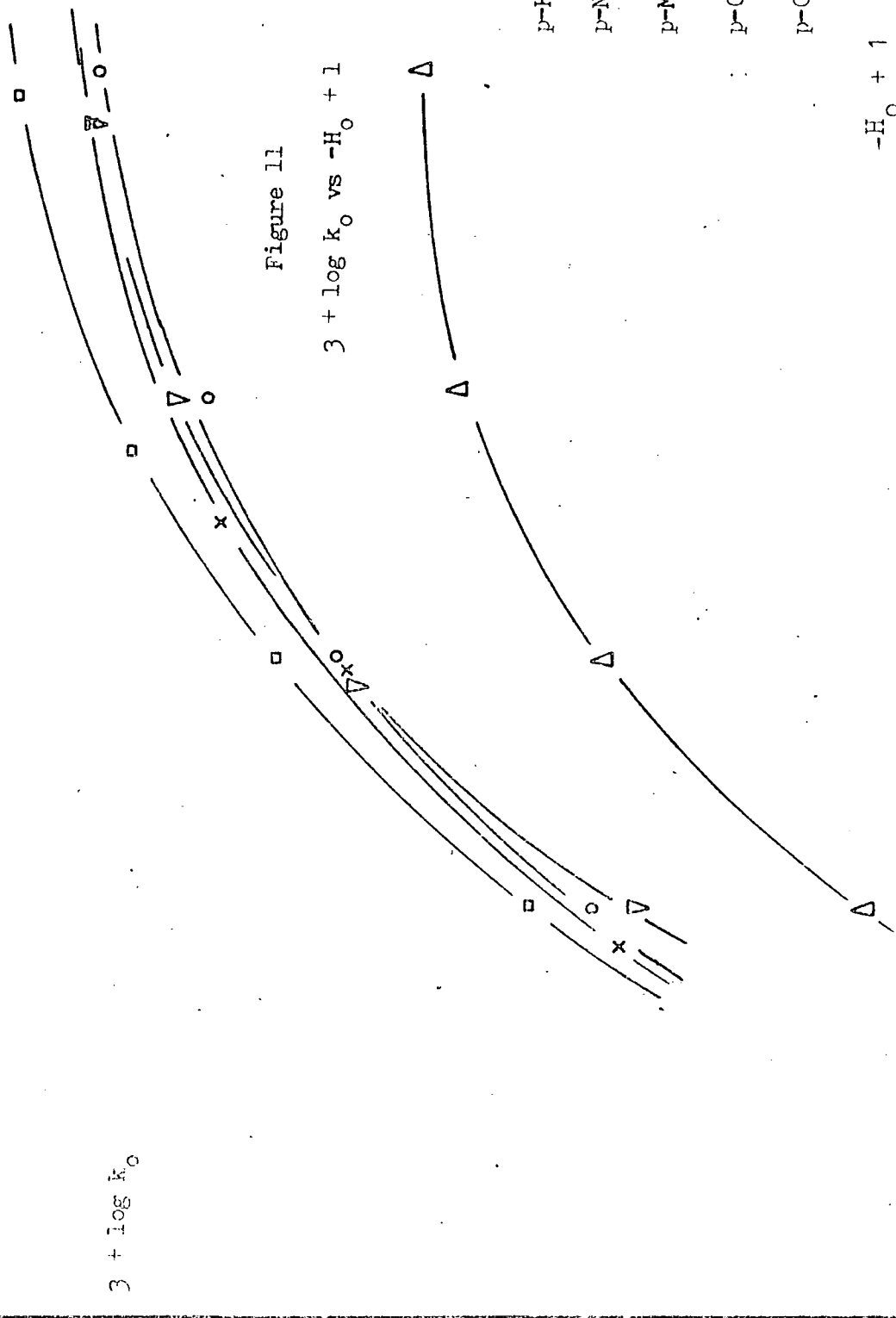
some while.

Consider the values below

p-substituent	$10^3 \ k_2/s^{-1}$
-H	10.8
$-NO_2$	2.33
-Cl	7.40
-Me	9.24
-OMe	8.78

The first point of note is that the substituent effect upon k_2 is small, perhaps smaller than we would have expected for our postulated "unopposed" effect. The values of k_2 whilst agreeing with those of Meyer[131] lie well below those values expected for the rate constant of a simple proton transfer reaction. It is also interesting to note that, whilst the substituent effects act upon k_2 for the p-substituted NMNA's, NMNA itself possesses an "anomalously" high reactivity. Continuing our investigation we plot $\log k_0$ vs H_0 for each analogue and arrive at figure 11. Clearly values of $\log k_0$ rise towards a maximum value for each analogue. It would seem appropriate to conduct an explanation along the following lines.

It is acknowledged in earlier discussion that our portrayal of the initial protonation of the nitrosamine as a simple one-stage process may be at fault. A possible alternative representation would seem to require a protonation at the nitroso-oxygen site followed by a rearrangement to give the N-protonated species.
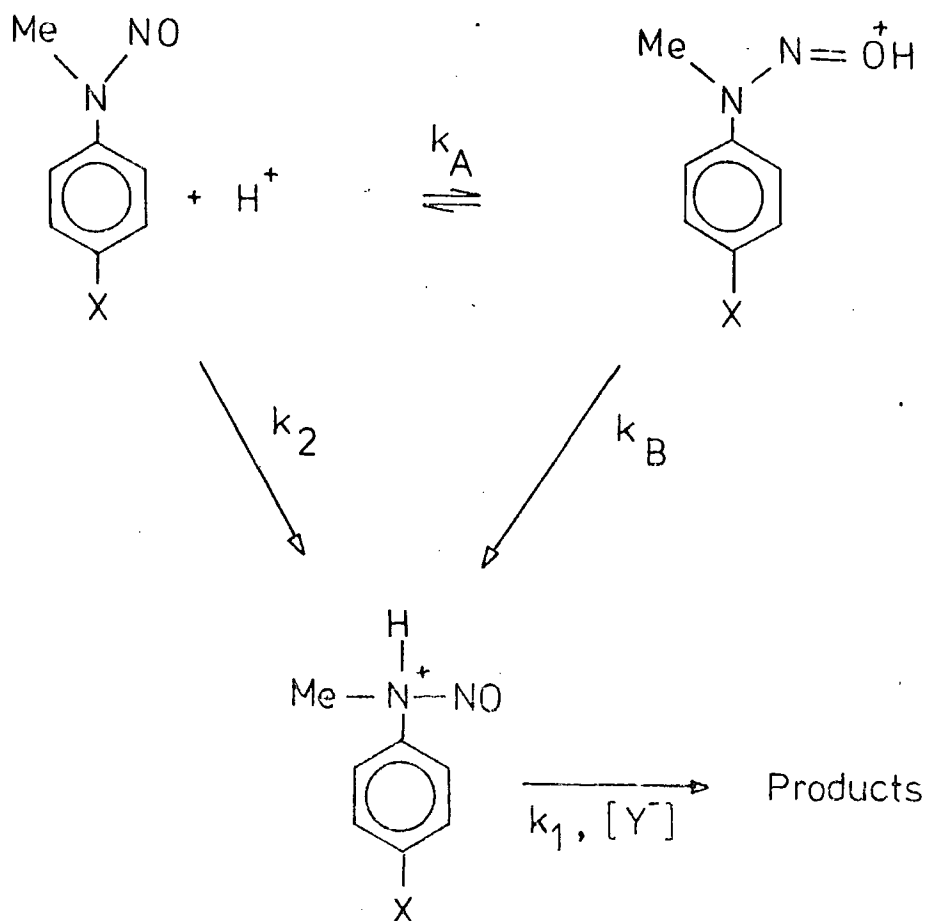
Note that under this scheme the derived rate-constant k_2 represents a composite rate constant for the protonation stage. It should not now be too surprising to recall that the measured values of k_2 for the p-substituted NMNA's fall short of the values expected for a proton-transfer reaction. Clearly the rearrangement stage governed by k_B represents the rate-determining stage of the N-protonation. In the high acidity region of our series of tests it would appear that the NMNA's are tending towards full O-protonation so that the rate of reaction is limited only by the rate of the rearrangement reaction. As the rate of this reaction is independent of the acidity of the medium values of log k_0 are seen to rise to a maximum with increasing $-H_0$ thereafter becoming primarily independent of the acidity.

4.3 Conclusion

Although the p-substituted N-methyl-N-nitrosoanilines afford confirmation of the effect of solvent changes upon the catalytic behaviour of the denitrosation reaction it must be reported that their detailed behaviour is not readily understood. It would appear that the experimental results are not easily rationalised except on the basis of a postulated two-stage protonation reaction. It is perhaps pertinent to suggest that any new study might start with a reappraisal of the variation of h_o in ethanolic solutions of HCl since it is acknowledged that some inaccuracy may derive from the use of the specific values presented by Braude. In the light of this acknowledgement it would seem unwise to place too much significance upon the unresolved anomaly concerning the reactivity of N-methyl-N-nitrosoaniline itself.

SECTION FIVE

THE EFFECT OF THE NATURE AND CONCENTRATION OF THE NUCLEOPHILE

THE REACTIONS OF A SERIES OF N-NITROSAMINES IN AQUEOUS ACIDIC SOLUTION IN THE PRESENCE OF HIGH CONCENTRATIONS OF NUCLEOPHILES

5.1 An Introduction

In earlier sections of this work we have discussed the effect of both nitroso-compound structure and choice of solvent upon the kinetic behaviour of the denitrosation reaction. We have also made mention of the effect of the nature and concentration of the nucleophile and in this present section we shall pursue this aspect in a little more detail.

Consider the denitrosation of an N-nitrosamine in an aqueous medium. Structural considerations would lead us to expect the rate-determining stage of the reaction to be represented by the attack of the nucleophile upon the protonated form of the nitrosamine and our choice of water as the solvent would reinforce this expectation. Such a reaction would be characterised as being subject to both acid and nucleophilic catalysis. The kinetic isotope effect demonstrated by the reaction may be expected to be of a fairly low magnitude. At low nucleophile concentrations these conditions are generally found to obtain and indeed the specific evidence of Thompson[105] and Williams[11,125] concerning the denitrosations of N-nitrosodiphenylamine and N-methyl-N-nitrosoaniline respectively has already been discussed. As the concentration of the nucleophilic species is increased we expect this situation to alter.

The disappearance of nucleophilic catalysis at high nucleophile concentrations which is so indicative of the change towards a rate-determining protonation was first reported by Challis and Osborne[132] for the reaction of N-nitrosodiphenylamine with HCl in mixed EtOH/H_2O solvent. The range of N-nitroso compounds studied at high nucleophile concentrations has increased steadily with similar results being obtained in each case. Thus whilst Thompson[106] has been able to confirm

the observations of Challis and Osborne for a 100% aqueous solvent Meyer[104] has moved on to study the reaction of N-methyl-N-nitroso-aniline. Using a 0.47 M aqueous H_2SO_4 solvent in the presence of an added excess of sodium azide the reaction was found to become independent of the concentration of the three nucleophiles Br^-, SCN^- and $SC(NH_2)_2$ respectively at high nucleophile concentrations. The measured kinetic isotope effect $k_{H_2O}:k_{D_2O}$ of 1.7 measured for PhNMeNO at a thiourea concentration of 0.42 M is taken as being indicative of a move towards a situation in which the protonation of the nitrosamine is the rate-determining step. Under such conditions we may refer to Section 2.1 and write the following expression for k_o, the observed first-order rate constant for the disappearance of the nitrosamine.

$$k_o = k_2 h_x$$

As k_o is dependent only upon k_2 and h_x, the appropriate acidity function, the limiting value of k_o at high nucleophile concentrations should be the same for any one nitrosamine irrespective of the nature of the associated nucleophile. Meyer's results confirm this expectation with a limiting value of $200 \times 10^{-4} \ s^{-1}$ being observed for both thiourea and the thiocyanate ion. As these two species do not possess identical nucleophilic reactivities we should not expect to reach the limiting value of k_o at the same concentration for each nucleophile and, indeed, whilst a 0.5 M concentration of thiourea is required to reach this limiting area a 0.9 M concentration of thiocyanate ion is required to bring about this same effect. We may further demonstrate this difference in nucleophilic reactivities as follows.

Consider the reaction scheme:

$$\underset{B}{\overset{A}{>}}\!NNO \;+\; H^{+} \quad \underset{k_{-2}}{\overset{k_{2}}{\rightleftharpoons}} \quad \underset{B}{\overset{A}{>}}\!\overset{+}{N}\!\underset{NO}{\overset{H}{<}}$$

$$Y^{-}\downarrow k_{1}$$

$$\underset{B}{\overset{A}{>}}\!N\!-\!H \;+\; NOY$$
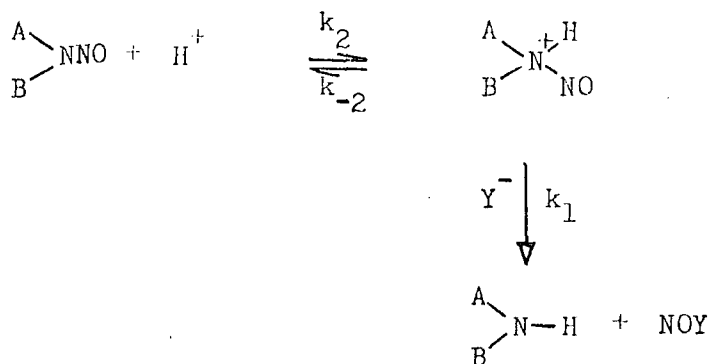
Reference to eqn. 10 of Section 2.1 leads us to presume that the reaction of NMNA at low nucleophile concentrations in an aqueous solvent proceeds according to the expression given below.

$$k_{o} \;=\; \frac{k_{1}k_{2}\,[H^{+}]\,[Y^{-}]}{k_{-2}}$$

when k_{o} is defined by

Rate $= k_{o}\,$ [N-methyl-N-nitrosoaniline]

At high concentrations of the nucleophilic species this same reaction is governed by the expression,

$$k_{o} \;=\; k_{2}\,[H^{+}]$$

At intermediate concentrations of the nucleophile we must refer to Section 2.1 for the full rate expression given by equation 9 whence,

$$k_{o} \;=\; \frac{k_{1}k_{2}\,[H^{+}]\,[Y^{-}]}{k_{-2} + k_{1}\,[Y^{-}]}$$

It is clear that if we take values of k_{o} obtained at these intermediate nucleophile concentrations then a plot of k_{o}^{-1} vs $[Y^{-}]^{-1}$

will lead to a straight line with an intercept equal to the value $1/k_2[H^+]$. Plots for the three nucleophiles employed by Meyer yield a common intercept of 50 s which leads to a value of 3.2×10^{-2} l mol^{-1} s^{-1} for k_2. The slopes of the three lines give an inverse measure of the reactivities of the nucleophiles so that Meyer was able to calculate the following relative reactivities.

Thiourea   :Thiocyanate   2.2

Thiocyanate:Bromide   124

These figures compare well with the values of 2.5 and 100 respectively calculated by Biggs et al.[60,68] from data obtained at low nucleophile concentrations. It should be noted in passing that Meyer's value for k_2 although lower than the value to be expected for a simple proton transfer reaction agrees favourably with the value calculated by the present author for the reaction in ethanol. It would seem that the two-stage protonation proposed for the ethanol reaction is also operative in aqueous solvents.

5.2 The Denitrosation of N-nitrosodiphenylamine at High Nucleophile Concentrations

Although Thompson[106] has demonstrated the removal of bromide catalysis for the reaction of N-nitrosodiphenylamine (NDPA) in aqueous H_2SO_4 at high nucleophile concentrations the existence of a common limiting value of k_o for all nucleophiles has not been proven. As the existence of this common limit is characteristic of the present rationale a series of exploratory kinetic experiments were carried out in the presence of various added concentrations of bromide ion and thiocyanate ion respectively. The data are given over, these same data being represented graphically in Figure 12.

$[H_2SO_4]$ = 0.277 M

$[NaN_3]$ = 0.16 M

$[NDPA]_o$ = 2.936 x 10^{-4} M
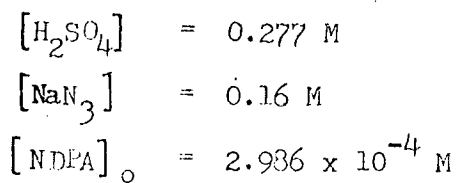
10^2 [KBr]/M	$10^2 k_o/s^{-1}$
4	1.050
8	1.639
12	1.839
16	2.051
20	2.216
24	2.347
28	2.447
32	2.708
36	2.778
40	2.887
50	2.981

10^3 [KSCN]/M	$10^2 k_o/s^{-1}$
4	0.568
8	0.973
12	1.279
16	1.508
20	1.731
24	1.822
28	1.978
32	2.175
36	2.275
40	2.283
50	2.490

Figure 12

$10^3 \ k_o/s^{-1}$

KBr

KSCN

k_o vs [nucleophile]

for denitrosation

of NDPA

10^2 [KBr]/M

or

10^3 [KSCN]/M
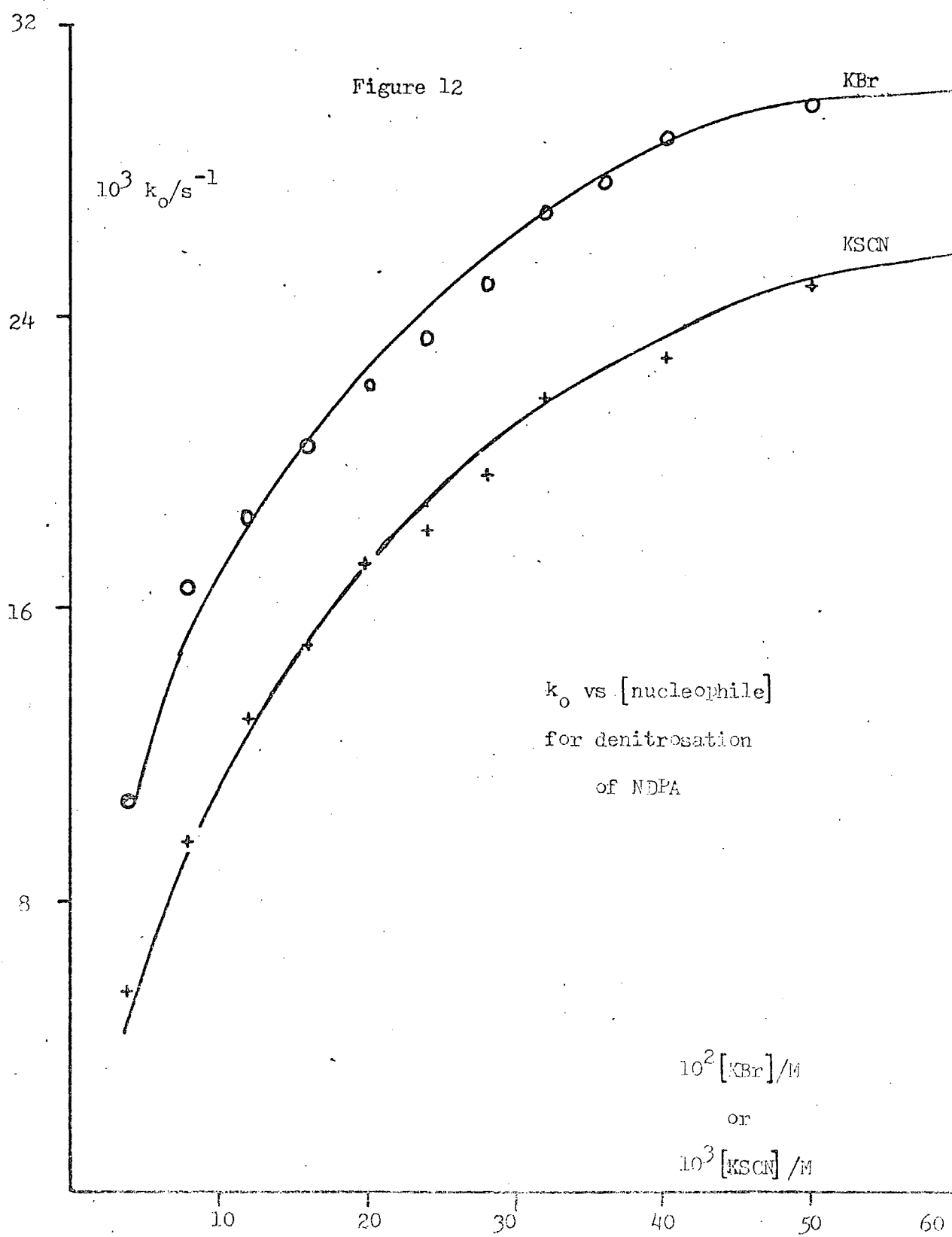
Clearly k_o tends to become independent of the nucleophile concentration at high nucleophile concentrations. We may perform a simple extrapolation to obtain the following limiting values for k_o.

$$k_o = 3.02 \times 10^{-2} \text{ s}^{-1} \quad \text{at} \quad [\text{KBr}] = 60 \times 10^{-2} \text{ M}$$
$$k_o = 2.58 \times 10^{-2} \text{ s}^{-1} \quad \text{at} \quad [\text{KSCN}] = 65 \times 10^{-3} \text{ M}$$

At first sight these limiting values of k_o do not seem to be in too good an agreement but it should be remembered that other factors operate. In particular we must correct the nominal acidity of the reaction medium to allow for the protonation of the azide anion and in the case of the thiocyanate anion to allow also for the protonation of the nucleophile. Assuming complete protonation of the azide we may write, to a close approximation,

$$[\text{H}_2\text{SO}_4]_{\text{Corr}} = [\text{H}_2\text{SO}_4] - \frac{[\text{NaN}_3]}{.2}$$

whence from tables we obtain

$[\text{H}^+]$ for Br^- experiment at limiting $[\text{Br}^-] = 0.259$ M

For the thiocyanate case we have a value[134] of pKa for the anion of -0.701 so that we may calculate the extent of protonation. Using this knowledge we may then arrive at a value for $[\text{H}^+]$ for the thiocyanate experiment such that,

$[\text{H}^+]$ for SCN^- experiment at limiting $[\text{SCN}^-] = 0.238$ M

On the assumption that

$$\text{Rate} = k_o [\text{NDPA}]$$

and

$$k_o = k_2 [\text{H}^+] \text{ at these high nucleophile concentrations}$$

then we may correct for the differences in acidity such that

$k_0(\text{KBr})$ (at $[\text{H}^+] = 0.295$ M) $= 3.02 \times 10^{-2}$ s^{-1}

$k_0(\text{KSCN})$ (at $[\text{H}^+] = 0.295$ M) $= \dfrac{2.58 \times 10^{-2}}{0.238} \times 0.295 = 3.19 \times 10^{-2}$ s^{-1}

Clearly the limiting value of k_0 at high nucleophile concentrations is independent of the nature of the nucleophile.

Proceeding further we note that at moderate nucleophile concentrations k_0 is given by

$$k_0 = \frac{k_1 k_2 [\text{H}^+][\text{Y}^-]}{k_{-2} + k_1 [\text{Y}^-]}$$

A plot of $1/k_0$ vs $1/[\text{Y}^-]$ should thus be linear with an intercept of $1/k_2[\text{H}^+]$.

Such a plot, given as figure 13 yields the following values for k_2 which should be dependent only upon the nature of the nitrosoamine.

$$k_2(\text{KBr}) = 1.5 \times 10^{-1} \text{ l mol}^{-1} \text{ s}^{-1}$$

$$k_2(\text{KSCN}) = 1.4 \times 10^{-1} \text{ l mol}^{-1} \text{ s}^{-1}$$

The slopes of the lines represent the values of

$$\frac{k_{-2}}{k_1 k_2 [\text{H}^+]}$$

Since k_2 and k_{-2} are identical for a particular nitrosoamine we may use the slopes to yield a value for the ratio $k_1(\text{SCN}^-)/k_1(\text{Br}^-)$ (which represents the ratio of the reactivities of the two nucleophiles in this reaction) by assuming that $[\text{H}^+]$ is equal in each case.

On this basis we have $k_1(\text{SCN}^-)/k_1(\text{Br}^-) = 5.4$. Considering our assumption concerning the acidities this value compares acceptably with that of 7 proposed by Thompson[140] for the reaction at low nucleophile concentrations. The low value of the present ratio compared with that

Figure 13

k_o^{-1} vs $[\text{nucleophile}]^{-1}$

for denitrosation of

NDPA
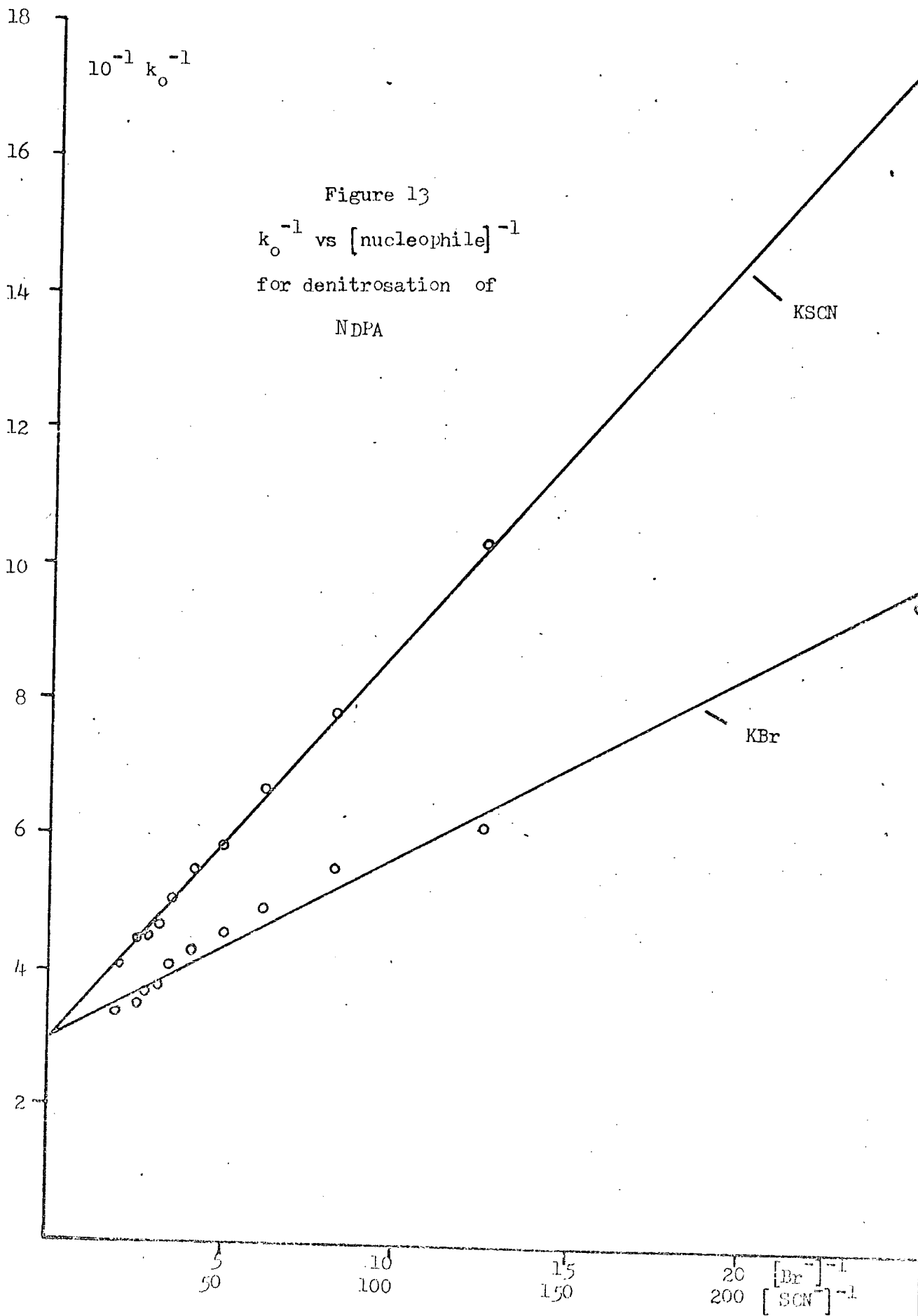
obtained by Meyer[104] for the reaction of NMNA clearly reflects the higher reactivity of NDPA which causes it to be less discriminating between the various nucleophiles.

5.3 The Denitrosation of a Series of para-substituted N-methyl-N-nitrosoanilines

In order to substantiate the generality of this behaviour at high nucleophile concentrations an investigation was carried out into the reaction of a series of p-substituted N-methyl-N-nitrosoanilines. The results of this investigation are given below.

$[H_2SO_4]$ = 0.407 M

$[Thiourea]$ = 0.792 M

$[Hydrazine Sulphate]$ (as nitrite trap) = 1.003 x 10^{-2} M

p-substituted NMNA	$10^4 k_o/s^{-1}$
-H	180
-Cl	145
-NO$_2$	71
-Me	209
-OMe	214

In each case a 20% increase in the thiourea concentration caused little or no increase in the value of k_o. The largest increase recorded, with an approximate value of 8% is comparable with the experimental error of ±5% and represents, at best a salt effect. It is clear that under the specified conditions the reaction is not subject to nucleophilic catalysis. In common with the effect on both NDPA and

NMNA the action of high nucleophile concentrations on the denitrosation of the p-substituted NMNA's is such as to render the initial protonation stage rate-determining so that,

$$k_o = k_2 h_x$$

where h_x represents an appropriate acidity function.

It is interesting to probe slightly deeper into the behaviour of the p-substituted analogues. Noting that the use of high nucleophile concentrations in an aqueous solvent brings about the same limiting condition as does the use of an ethanolic solvent, it should be possible to rationalise the effects of substitution in a manner analogous to that employed in Section 4. With the opposing effect upon k_1 removed we might expect to see the substituent effects upon k_2 reflected by a wide variation in k_o across the various analogues. Whilst this variation does operate in the expected direction it shares a common feature with that observed for the ethanol reaction in that it is of a low magnitude. The explanation is almost certainly as per Section 4 in that we are seeing evidence of a two-stage protonation. After allowing for the protonation of the thiourea (pKa = -1.19) we may calculate values of k_2

p-substituted NMNA	$10^2 k_2/s^{-1} l^{-1} mol$
-H	3.22
-Cl	2.69
-NO$_2$	1.27
-Me	3.74
-OMe	3.83

Clearly these values lie far below those expected for a simple single-stage protonation reaction.

5.4 Conclusion

At high concentrations of added nucleophiles we expect the denitrosation of the N-nitrosamines in aqueous solution to proceed with a characteristic independence of both the nature and the concentration of the nucleophile. Certainly I find no exception amongst the compounds studied here and our proposal of this effect as being general for all N-nitrosamines gains support.

It is interesting to note that here again we have evidence of the existence of a two-stage protonation process so that we are drawn towards the views of Challis and Osborne[132] who propose that an initial o-protonation at the nitroso-oxygen site is followed by a rearrangement to give the N-protonated form. As of now however no conclusive evidence concerning this point exists.
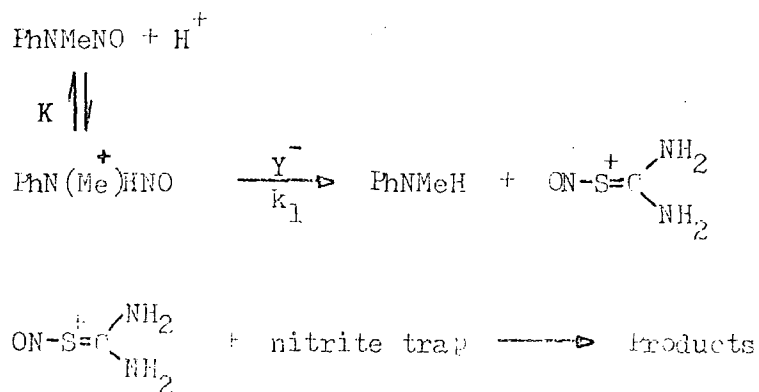
SECTION SIX

THE REACTION OF N-METHYL-N-NITROSOANILINE

WITH SULPHUR-CONTAINING NUCLEOPHILES

IN ACIDIC SOLUTION

0

6.1 An Introduction

It has been apparent for some time that the denitrosation of N-nitrosamines in aqueous acid solution may be catalysed by the addition of moderate concentrations of various nucleophilic agents. Whilst these nucleophilic agents have classically taken the form of the halide ions this list is by no means exhaustive and Section 1.7 of the present work has allowed us to consider one alternative series of nucleophiles; the sulphur nucleophiles. With the current interest in the biological action of the N-nitrosamines and given the abundant supply of sulphur nucleophiles in the body, the interaction of the two takes on a high biological significance. With this significance in mind we shall develop the subject further.

Earlier, in Section 1.7, we made mention of a study by Williams[68] which centered around the reaction of thiourea. The following scheme is proposed for reaction in the presence of a nitrite trap.

PhNMeNO + H$^+$

$K \Updownarrow$

PhN$^+$(Me)HNO $\xrightarrow[k_1]{Y^-}$ PhNMeH + ON-S=C$\begin{smallmatrix} NH_2 \\ NH_2 \end{smallmatrix}$

ON-S=C$\begin{smallmatrix} NH_2 \\ NH_2 \end{smallmatrix}$ + nitrite trap $\longrightarrow$ Products

It is clear from the results of the kinetic and product studies that the rate determining stage of this reaction under the conditions stated is represented by the nucleophilic attack of the thiourea upon the protonated form of the nitrosamine. Given that,

$$\text{Rate} \quad = \quad \frac{-d[NMNA]}{dt} \quad = \quad k_o[NMNA]$$

we may write

$$k_o \quad = \quad k_1 K h_o [Y^-]$$

A plot of k_o vs [Thiourea] thus leads to a value of $k_1 K$ which demonstrates the nucleophilic reactivity of thiourea to be comparable with that of the iodide ion.
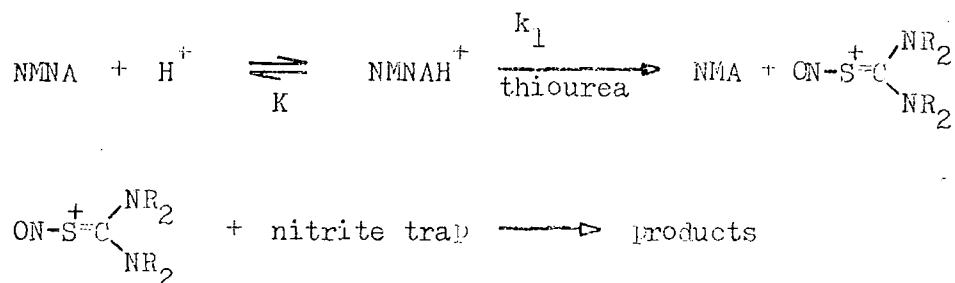
Similar results have been obtained for the reaction of thiourea with N-nitrosodiphenylamine[125].

6.2 The Reaction of N-methyl-N-nitrosoaniline with Thiourea and the N-alkyl Thioureas

6.2.1 An Introduction

In examining the reactions of the N-nitrosamines in acidic solution it is advantageous to carry out all investigations in the presence of an excess concentration of an added nitrite trap such as sodium azide or hydrazine sulphate. In such circumstances the inherent reversibility of the denitrosation reaction is removed so that the kinetic behaviour of the system is considerably simplified. In the case of reaction in the presence of the thioureas it appears that the choice of the nitrite trap must be a judicious one. Whilst hydrazine sulphate behaved satisfactorily over the entire range of thio-substrates there was evidence of extensive side reactions with some of the traps examined and in particular the combination of sulphamic acid and tetramethylthiourea lead to a reproducible s-shaped curve for a plot of k_o vs [tetramethylthiourea]. Consequently hydrazine sulphate was employed throughout the present investigations.

In such circumstances we expect the reaction scheme due to Williams, which is depicted, above to apply. Whilst this scheme has been proven for the reaction of thiourea, (Section 1.7), it would be unwise to apply it blindly to the reaction of the alkyl-substituted analogues without some previous analysis of the products. I have carried out such a series of investigations whose results are described fully in Section 8. Suffice it to say that I find no evidence in these results to suggest any reaction which lies outside of the scheme proposed by Williams; neither do I find evidence amongst the results of the kinetic investigations save in the one instance noted above. It is clear that thioureas react with NMNA according to the following scheme.

$$NMNA + H^+ \underset{K}{\rightleftharpoons} NMNAH^+ \xrightarrow[\text{thiourea}]{k_1} NMA + ON-\overset{+}{S}=C \begin{smallmatrix} NR_2 \\ NR_2 \end{smallmatrix}$$

$$ON-\overset{+}{S}=C\begin{smallmatrix} NR_2 \\ NR_2 \end{smallmatrix} + \text{nitrite trap} \longrightarrow \text{products}$$

If we assume that the concentration of the thiourea remains constant during any one kinetic run then we may define the value k_o, which represents the observed first-order rate constant for the reaction, as above. Referring to the work of Williams[68] and acting on the assumption that the initial protonation has some dependence upon the Hammett acidity function, h_o, we may write

$$k_o = k_1 K h_o^x [Y^-]$$

where x represents the experimentally determined order in h_o expected to be close to unity.

6.2.2 The Variation of k_0 with [Hydrazine Sulphate]

A series of investigations were carried out to ascertain the concentration of hydrazine sulphate which must be added to the reaction system to bring about the complete subjugation of the reverse reaction described above. Basically the reaction of each system to increasing concentrations of hydrazine sulphate is identical so that we will not dwell upon a detailed discussion of each. Nevertheless the results obtained with each thiourea at each of two acidities are given below for reference.

Thiourea

$$[\text{Thiourea}] = 1.801 \times 10^{-3} \text{ M}$$
$$[\text{NMNA}]_0 = 1.348 \times 10^{-4} \text{ M}$$
$$[\text{H}_2\text{SO}_4] = 0.78 \text{ M}$$

$10^4[\text{N}_2\text{H}_5^+\text{HSO}_4^-]$ /M	$10^3 k_0$/s^{-1}
0	13.10
0.25	5.40
1.25	1.52
2.50	1.70
7.50	1.82
12.75	1.90
25.00	1.87
50.00	1.90
100.00	2.00

$[H_2SO_4] = 3.94$ M

$10^4[N_2H_5^+HSO_4^-]$/M	10^2k_o/s^{-1}
0	2.75
0.25	2.28
1.25	2.16
2.50	2.30
7.50	2.76
12.50	2.72
25.00	2.88
50.00	2.92
100.00	2.86

N-Methylthiourea

$[\text{N-methylthiourea}] = 1.802 \times 10^{-3}$ M

$[\text{NMNA}]_o = 1.297 \times 10^{-4}$ M

$[H_2SO_4] = 0.78$ M

$10^4[N_2H_5^+HSO_4^-]$/M	10^3k_o/s^{-1}
0	7.71
0.25	3.35
1.25	1.49
2.50	1.57
7.50	1.66
12.50	1.76
25.00	1.78
50.00	1.82
100.00	1.81

$[H_2SO_4] = 3.94$ M

$10^4[N_2H_5^+HSO_4^-]/M$	$10^2k_0/s^{-1}$
0	2.36
0.25	2.01
1.25	1.72
2.50	1.81
7.50	2.30
12.50	2.34
17.50	2.34
50.00	2.53

N,N'-dimethylthiourea

$[N,N'-dimethylthiourea] = 1.187 \times 10^{-3}$ M

$[NMNA]_0 = 1.297 \times 10^{-4}$ M

$[H_2SO_4] = 0.78$ M

$10^4[N_2H_5^+HSO_4^-]/M$	$10^4k_0/s^{-1}$
0	95.10
0.25	17.10
1.25	8.68
2.50	9.64
7.50	10.20
12.50	10.34
25.00	10.56
50.00	10.04
100.00	10.43

$[H_2SO_4] = 3.94$ M

$10^4[N_2H_5^+ HSO_4^-]/M$	$10^2 k_o/s^{-1}$
0	1.85
0.25	2.51
1.25	1.57
2.50	1.41
7.50	1.72
12.50	1.77
25.00	1.82
50.00	1.83

Trimethylthiourea

$[Trimethylthiourea] = 1.210 \times 10^{-3}$ M

$[NMNA]_o = 1.297 \times 10^{-4}$ M

$[H_2SO_4] = 0.78$ M

$10^4[N_2H_5^+ HSO_4^-]/M$	$10^4 k_o/s^{-1}$
0	127.90
0.25	21.71
1.25	8.83
2.50	8.08
7.51	8.60
12.52	8.80
25.04	8.60
50.08	8.60
100.20	8.40

$[H_2SO_4]$ = 3.94 M

$10^4[N_2H_5^+ HSO_4^-]/M$	$10^2 k_0/s^{-1}$
0	3.72
0.25	1.63
1.25	1.36
2.50	1.76
7.51	2.10
12.52	2.10
25.04	2.12
50.08	2.13

Tetramethylthiourea

$[Tetramethylthiourea]$ = 1.206 x 10^{-3} M

$[NMNA]_0$ = 1.297 x 10^{-4} M

$[H_2SO_4]$ = 0.78 M

$10^4[N_2H_5^+ HSO_4^-]/M$	$10^4 k_0/s^{-1}$
0	126.30
0.25	15.58
1.25	7.82
2.50	8.77
7.50	8.99
12.50	9.06
25.00	8.78
50.00	8.93
100.00	8.68

$$[H_2SO_4] = 3.94 \ M$$

$10^4[N_2H_5^+HSO_4^-]/M$	$10^3k_o/s^{-1}$
0	11.70
0.25	4.30
1.25	3.80
2.50	4.94
7.50	5.83
12.50	5.90
25.00	5.58
50.00	6.11

If we were to plot k_o vs [hydrazine sulphate] for each set of data we would obtain a series of graphs which shared certain common features. Consider figure 14 which uses the data collected for N-methylthiourea at low acidity as an illustration. It should be noted that values of k_o fall initially from a maximum in the absence of hydrazine sulphate towards a minimum value at low trap concentrations. We have observed such behaviour before in the reaction of N-methyl-N-nitrosourea, Section 3.2.3 and apply the same explanation here. These high values of k_o which arise at low trap concentrations are probably due to our application of first-order guggenheim treatments to a reaction which, under the conditions operating in that area of the graph, are not first order. Being of little significance these values have been omitted from figure 14. As the concentration of hydrazine sulphate is raised further k_o is observed to rise from its minimum value towards a limiting value whose exact magnitude is not unexpectedly

Figure 14

k_o vs [Hydrazine Sulphate]
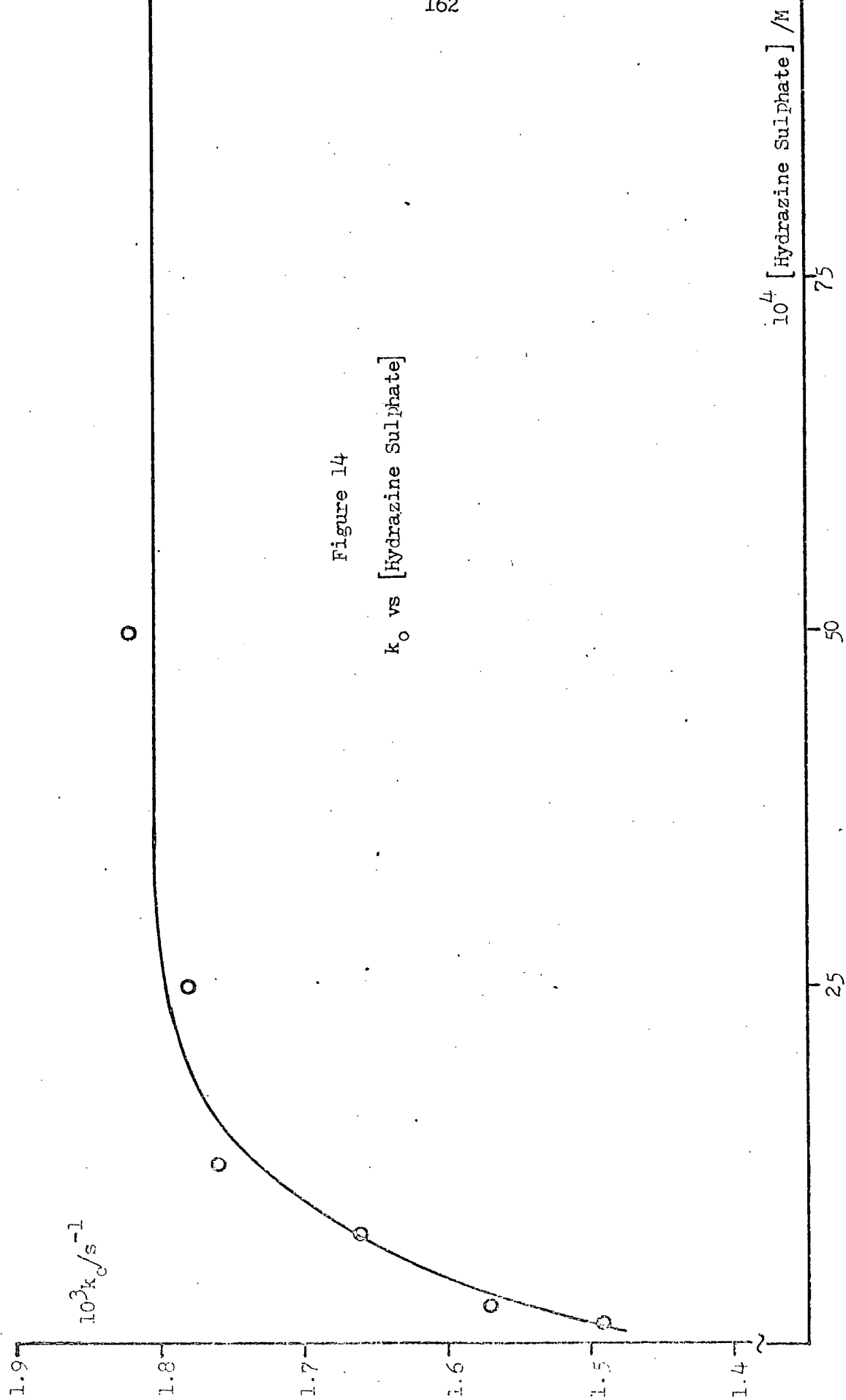
dependent upon which particular thiourea is in use. At these
limiting values of k_o the back reaction has been successfully
suppressed. In all cases we find that a hydrazine sulphate concentration
of 1×10^{-3} M or more guarantees the operation of these limiting
conditions. Further increases in hydrazine sulphate effect no
further increase in k_o and we may assume that no direct reaction occurs
between hydrazine sulphate and NMNA.

6.2.3 The Variation of k_o with [Thiourea]

An extensive series of investigations was carried out using each
of the thioureas in turn to ascertain the manner in which k_o varies
with the concentration of the nucleophile. In each case hydrazine
sulphate was present at a concentration in excess of 1×10^{-3} M so
that the limiting conditions described above were in operation and
good first-order behaviour was observed. With each thiourea being
the subject of study at a number of acidities the investigations
generated the following data.

Thiourea

$[H_2SO_4] = 0.78$ M

$10^3[\text{Thiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
3.03	2.87
6.06	4.90
9.08	6.26

$[H_2SO_4]$ = 1.57 M

$10^3[\text{Thiourea}]/M$	$10^3k_o/s^{-1}$
0	0
3.03	9.00
6.06	16.00
9.09	20.74

$[H_2SO_4]$ = 2.36 M

$10^3[\text{Thiourea}]/M$	$10^3k_o/s^{-1}$
0	0
1.21	8.42
2.42	16.25
3.63	22.30

$[H_2SO_4]$ = 3.14 M

$10^3[\text{Thiourea}]/M$	$10^3k_o/s^{-1}$
0	0
1.20	14.08
2.40	26.44
3.60	36.86

$[H_2SO_4]$ = 3.92 M

$10^3[\text{Thiourea}]/M$	$10^3k_o/s^{-1}$
0	0.34
0.60	10.24
1.20	19.75
1.80	28.70

N-methylthiourea

$[H_2SO_4]$ = 0.85 M

$10^3[\text{N-methylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
3.00	3.11
6.01	5.48
9.01	7.43

$[H_2SO_4]$ = 1.70 M

$10^3[\text{N-methylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
3.00	9.67
6.01	17.35
9.01	23.36

$[H_2SO_4]$ = 2.56 M

$10^3[\text{N-methylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
1.20	8.73
2.40	16.47
3.61	22.13

$[H_2SO_4]$ = 3.42 M

$10^3[\text{N-methylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
1.20	14.21
2.40	26.04
3.61	36.46

$[H_2SO_4] = 4.24$ M

$10^3[\text{N-methylthiourea}]/\text{M}$	$10^3 k_o/\text{s}^{-1}$
0	0.58
0.60	9.37
1.20	17.12
1.80	25.74

N,N'-dimethylthiourea

$[H_2SO_4] = 0.85$ M

$10^3[\text{N,N'-dimethylthiourea}]/\text{M}$	$10^3 k_o/\text{s}^{-1}$
0	0
1.93	1.88
3.96	3.40
5.94	4.79

$[H_2SO_4] = 1.70$ M

$10^3[\text{N,N'-dimethylthiourea}]/\text{M}$	$10^3 k_o/\text{s}^{-1}$
0	0
1.93	6.35
3.96	11.72
5.94	16.57

$[H_2SO_4] = 2.56$ M

$10^3[\text{N,N'-dimethylthiourea}]/\text{M}$	$10^3 k_o/\text{s}^{-1}$
0	0
0.79	6.26
1.58	11.89
2.38	17.31

$[H_2SO_4]$ = 3.42 M

$10^3[N,N'-dimethylthiourea]/M$	$10^3k_o/s^{-1}$
0	0
0.79	10.52
1.58	20.34
2.38	29.61

$[H_2SO_4]$ = 4.24 M

$10^3[N,N'-dimethylthiourea]/M$	$10^3k_o/s^{-1}$
0	0.58
0.40	7.33
0.79	13.14
1.19	19.40

Trimethylthiourea

$[H_2SO_4]$ = 0.85 M

$10^3[Trimethylthiourea]/M$	$10^3k_o/s^{-1}$
0	0
2.02	1.61
4.03	2.94
6.05	4.17

$[H_2SO_4]$ = 1.70 M

$10^3[Trimethylthiourea]/M$	$10^3k_o/s^{-1}$
0	0
2.02	5.70
4.03	10.95
6.05	15.24

$[H_2SO_4] = 2.56$ M

$10^3 [\text{Trimethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
0.81	6.11
1.61	11.95
2.42	17.40

$[H_2SO_4] = 3.42$ M

$10^3 [\text{Trimethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
0.81	12.09
1.61	22.56
2.42	34.05

$[H_2SO_4] = 4.24$ M

$10^3 [\text{Trimethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0.58
0.40	8.13
0.81	15.12
1.21	22.63

Tetramethylthiourea

$[H_2SO_4] = 0.85$ M

$10^3 [\text{Tetramethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
2.01	1.61
4.02	3.20
6.03	4.19

$[H_2SO_4] = 1.70$ M

$10^3[\text{Tetramethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
2.01	5.05
4.02	9.40
6.03	12.72

$[H_2SO_4] = 2.56$ M

$10^3[\text{Tetramethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
0.80	3.49
1.61	6.90
2.41	9.97

$[H_2SO_4] = 3.42$ M

$10^3[\text{Tetramethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0
0.80	4.21
1.61	7.98
2.41	11.18

$[H_2SO_4] = 4.24$ M

$10^3[\text{Tetramethylthiourea}]/M$	$10^3 k_o/s^{-1}$
0	0.58
0.40	2.55
0.80	4.34
1.21	6.10

When this data is portrayed graphically, as for example in Figures 15 to 19, we obtain good linear plots of k_o vs [Thiourea] for all of the thioureas at each of the acidities studied. It is clear that the reaction is first-order with respect to the concentration as we might have expected. In each case the plot depicts a small positive intercept on the y-axis which represents the value of k_o for the water catalysed reaction at that particular acidity. Where this value is negligibly small compared with the values obtained for the thiourea catalysed reaction it is set equal to zero.

The slopes of these plots are given below.

Thiourea

$[H_2SO_4]$/M	slope	corrected slope *
0.78	0.69	0.75
1.57	2.29	2.94
2.36	6.17	10.94
3.14	10.25	29.80
3.92	15.77	85.71

N-methylthiourea

$[H_2SO_4]$/M	slope	corrected slope *
0.85	0.82	0.91
1.70	2.59	3.59
2.56	6.17	13.10
3.42	10.11	40.93
4.24	13.86	111.75

Figure 15

k_o vs [N-methylthiourea]

$[H_2SO_4]$ = 0.85 M

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Figure 16

k_o vs [N-methylthiourea]

$[H_2SO_4] = 1.70$ M

Figure 17

k_o vs [N-methylthiourea]

$[H_2SO_4]$ = 2.56 M

Figure 18

k_0 vs [N-methylthiourea]

$[H_2SO_4] = 3.42$ M

Figure 19

k_o vs [N-methylthiourea]

$[H_2SO_4] = 4.24$ M
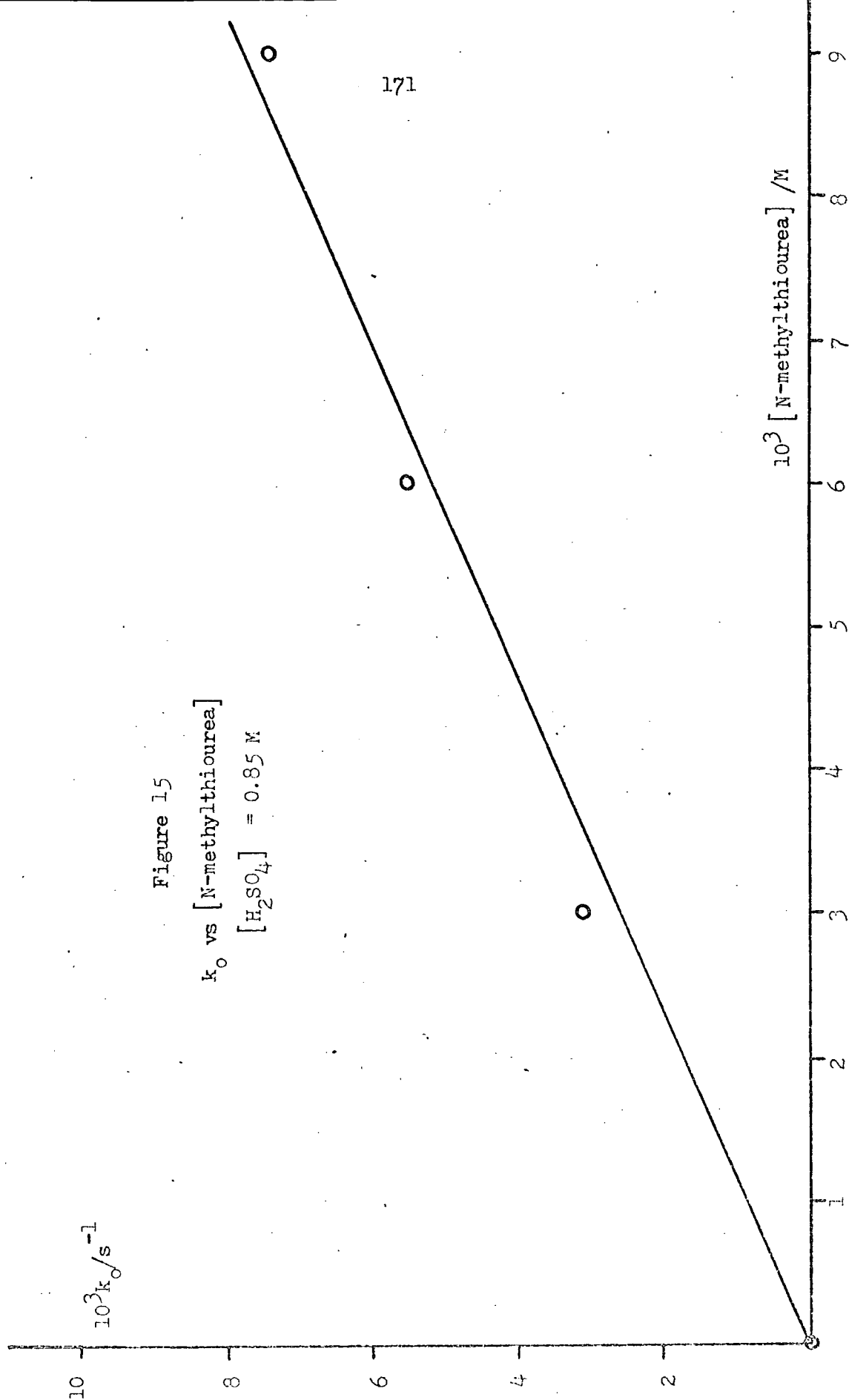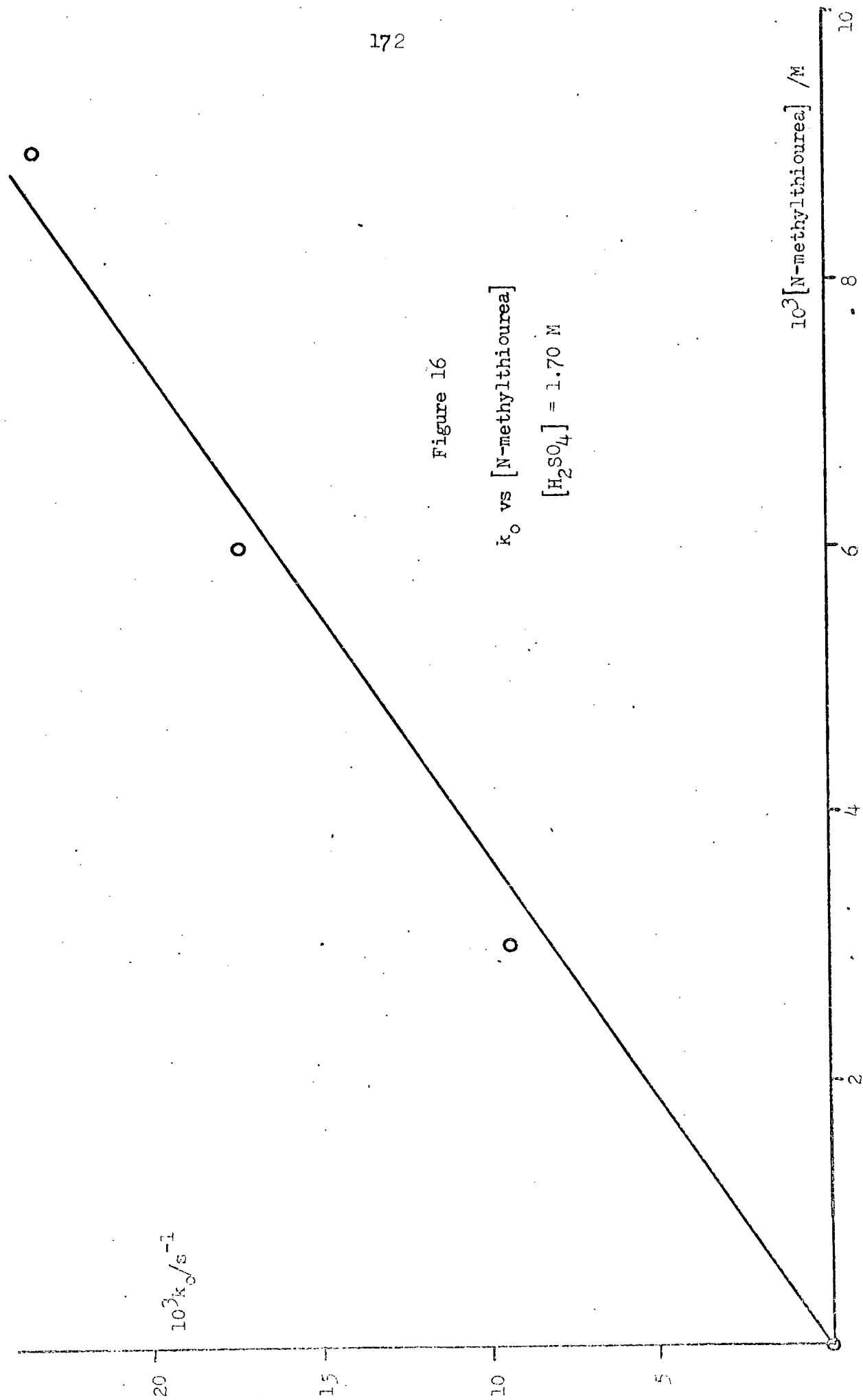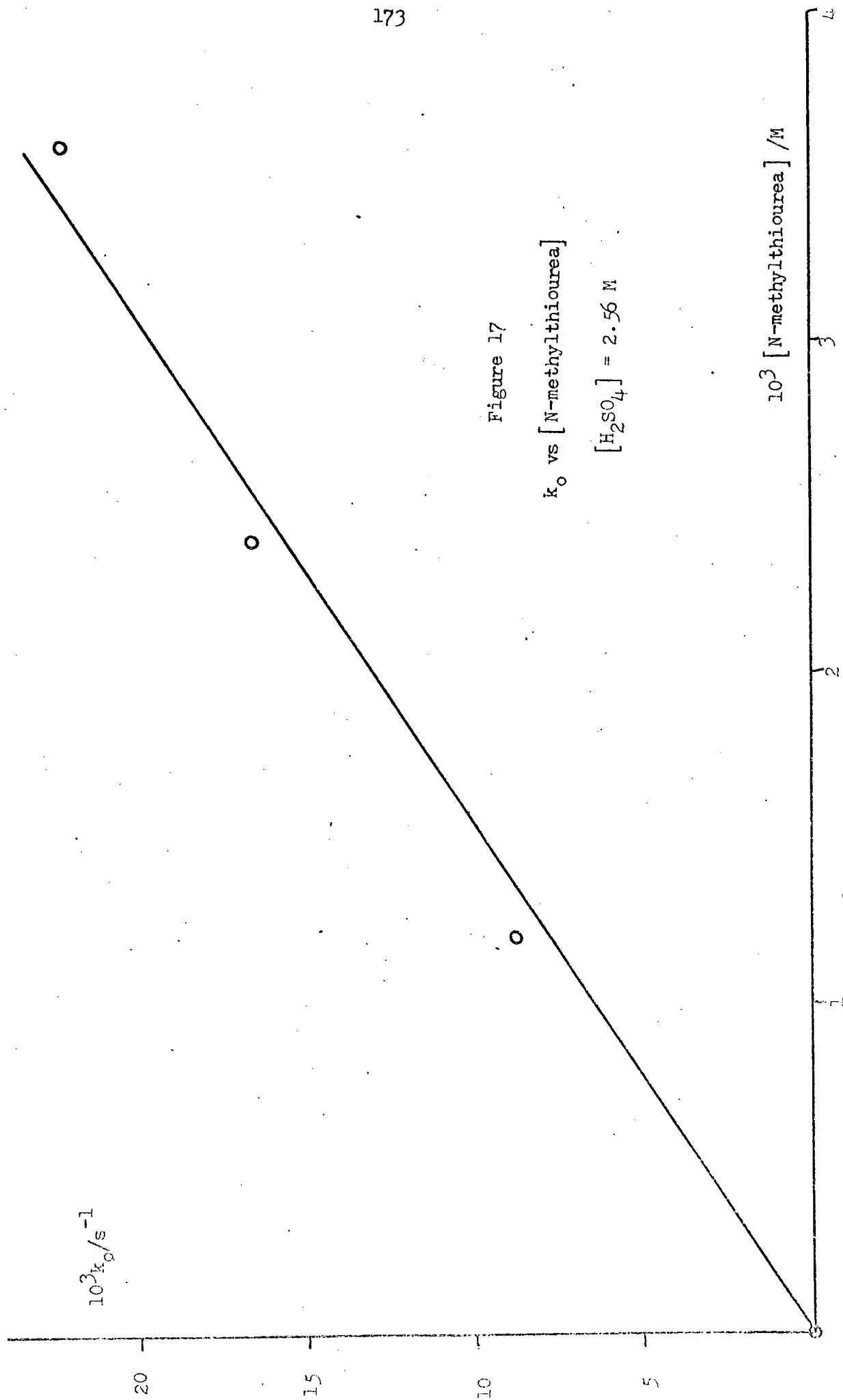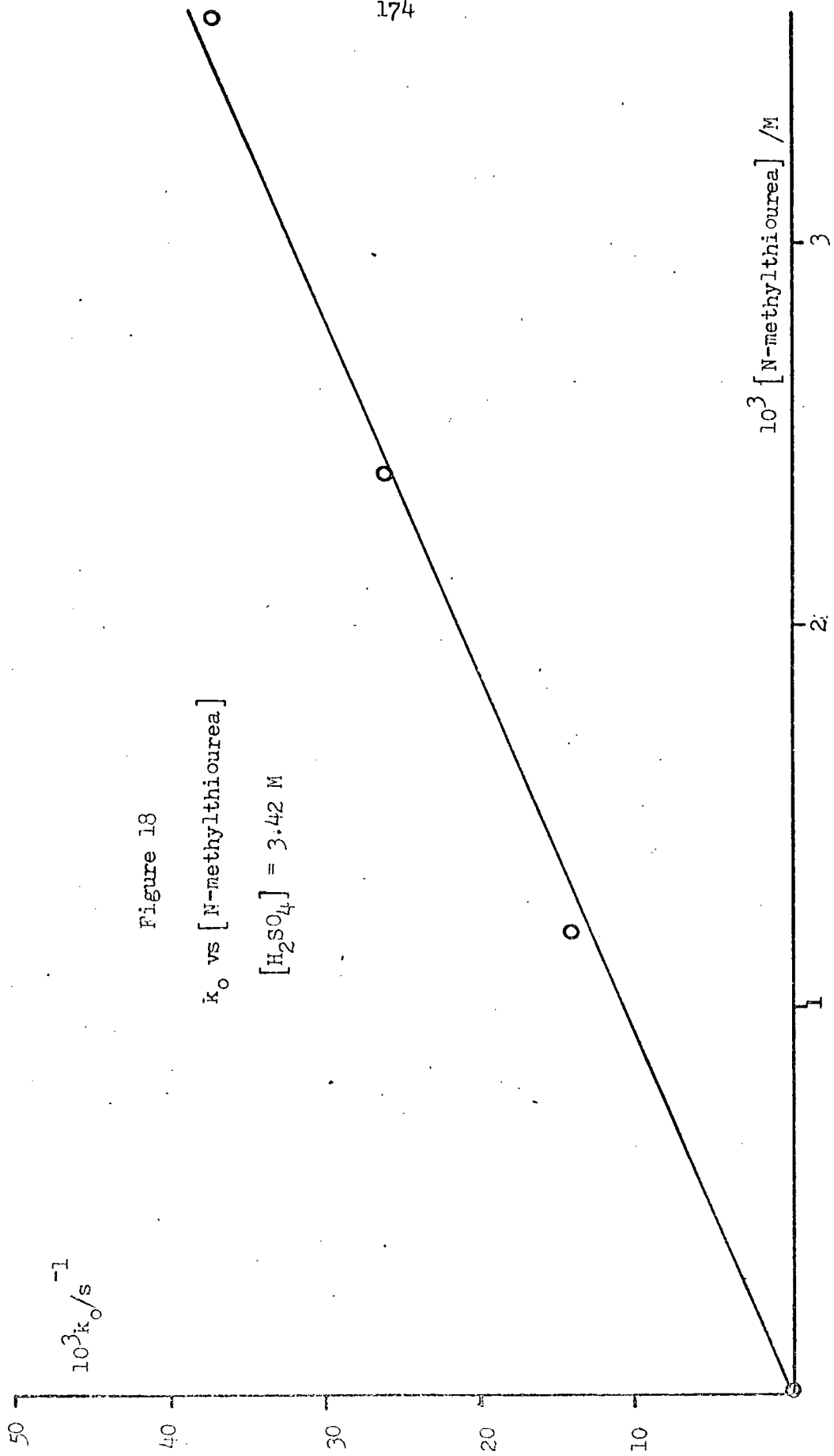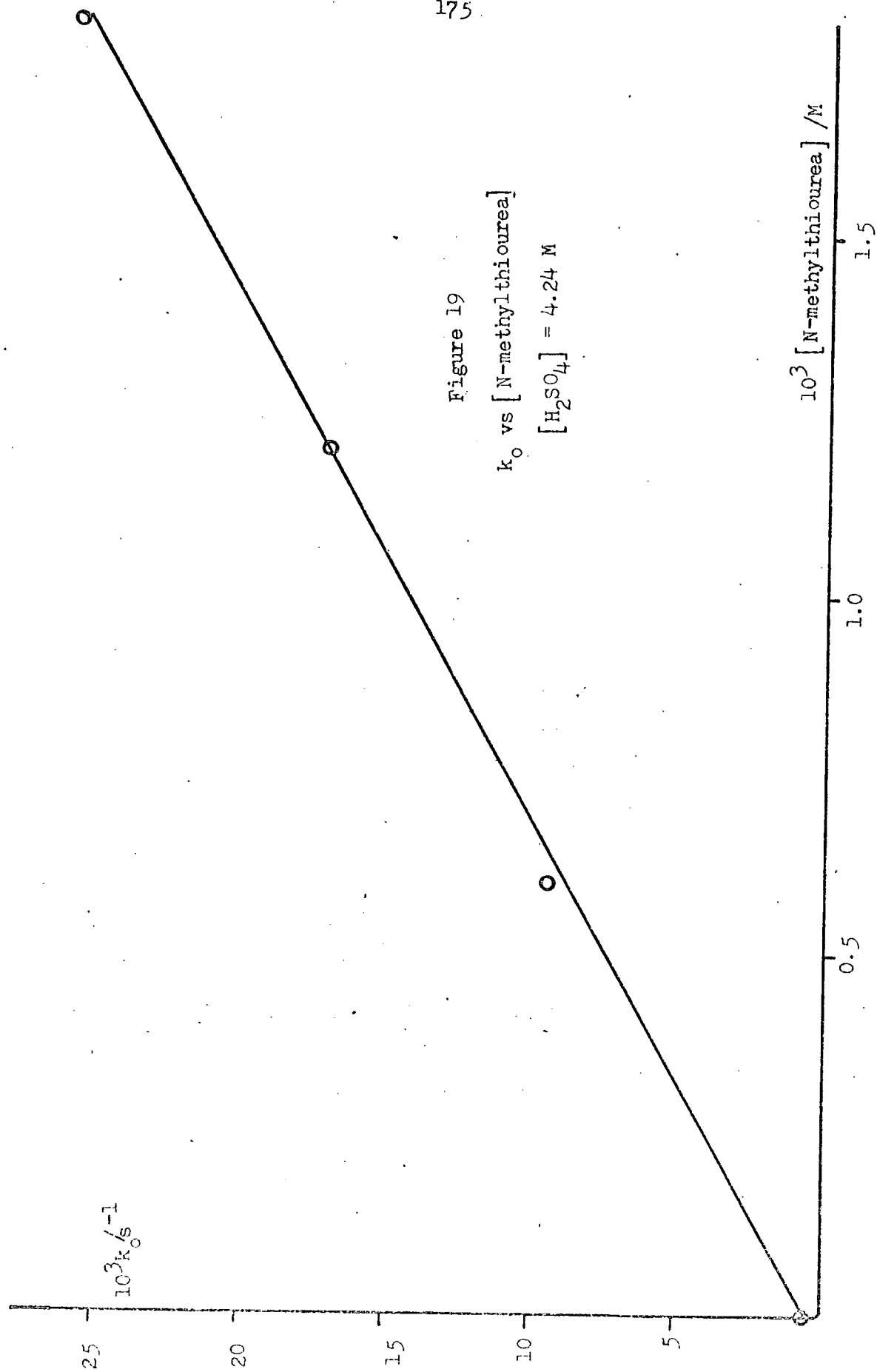
N,N'-dimethylthiourea

$[H_2SO_4]$/M	slope	corrected slope *
0.85	0.80	0.86
1.70	2.77	3.44
2.56	7.27	12.41
3.42	12.46	36.43
4.24	15.74	86.01

Trimethylthiourea

$[H_2SO_4]$/M	slope	corrected slope *
0.85	0.60	0.72
1.70	2.53	2.91
2.56	7.20	10.35
3.42	13.97	30.57
4.24	18.14	68.20

Tetramethylthiourea

$[H_2SO_4]$/M	slope	corrected slope *
0.85	0.70	0.80
1.70	2.12	3.19
2.56	4.14	10.27
3.42	4.64	23.32
4.24	4.56	47.01

*see text.

There is much evidence to suggest that the thioureas are subject to a significant degree of protonation at these acidities[135,136,137],

probably at the sulphur atom and since the reaction is presumed to proceed via the agency of the unprotonated species the values of the slopes have been corrected to allow for the protonation of the nucleophile. Values of pK_a based on the H_o scale are obtained for the thioureas from the work of Janssen[138] whence

$$K_b = \frac{1}{K_a}$$

$$\frac{[\text{Thiourea}]}{[\text{Thiourea}]_{TOTAL}} = \frac{1}{(1 + K_b h_o)}$$

and

$$\text{corrected slope} = \text{slope} \times \frac{[\text{Thiourea}]_{TOTAL}}{[\text{Thiourea}]}$$

It is clear that these corrected slopes represent the values of $k_1 Kh_o{}^X$. If we prepare plots of $\log k_1 Kh_o{}^X$ vs $\log h_o$ we may determine the value of x which is expected to lie close to unity. These plots, prepared for each of the thioureas are given as figures 20 to 24. Good straight lines are obtained in each case with the calculated values of x being as tabulated.

Substrate	Order in h_o
Thiourea	1.20
N-methylthiourea	1.16
N,N'-dimethylthiourea	1.12
Trimethylthiourea	1.11
Tetramethylthiourea	0.98

Clearly the Hammett acidity function, H_o, represents an adequate description of the acidity dependence of the nitrosamine protonation.

Figure 20

$\log k_1 K h_o^{x}$ vs $\log h_o$

for Thiourea

Figure 21

$\log k_1 K n_o^x$ vs $\log h_o$

for N-methylthiourea

Figure 22

$\log k_1 K h_o^x$ vs $\log h_o$

for N,N'-dimethylthiourea

Figure 23

$\log k_1 K h_o{}^x$ vs $\log h_o$

for Trimethylthiourea

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Figure 24

log $k_1 K h_o^x$ vs log h_o
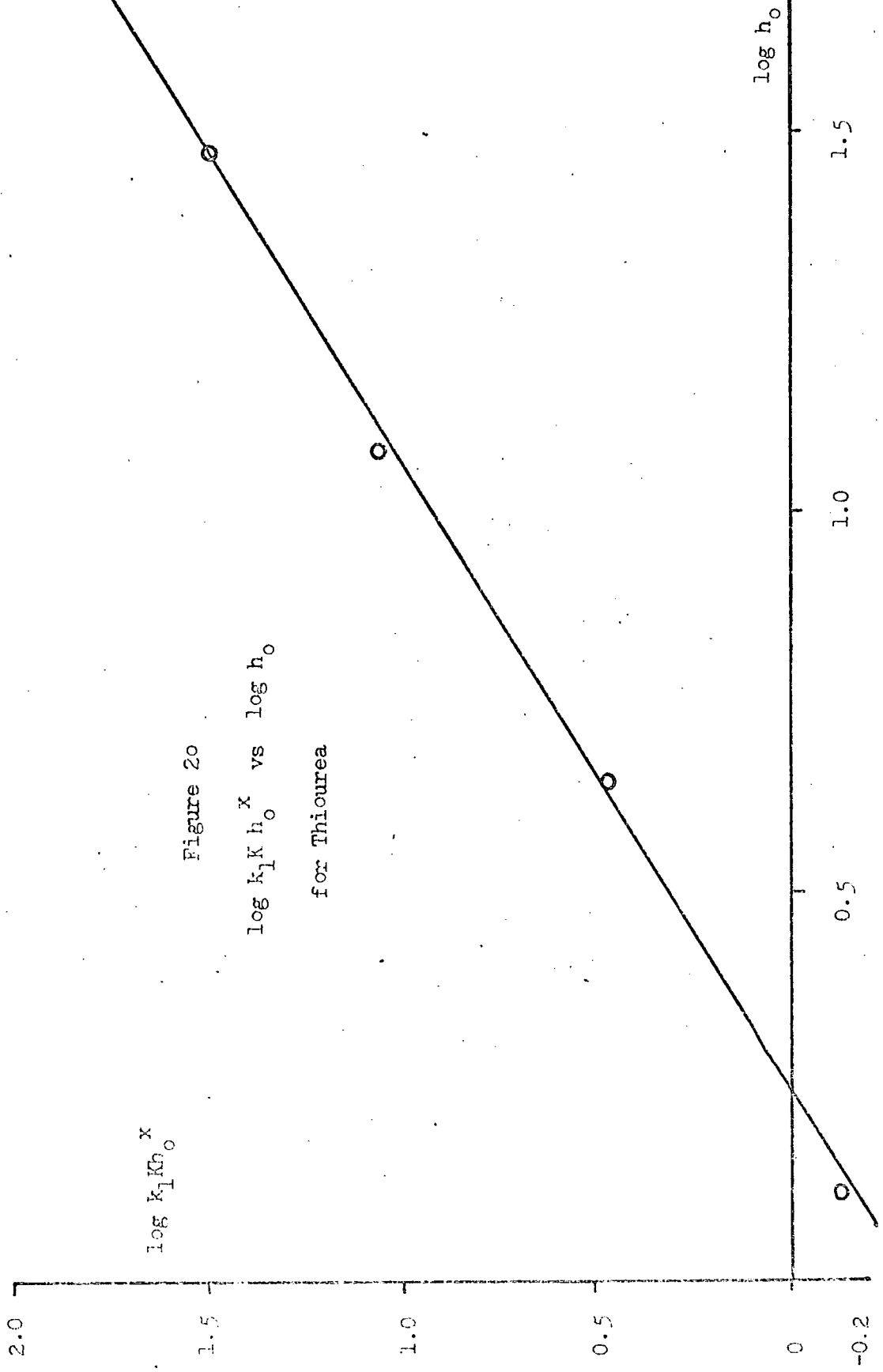
for Tetramethylthiourea
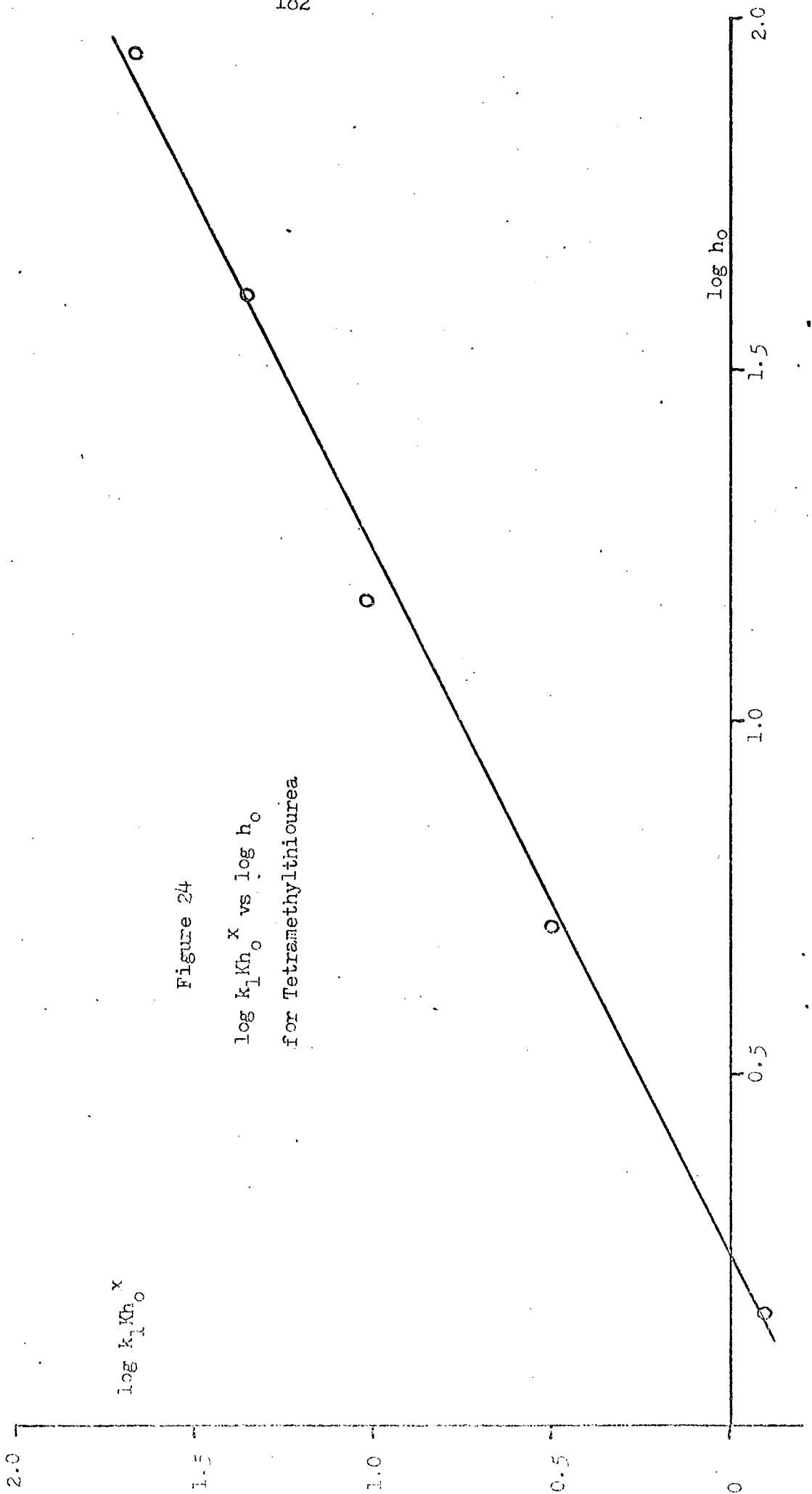
For all of the thioureas, other than tetramethylthiourea, the rate-dependence upon h_o is to the power of between 1.11 and 1.21. For the tetramethyl substituted compound the value is a little lower at 0.98. Obviously the value of x for a particular nitrosamine should be independent of the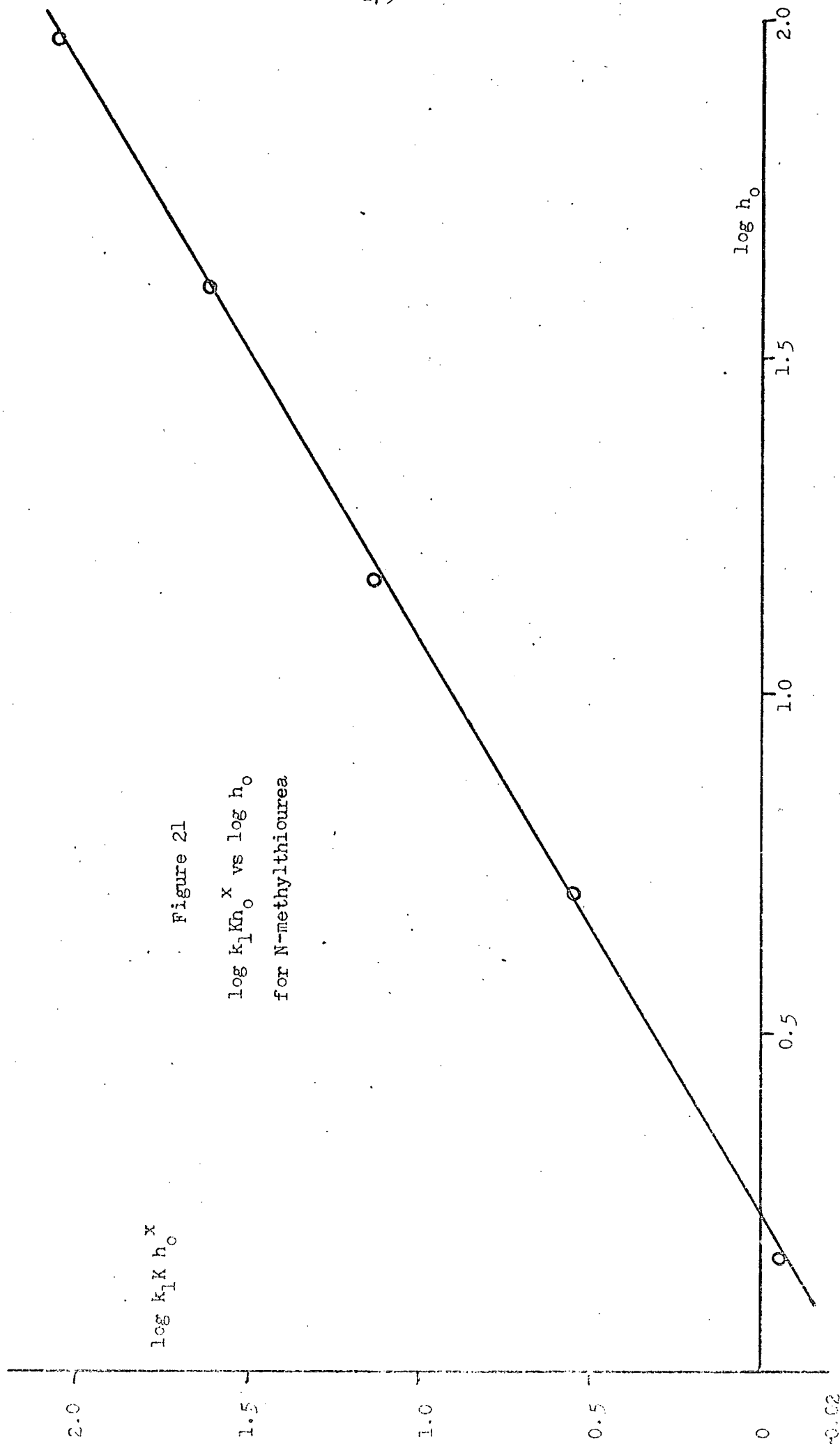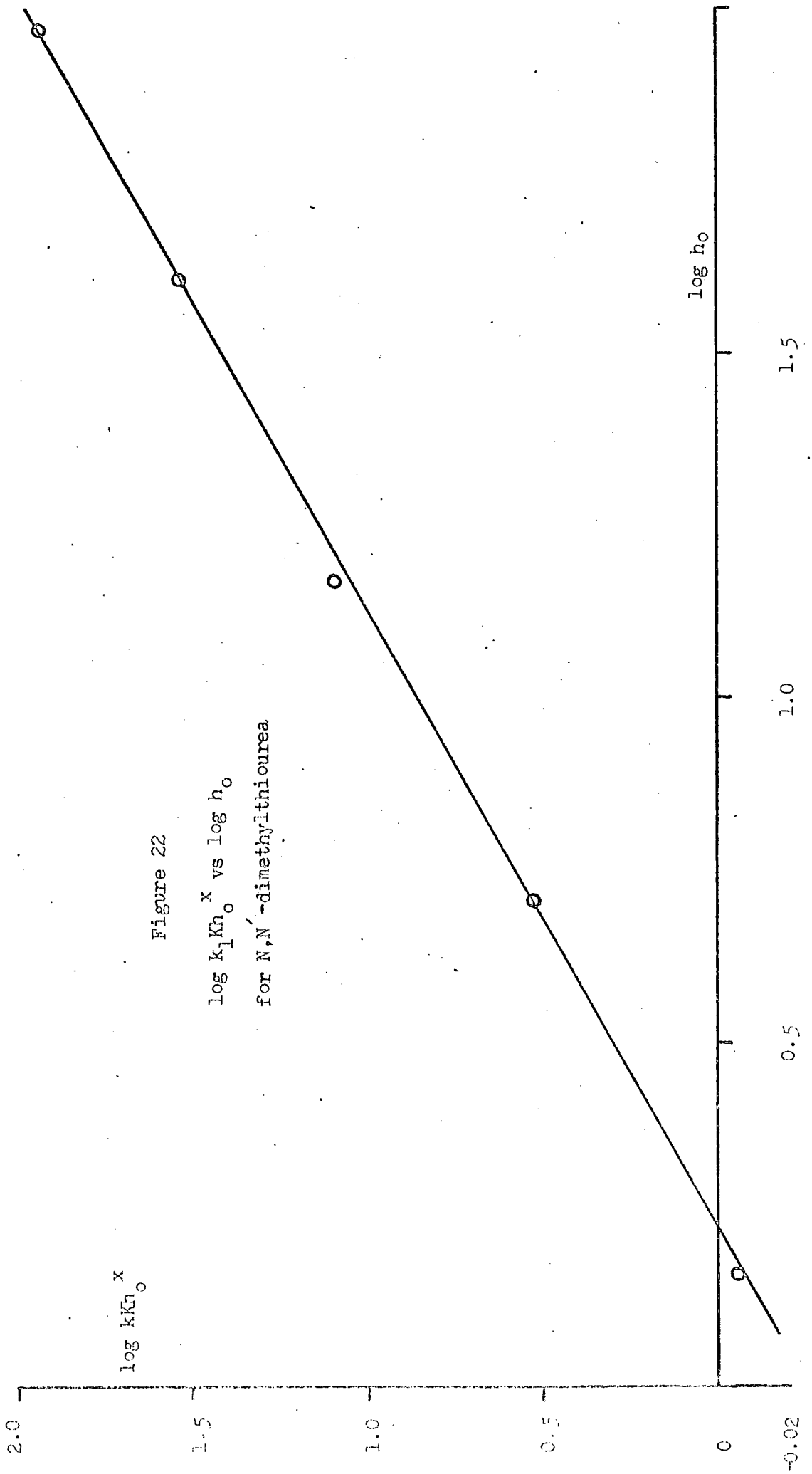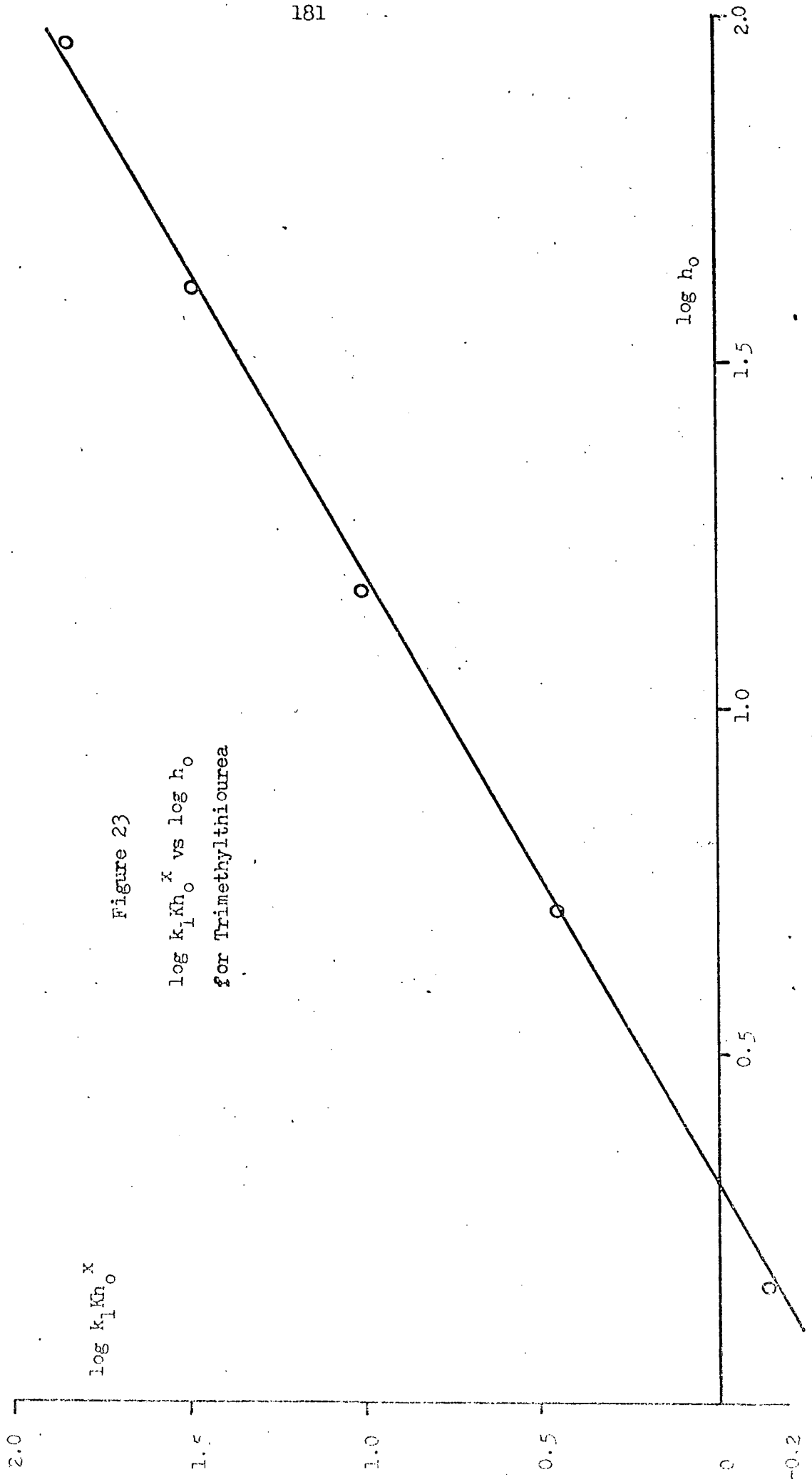 particular nucleophile employed and, in essence, the figures quoted above reflect a dependence upon h_o to the power of 1.00 $\pm$ 0.02.

With the data presently available to us we may calculate values of $k_1 K$ for the thioureas at each of the given acidities.

Thiourea

$[H_2SO_4]/M$	$k_1 K$
0.78	0.55
1.57	0.50
2.36	0.56
3.14	0.52
3.92	0.54

N-methylthiourea

$[H_2SO_4]/M$	$k_1 K$
0.85	0.55
1.70	0.55
2.56	0.58
3.42	0.57
4.24	0.53

N,N′-dimethylthiourea

$[H_2SO_4]/M$	k_1K
0.85	0.56
1.70	0.56
2.56	0.62
3.42	0.59
4.24	0.55

Trimethylthiourea

$[H_2SO_4]/M$	k_1K
0.85	0.47
1.70	0.48
2.56	0.53
3.42	0.52
4.24	0.45

Tetramethylthiourea

$[H_2SO_4]/M$	k_1K
0.85	0.54
1.70	0.60
2.56	0.65
3.42	0.50
4.24	0.57

The values of k_1K reflect the expected independence of acidity so that we may calculate the following mean values.

| Substrate	Mean Value of k_1K
Thiourea	0.53
N-methylthiourea	0.57
N,N'-dimethylthiourea	0.58
Trimethylthiourea	0.49
Tetramethylthiourea	0.57

The striking feature of these values is their similarity. We know from our earlier discussion of halide catalysis, (Section 1.7), that the reaction is very sensitive towards the reactivity of the nucleophile and indeed the Pearson plot of log k_1K vs n possesses a slope[125] of 1.41: clearly the similarity of the values of k_1K depicted above demonstrates that the thioureas possess almost identical reactivities. Stedman et al.[69,73] report a similar observation for the thioureas in their reaction with free nitrous acid and it is tempting to apply their reasoning in the present case. Arguing on the basis of the similarity which exists between the rate constants obtained for the thiourea/nitrous acid reaction and those obtained for the diazotisation of aniline derivatives[62,119] they believe that the kinetics of both reactions are governed by the encroachment of a diffusion-controlled limit. Under such conditions as these we would not expect a great discrimination between the various thioureas. It would appear however that this rationale does not apply in the present case. If we assume that k_1 does approach the diffusion-controlled limit then we must assign a value of circa 1×10^{10} l mol^{-1} s^{-1}. It therefore follows that K must have a value of around 5×10^{-11} which corresponds to a pK_a value of around -10 for the nitrosamine. Whilst this value has never been measured directly it has been estimated[64] as being of the order of -2. Further, when we consider that the Pearson plot of log k_1K vs n (a) shows no indication of levelling off at n=7.3 for thiourea and (b) depicts a value of 0.63 for

the iodide ion, (n=7.4), then we realise that we can not apply Stedman's rationale here.

It would appear that an explanation must proceed along the following lines. Let us assume that, in the transition state for the nucleophilic attack, little if any of the positive charge is delocalised to the thioureas' amino-nitrogen atoms. Thus, if we represent the formation of the transition state as below, canonical IV makes no significant contribution.

I

II

III

IV
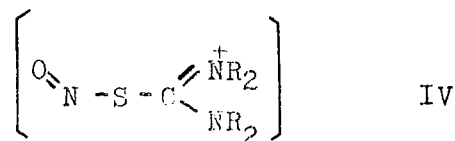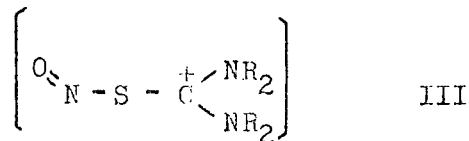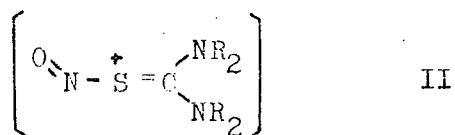
The important contributions thus come from the structures labelled I, II and III where the positive charge is located on carbon and sulphur atoms respectively. In such circumstances an increasing degree of N-methyl substitution is not expected to stabilise the transition state to any great extent and the thioureas will possess similar reactivities.
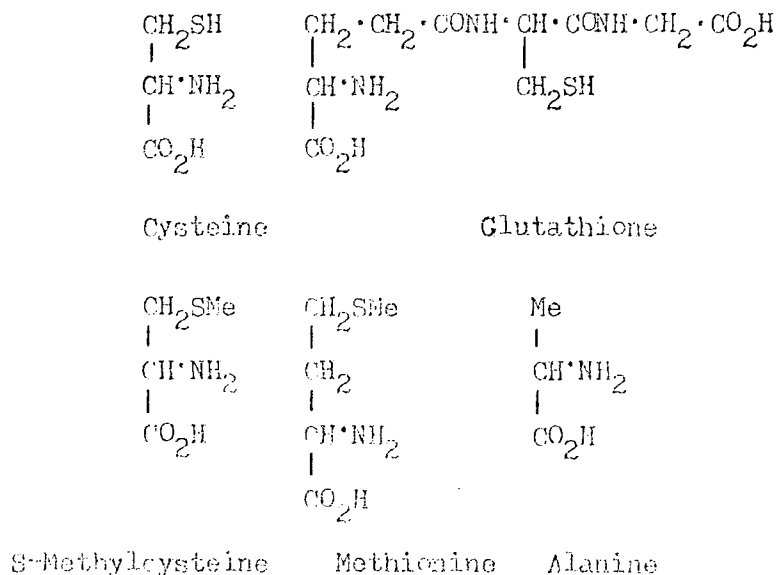
6.3 The Reaction of N-methyl-N-nitrosoaniline with Cysteine, Glutathione, S-methylcysteine and Methionine.

6.3.1 An Introduction

In the preceeding section we discussed the remarkable reactivity which the thioureas exhibit in their reactions with N-nitrosamines. A valid question now might be; "is this reactivity common to all neutral sulphur sites?" Certainly the question is an interesting one especially if we turn our attention towards the sulphur sites of such biologically important species as the amino acids. The possibility of reaction between these species and the N-nitrosamines would necessitate a fairly radical revision of the schemes proposed for the behaviour of the N-nitrosamines in the human body. At first sight it appears that this possibility is indeed a reality since the reaction of N-methyl-N-nitrosotoluene-p-sulphonamide with cysteine has already been reported[71].

6.3.2 The Variation of k_o with [Naturally Occuring Sulphur Nucleophiles]

In an attempt to discover whether the neutral sulphur sites of biologically important substances do possess an appreciable reactivity in this context the following five substances were chosen for study.

$$CH_2SH$$
$$|$$
$$CH \cdot NH_2$$
$$|$$
$$CO_2H$$

$$CH_2 \cdot CH_2 \cdot CONH \cdot CH \cdot CONH \cdot CH_2 \cdot CO_2H$$
$$|\qquad\qquad\qquad\quad |$$
$$CH \cdot NH_2 \qquad\qquad CH_2SH$$
$$|$$
$$CO_2H$$

Cysteine   Glutathione

$$CH_2SMe$$
$$|$$
$$CH \cdot NH_2$$
$$|$$
$$CO_2H$$

$$CH_2SMe$$
$$|$$
$$CH_2$$
$$|$$
$$CH \cdot NH_2$$
$$|$$
$$CO_2H$$

$$Me$$
$$|$$
$$CH \cdot NH_2$$
$$|$$
$$CO_2H$$

S-Methylcysteine   Methionine   Alanine

The inclusion of alanine which does not possess a sulphur centre is important in that it provides us with a valuable control. As with the thioureas all kinetic experiments were carried out in aqueous solution in the presence of a high concentration of hydrazine sulphate so that the complication of the denitrosation reaction's reversibility is removed. Under the conditions employed the reaction was observed to follow first-order kinetics so that we may define an observed first-order rate constant, k_o, such that,

$$\frac{-d[NMNA]}{dt} = k_o[NMNA]$$

where $\quad k_o = k_1 Kh_o^x [\text{nucleophile}]$

Values of k_o as a function of the concentration of each of the nucleophiles were recorded as follows. The data are presented graphically as figures 25 to 28.

$[H_2SO_4] = 4.48$ M

10^3 [Cysteine] /M	$10^4 k_o/s^{-1}$
0	8.41
2.05	9.63
6.14	9.89
10.23	10.52

10^3 [Glutathione] /M	$10^4 k_o/s^{-1}$
0	8.41
1.99	8.40
6.00	9.80
9.92	10.85

Figure 25

k_o vs [Cysteine]

Figure 26

k_o vs [Glutathione]

Figure 27

k_o vs [S-methylcysteine]

Figure 28

k_o vs [Methionine]

x-axis: 10^3 [Methionine] /M

y-axis: $10^4 k_o / s^{-1}$
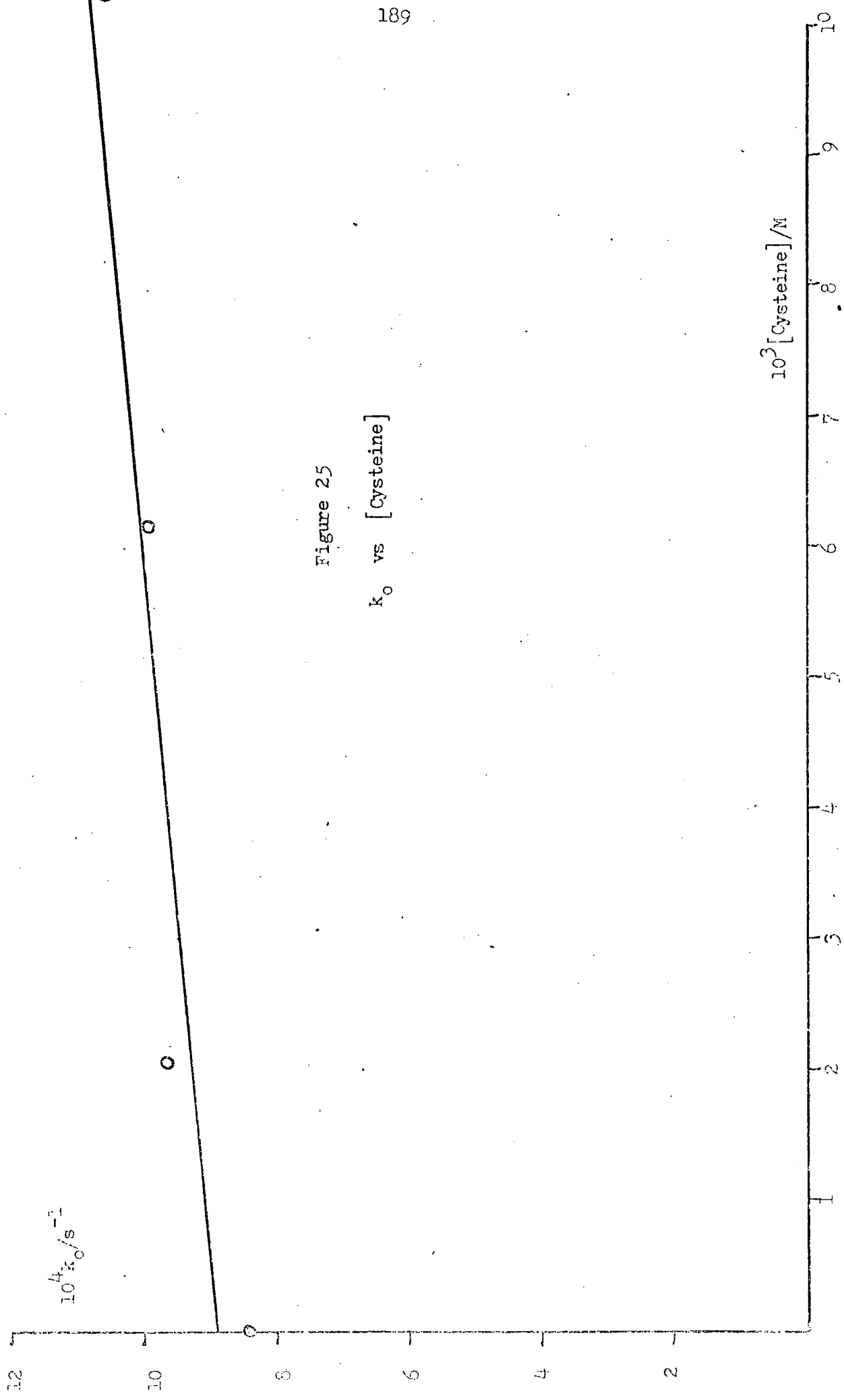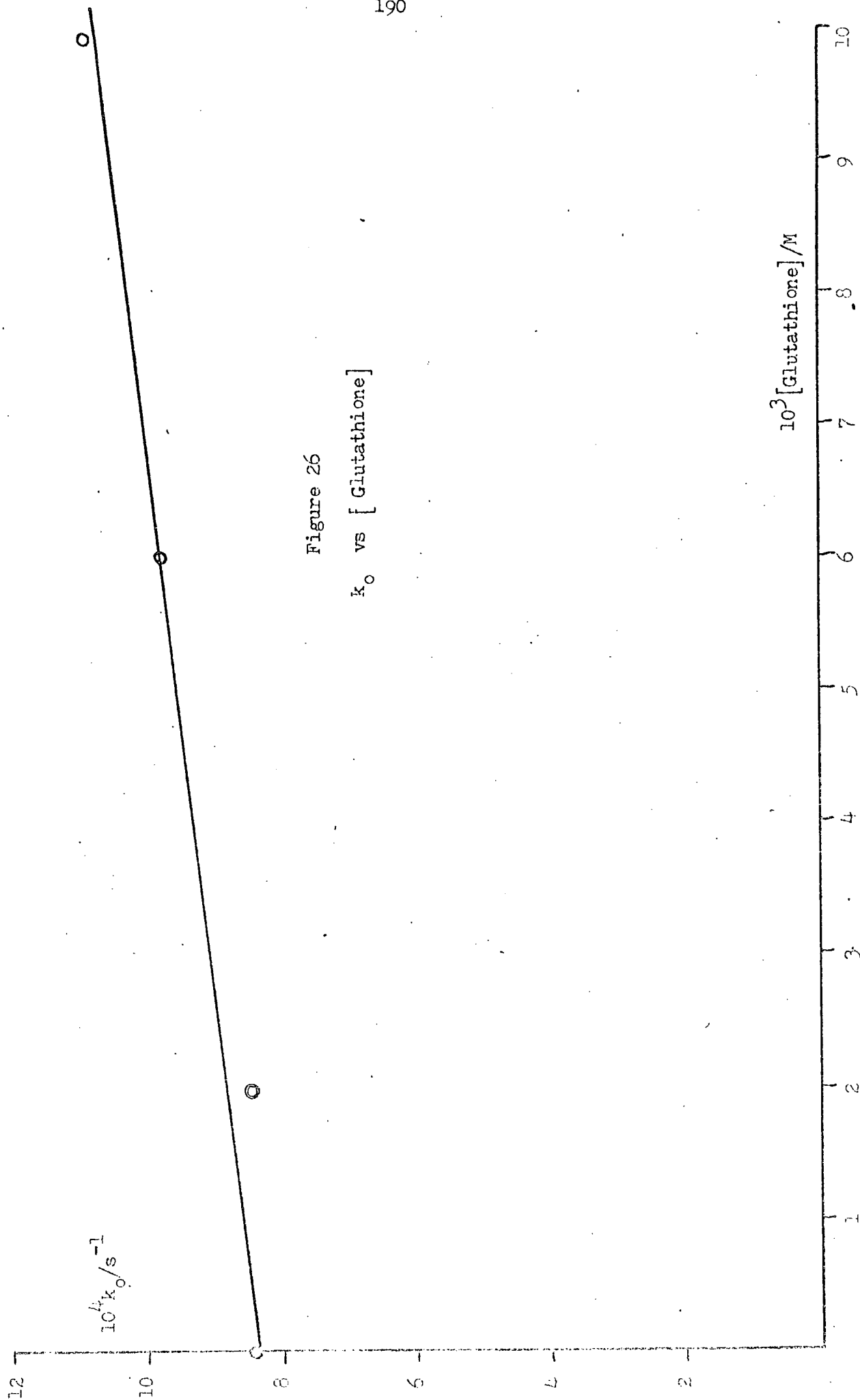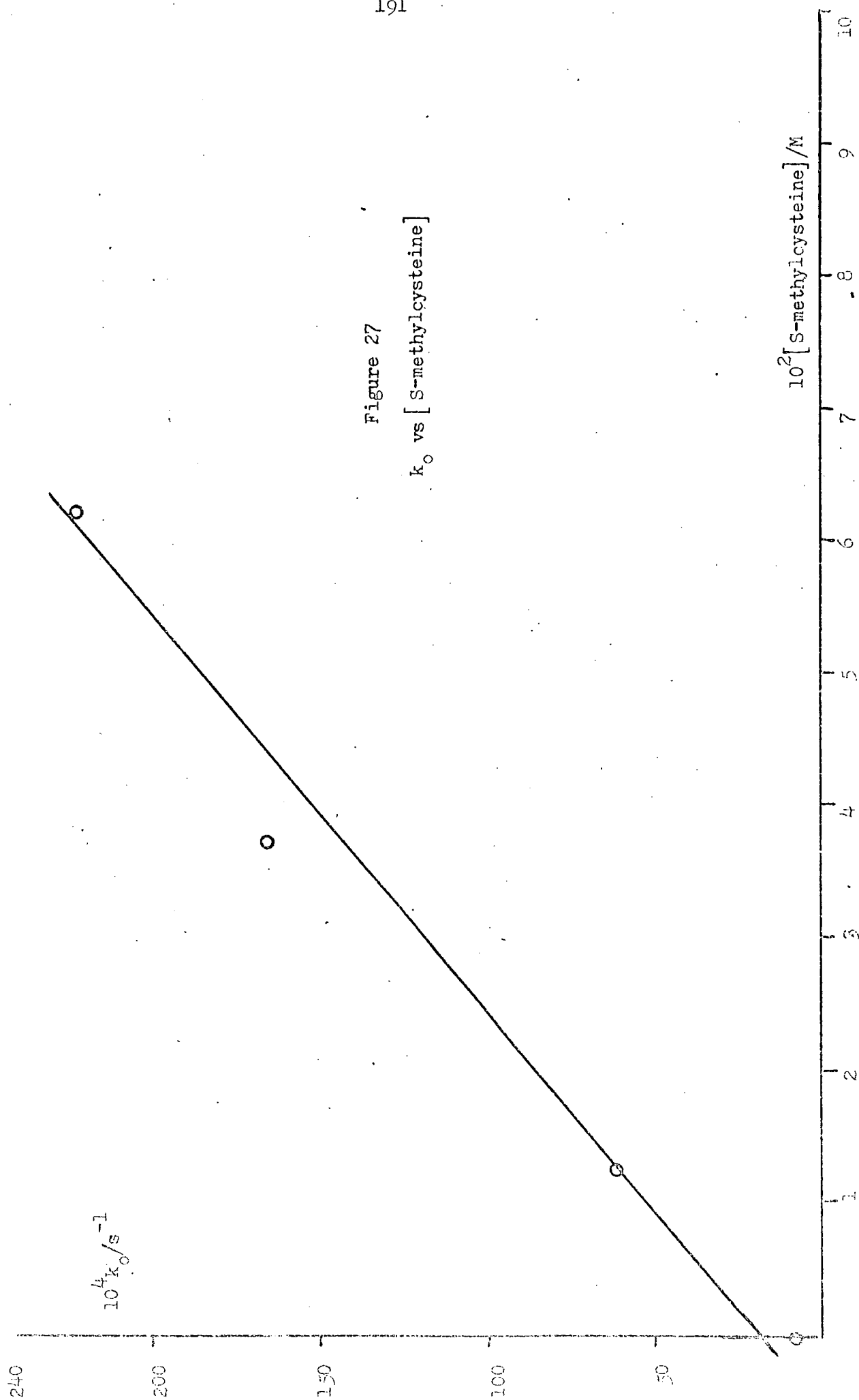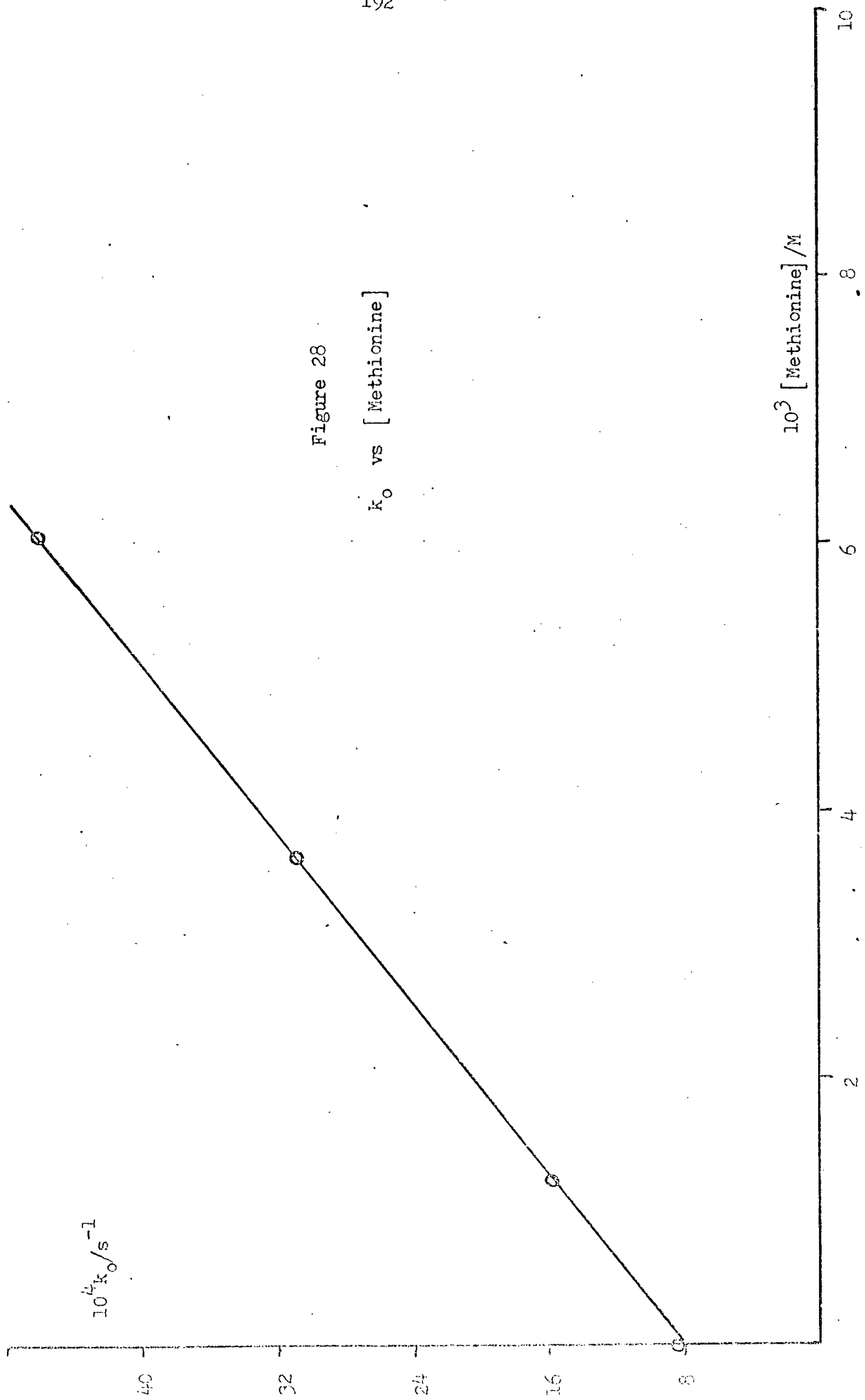
10^2 [S-methylcysteine]/M	$10^4 k_o / s^{-1}$
0	8.41
1.24	62.80
3.73	153.80
6.22	221.90

10^3 [Methionine]/M	$10^4 k_o / s^{-1}$
0	8.41
1.21	15.83
3.64	31.21
6.06	46.14

10^3 [Alanine]/M	$10^4 k_o / s^{-1}$
0	8.41
8.69	8.26
26.11	8.48
43.52	8.90

It is clear that for cysteine and glutathione (which contain the $-CH_2SH$ group) reactivity towards the nitrosamine is very small. Nevertheless the significant increases in k_o observed with increasing concentrations of both substrates would seem to indicate the existence of a direct reaction between the nitrosamine and the nucleophiles. It should be noted that the presence of the nitrite trap precludes the operation of an alternative explanation which would involve a solvent-promoted denitrosation coupled with the subsequent nitrosation of the sulphur nucleophile by free nitrous acid. Since alanine reveals itself to be singularly unreactive in the present context the possibility of a nitrosation at the $-NH_2$ (or $-NH_3^+$) site is also excluded. Clearly we are dealing here with a direct nitrosation

of the sulphur site by NMNA to yield an $ON-\overset{+}{S}\!\overset{\diagup}{\diagdown}$ intermediate which is irreversibly removed by reaction with the nitrite trap.

If we assume that

$$k_o = k_1 Kh_o^{1.11} \left[\text{nucleophile} \right]$$

where 1.11 represents the mean of the values of x calculated for the thioureas then we may proceed to calculate $k_1 K$ for each of the sulphur nucleophiles. Values for $k_1 Kh_o^{1.11}$ are of course provided by the slopes of the k_o vs $\left[\text{nucleophile} \right]$ plots whence we arrive at the following table.

Nucleophile	$k_1 K$
Cysteine	9×10^{-5}
Glutathione	1.6×10^{-4}
S-methylcysteine	1.7×10^{-3}
Methionine	3.2×10^{-3}

If this table is viewed in conjunction with the data provided by Williams which are given below then we produce a fruitful summary.

Nucleophile	$k_1 K$
Cl^-	4×10^{-5}
Br^-	2.2×10^{-3}
I^-	0.63

Clearly the $-CH_2SH$ group (which is present in both cysteine and glutathione) possesses a reactivity towards NMNA which is comparable with that of the chloride ion. This low reactivity may be considerably increased by the inclusion of an S-methyl group so that the $-CH_2SCH_3$ group (present in both S-methylcysteine and methionine) exhibits a reactivity close to that of the bromide ion.

6.4 Conclusions

In the present sections we have considered evidence which has high-
lighted the nucleophilic reactivity of neutral sulphur sites. It is
clear that the alkyl-thioureas parallel thiourea itself in reactity with
NMNA to yield, initially, the S-nitroso adduct, their reactivity being
comparable with that of the iodide ion. In establishing the existence
of a reaction between NMNA and a series of sulphur-containing amino acids
a question is raised which goes beyond the scope of the present work:
"How important is the sulphur pathway in directing the metabolic
processes of N-nitrosamines within the human body?"

SECTION   SEVEN

THE INTERACTION OF N-METHYL-N-NITROSOANILINE

WITH METAL IONS IN AQUEOUS

ACIDIC SOLUTION.

7.1 An Introduction

This section contains the results of a preliminary investigation into the nature of nitrosoamine/metal interactions. The results are by no means conclusive, indeed they raise more questions than they answer, but I believe that there is evidence to support the existence of an interaction between NMNA and certain transition metal ions. The data are given here in the hope that they may prove useful to others who venture into this most interesting aspect of nitrosoamine chemistry.

Oxidation of the ferrous ion by nitrous acid in acidic solution has been widely reported[139] and is known to be due to simultaneous oxidation by nitrogen dioxide (from the decomposition of the nitrous acid) and the nitrosonium ion (or nitrous acidium ion). The overall reaction which has the stoichiometry,

$$Fe^{2+} + HNO_2 + H^+ \rightleftharpoons Fe^{3+} + NO + H_2O$$
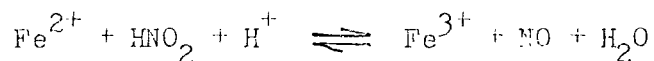
is governed by an equilibrium constant K where, at $25^\circ C$,

$$K = \frac{[Fe^{3+}] \, P_{NO}}{[Fe^{2+}][HNO_2][H^+]} = 9.2 \times 10^3 \text{ atm mol}^{-2} \, l^{-2}$$

From the investigations of Abel et al.[139] it is clear that the rate of appearance of the ferric ion may be represented as follows

$$\frac{d[Fe^{3+}]}{dt} = \left[k_1 + k_2[H^+] + \frac{k_3[HNO_2]}{P_{NO}} \right] [Fe^{2+}][HNO_2]$$

where

$$k_1 = 7.83 \times 10^{-3} \; l \; mol^{-1} \; s^{-1}$$

$$k_2 = 2.27 \times 10^{-1} \; l^2 \; mol^{-2} \; s^{-1}$$

$$k_3 = 4.00 \qquad l^2 \; atm \; mol^{-2} \; s^{-1}$$

On the basis of these results the reaction scheme depicted below has been proposed for reaction in a nitrogen atmosphere.

$$2HNO_2 \rightleftharpoons NO_2 + NO + H_2O$$

$$Fe^{2+} + NO_2 \longrightarrow Fe^{3+} + NO_2^{-}$$

$$HNO_2 + H^+ \longrightarrow H_2O + NO^+$$

$$Fe^{2+} + NO^+ \longrightarrow Fe^{3+} + NO$$
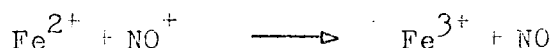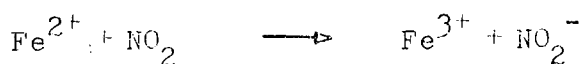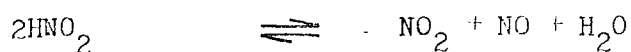
In the light of the established reactivity between the ferrous ion and nitrous acid it was decided to investigate the use of Fe^{2+} as a nitrite scavenger or trap in the nucleophilic denitrosation of NMNA. If the ferrous ion was to prove successful in this context then might it not be that this reactivity could be extended to cover other metal ions of low oxidation state.

7.2 The Interaction of the Ferrous Ion and N-methyl-N-nitrosoaniline

From the experience of the previous sections we might prepare the following reaction scheme.

REARRANGEMENT

$$NOY + Fe^{2+} \xrightarrow[k_3]{} Products$$
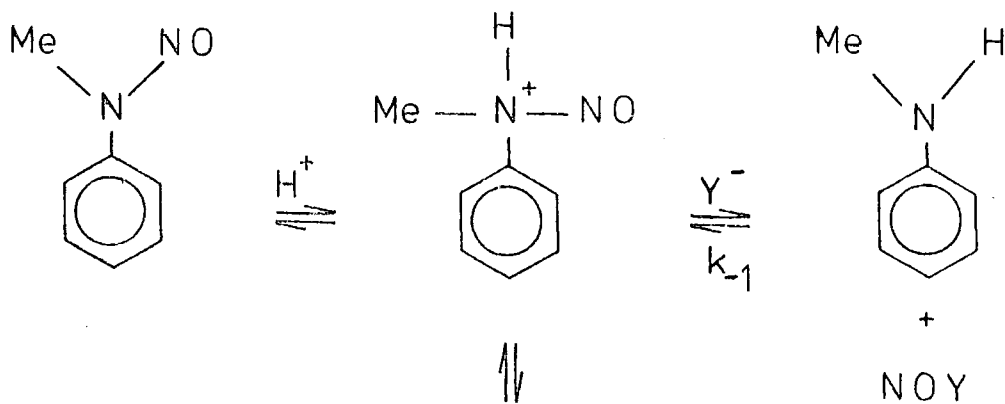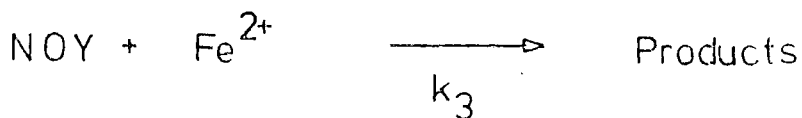
If this scheme is indeed operative then it is clear that at the end of the reaction the initial NMNA will be present in the form of no more than four components: (a) unchanged NMNA (or NMNAH$^+$),   (b) N-methylaniline,   (c) the rearrangement product, p-nitrosoaniline and possibly   (d) the "yellow product" described in Section 1.6. Preliminary experiments indicated that it might be possible to demonstrate nitrite scavenging activity on the part of Fe^{2+} if it were present in high concentrations relative to the N-Nitrosamine. Consequently a series of qualitative investigations were carried out under these conditions in order that any reaction outside of the proposed scheme might be detected. It must be said that these investigations met with mixed success.

The initial approach was to prepare an aqueous reaction mixture containing, sulphuric acid, NMNA and Fe^{2+} in the form of its ammonium sulphate and to extract the organic products so that they

might be subject to the scrutiny of G.L.C. and mass spectrometry.
This approach proved unsuccessful probably for the reasons discussed
in Section 8. An attempted investigation using T.L.C. was a little
more successful in yielding the results depicted in Figures 29 to
32.

If we assume that spots with R_f values which differ by less than
0.02 units are derived from the same component then we may summarise
the results as below.

COMPONENT	R_f value	IDENTITY
0 *	0	NMA oxidation product
1	0.07	ditto
2 *	0.20	?
3	0.30	?
4 *	0.44	?
5	0.53	?
6	0.73	NMA
7	0.85	NMA
8	0.93	NMA/NMNA or R/F

* observed only under one method of detection

Even if we discount those spots which have only been observed
under one method of detection it is clear that the chromatogram
exhibits at least 5 spots. Of the 8 spots observed the three of
highest R_f value (R_f = 0.73, 0.85 and 0.93 respectively) correspond
to combinations of NMA, NMNA and the rearrangement product and
therefore represent products predicted within the framework of the
proposed reaction scheme. The spots at R_f=0 and R_f=0.07 respectively

FIGURE 29   T.L.C. FOR SECTION 7 VIEWED IN DAYLIGHT

NMA Stock	NMA Fresh	NMA Stock	Rearrangement Product	Phenyl-hydrazine	Sample	Component Identities
purple						
yellow		yellow	yellow / yellow	yell/brown / yell/brown	NONE	?
yellow	yellow	yellow				

FIGURE 30   T.L.C. FOR SECTION 7 VIEWED UNDER SHORT-WAVE U.V. LIGHT

FIGURE 31   T.L.C. FOR SECTION 7 VIEWED UNDER LONG-WAVE U.V. LIGHT

FIGURE 32   T.L.C. FOR SECTION 7 VIEWED AFTER EXPOSURE TO IODINE VAPOUR

are probably due to NMA oxidation products since they are shown by a stock sample of NMA but not by one that has been purified before use. The other spots must be the cause for some concern.

It has not been possible to obtain a T.L.C. of one of our expected products, the yellow product of Section 1.6 and so it may well be that this together with its oxidation products accounts for some if not all of the unassigned spots. There exists also the possibility of direct reaction between the ferrous ion and the nitrosamine to give the corresponding hydrazine; in this case the N-methyl-N-phenyl compound. Although a sample of this was not available at the time of the study a sample of phenylhydrazine did yield spots of a similar R_f value to the unassigned spots on the chromatogram. Although these latter reactions must remain a possibility we have obtained evidence concerning the percentage conversion of NMNA to NMA which suggests that they are of little significance. It should be noted, however, that the present work does not shed any light upon the extent of any direct reaction between Fe^{2+} and NMNA which would yield NMA as its principle product. In the absence of definite evidence to the contrary we will assume that the reaction scheme depicted above is valid.

In the past[11] the ability of a substance, X, to act as a nitrite trap in the denitrosation of NMNA has been tested by carrying out a series of kinetic runs at increasing $[X]$. The first-order rate constant k_o obtained for each run is then plotted against $[X]$. If X is acting as a nitrite trap the plot of k_o vs $[X]$ will behave characteristically by rising to a limiting value whereafter the reaction is zero-order in $[X]$. Observation of k_o as a function of added NMA concentrations leads to a value for $k_{-1}:k_3$ which is seen

as a measure of the efficiency of a material X in removing the NO species. Whilst this approach has proved successful in the study of the more conventional nitrite traps such as urea and hydrazine sulphate it may not be employed for the ferrous ion. Preliminary experiments demonstrated the production of a large U.V. absorption peak at $\sim$ 300 nm which has been attributed to the ferric ion formed during the reaction. The extinction coefficient of this peak is such as to preclude attempts to monitor the disappearance of the NMNA peak at 270 nm; consequently values of k_o are unobtainable.

In an attempt to determine whether Fe^{2+} does act as a nitrite trap the following approach was employed. In the absence of a nitrite trap the denitrosation system described above moves towards an equilibrium. NMNA, NMA, the rearrangement product and an unidentified yellow product are observable components of the equilibrium mixture, their relative concentrations being dependent on the nature of the nucleophile Y employed in the reaction. When an excess concentration of a nitrite trap such as sodium azide or hydrazine sulphate is included in the reaction mixture the species NOY is rapidly removed so that the equilibrium depicted below proceeds to the right with the back reaction being suppressed.

$$NMNAH^+ \; + \; Y \; \rightleftharpoons \; NMA \; + \; NOY$$
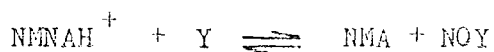
Clearly the addition of a nitrite trap to the reaction mixture should have a notable action in increasing the concentration of NMA present at the end of the reaction. A consequent decrease in the concentration of NMNA, the rearrangement product and the yellow component should also be observed. If a reaction mixture containing Fe^{2+} is allowed

to go to completion a comparison of the NMA, NMNA, Rearrangement Product and Yellow Product concentrations in the final mixture with those expected on the basis of the above considerations should indicate whether the ferrous ion is acting as a nitrite trap. In the present work, carried out according to the proceedure detailed in Section 8, the final equilibrium mixtures were neutralised so that U.V. absorption spectra such as those depicted in Figures 33 to 35 could be recorded. The comparison of the final concentrations was then carried out on the basis of the collected U.V. spectra. The results are summarised below. Note that in certain cases where the absorption peak at 237 nm is observed to be due to both NMNA and NMA in undetermined proportions it has been possible to provide only a maximum value for the final concentration of NMA.

Using water as the nucleophile the following data were obtained.

$$[H_2SO_4] \; = \; 3.512 \; M \qquad\qquad [NMNA]_o \; = \; 5.012 \times 10^{-4} \; M$$

In Air

Nitrite Trap	Final Concentration of NMA/M
None	$\leqslant 2.3 \times 10^{-4}$
Hydrazine Sulphate (4×10^{-3} M)	4.7×10^{-4}
Ferrous Ammonium Sulphate (4×10^{-3} M)	$\leqslant 2.6 \times 10^{-4}$
Ferrous Ammonium Sulphate (4×10^{-2} M)	4.3×10^{-4}

Figure 33

U.V. absorption spectrum for

Section 7.2 with no nitrite

trap present.

Figure 34

U.V. absorption spectrum for
Section 7.2 with hydrazine
sulphate as the nitrite trap
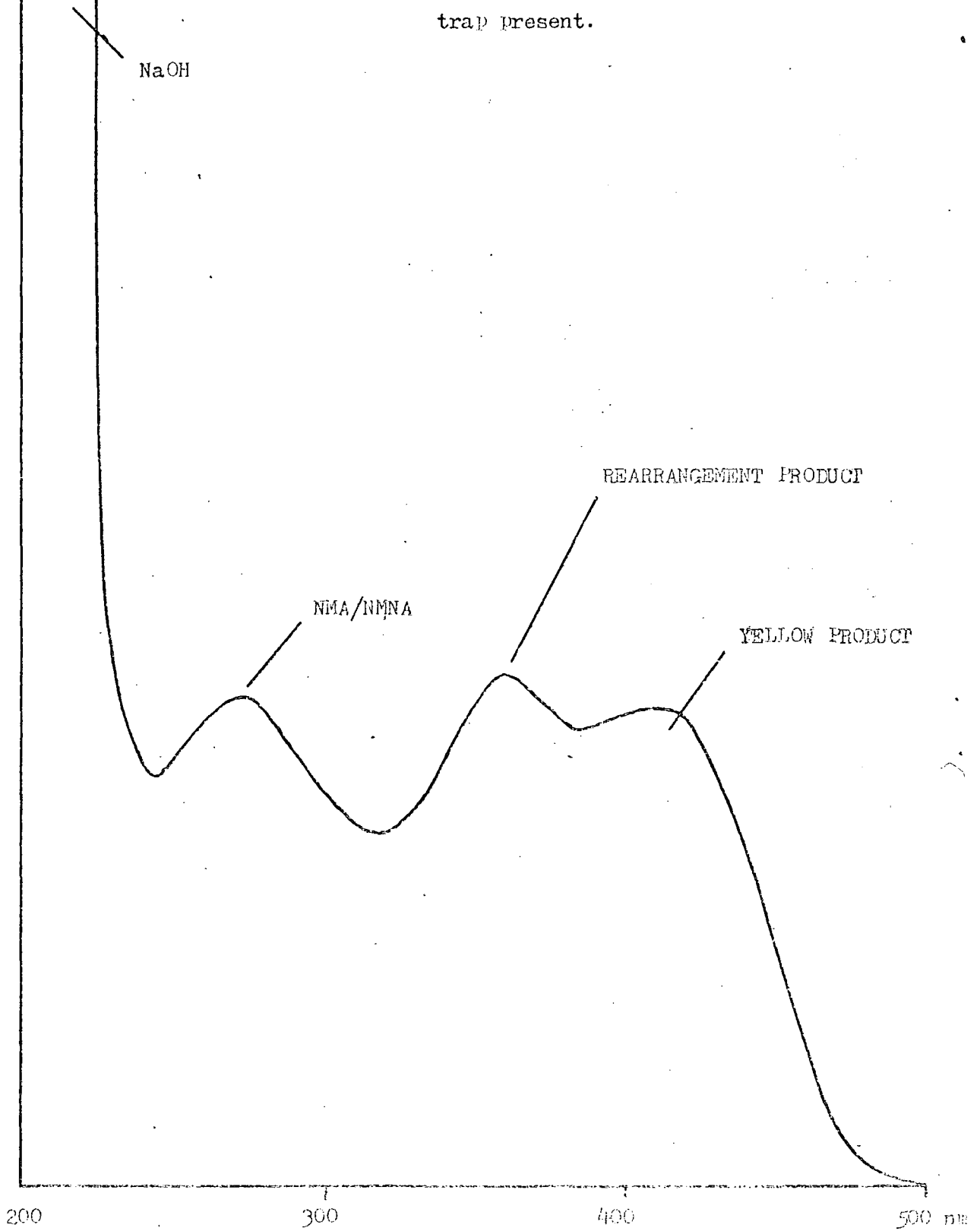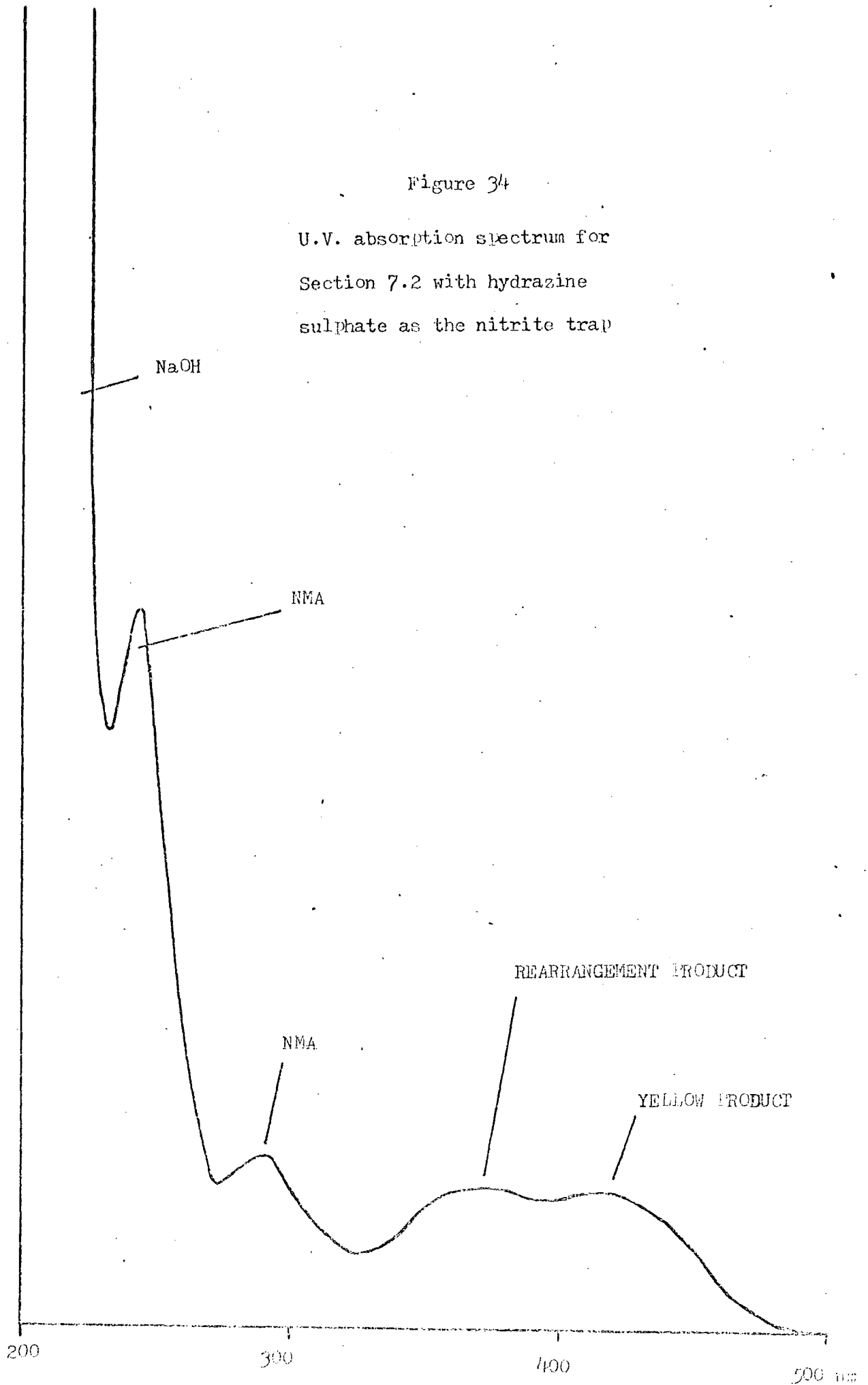
Figure 35

U.V. absorption spectrum for
Section 7.2 with a 4×10^{-2} M
concentration of Ferrous Ammonium
Sulphate as the nitrite trap.
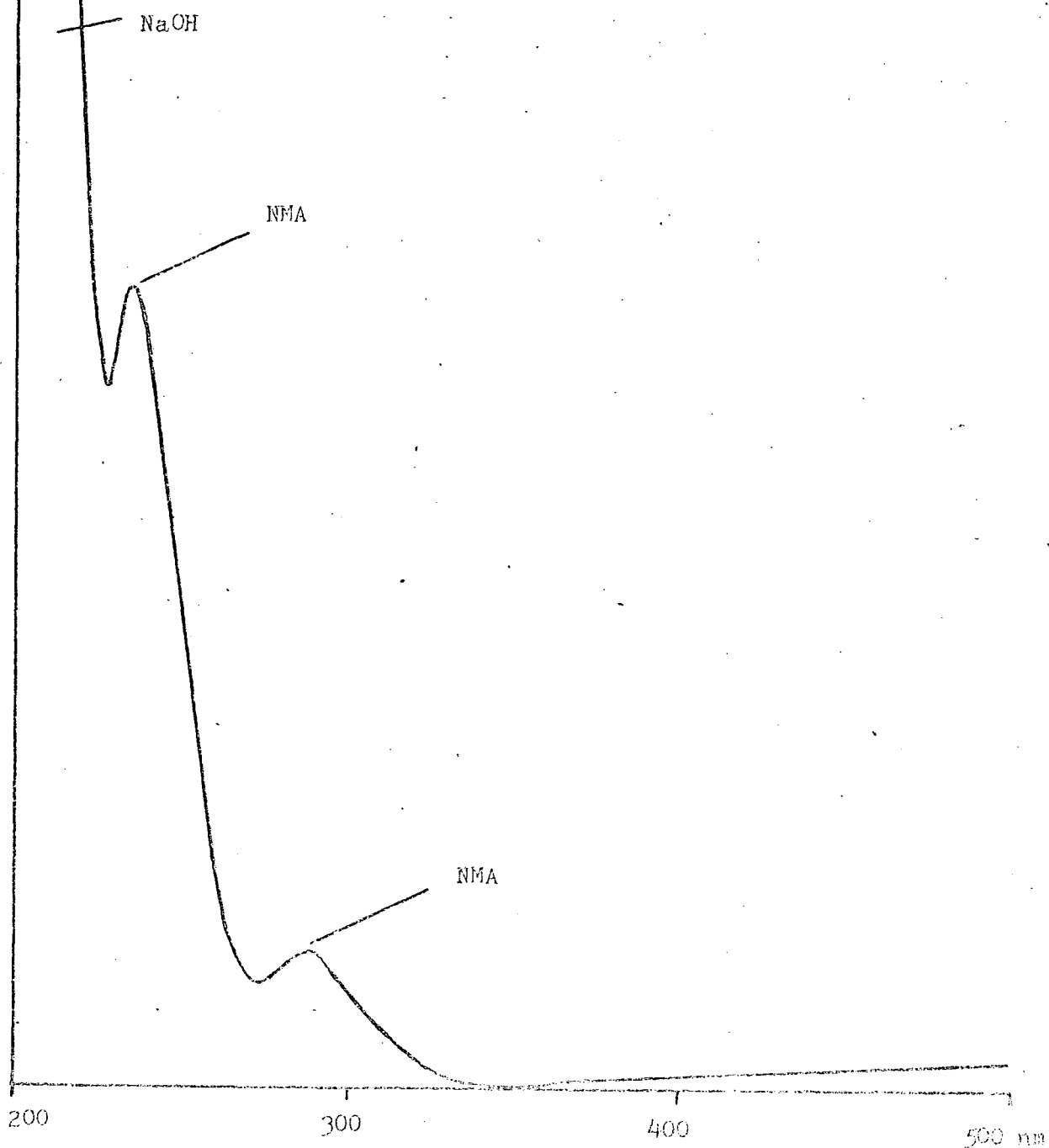
Under N$_2$

Nitrite Trap	Final Concentration of NMA/M
Ferrous Ammonium Sulphate (4 x 10^{-3} M)	$\leqslant$ 2.9 x 10^{-4}
Ferrous Ammonium Sulphate (4 x 10^{-2} M)	4.6 x 10^{-4}

At a concentration of 4 x 10^{-3} M the ferrous ion shows only slight evidence of its acting as a nitrite trap. The U.V. spectrum indicates that the final concentrations of NMNA, the rearrangement product and the yellow product are reduced relative to those concentrations obtained in the absence of a trap. Conversely the yield of NMA does seem to be slightly higher with a discrete NMA peak at 237 nm being just visible for the run containing the ferrous ion. The effect is small however and certainly far smaller than the effect brought about by a similar concentration of hydrazine sulphate.

At an Fe^{2+} concentration of 4 x 10^{-2} M however the observations are more conclusive. In comparison with the results obtained for the run in the absence of a trap the final concentrations of the yellow product, the rearrangement product and NMNA have been reduced whilst that of NMA has been increased, almost by a factor of 2. It is interesting to note that the concentrations of the yellow product and the rearrangement product are lower even than those values obtained in the presence of hydrazine sulphate and it is tempting to infer that the ferrous ion is the more efficient of the two traps at these respective concentrations. A consideration of the recorded NMA concentrations shows however that this is not so and we must look elsewhere for an explanation of the low yields attained by these

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products in the presence of the ferrous ion. The most obvious
possibility concerns a direct reaction between the ferrous ion and
these two compounds which serves to lessen their concentration in
the equilibrium mixture. It should be noted however that a direct
reaction of the metal ion with NMNA, NMNAH$^+$ or NMA would yield
similar observations.

The two kinetic runs involving Fe^{2+} were repeated under a
nitrogen atmosphere in accordance with the details given in Section 8
so that the importance of atmospheric oxygen in the reaction scheme
might be established. Whilst the reaction at high $[Fe^{2+}]$ yielded
results comparable with those obtained in air the reaction at low
$[Fe^{2+}]$ behaved surprisingly in that the yield of NMA was increased
that of rearrangement product decreased, and that of the yellow
product unaltered. It appears that the presence of air adversely
affects the ability of Fe^{2+} to act as a nitrite trap. This may be
due to one of two factors:

(a) a retardation by air of the reaction of the ferrous ion with
the NOY species

or

(b) a reduction of the effective Fe^{2+} concentration due to atmospheric
oxidation.

With regard to the 2nd possibility I have studied the decomposition
of neutral solutions of ferrous ammonium sulphate and report
percentage decompositions of 0.13% and 0.12% over six hours for the
solution in air and nitrogen respectively. Since the corresponding
figures for solutions in 3.52 M H$_2$SO$_4$ are 0.02% and 0% respectively
it appears that we must rule out explanation (b). Although the
possibility of the oxidation reaction being catalysed by one of the
organic components of the run may not be discounted it seems to me

that the explanation lies with our first proposal. In the absence
of air the reaction steps leading to the removal of nitrous acid
may be summarised as follows

$$HNO_2 + Fe^{2+} + H^+ \rightleftharpoons Fe^{3+} + NO + H_2O$$
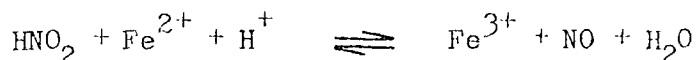
In air however the NO is readily oxidised by atmospheric oxygen to
give NO_2 which acts to lower the Fe^{2+} concentration according to the
following reaction.

$$Fe^{2+} + NO_2 \rightleftharpoons Fe^{3+} + NO_2^-$$
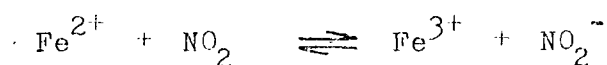
The observed reduction in the trap efficiency of ferrous iron in the
presence of air is thus explained. Presumeably the effect is not
noted at the higher Fe^{2+} concentration because of the presence of a
vast excess of the ferrous ion.

I have repeated these investigations using a 0.1 M concentration
of the bromide ion in place of water as the nucleophile and report
that the results are essentially similar to those obtained for H_2O.
Whilst we shall not enter into a detailed discussion the data is
given below for reference.

$$[H_2SO_4] = 3.512 \text{ M} \qquad [NMNA]_o = 5.012 \times 10^{-4} \text{ M}$$

In Air

Nitrite Trap	Final Concentration of NMA/M
None	$\leqslant 2.9 \times 10^{-4}$
Hydrazine Sulphate (4×10^{-3} M)	5.3×10^{-4}
Ferrous Ammonium Sulphate (4×10^{-3} M)	$\leqslant 3.0 \times 10^{-4}$

Ferrous Ammonium Sulphate
(4×10^{-2} M) 4.9×10^{-4}

Under N_2

Nitrite Trap	Final Concentration of NMA/M
Ferrous Ammonium Sulphate (4×10^{-3} M)	$\leqslant 3.5 \times 10^{-4}$
Ferrous Ammonium Sulphate (4×10^{-2} M)	4.9×10^{-4}

Clearly the ferrous ion does show evidence of acting as a nitrite trap. As an extension of the present investigation the experiments described above have been repeated using equal concentrations of a whole series of nitrite traps. Consider the data given below collected in the absence of an added nucleophile.

$$[H_2SO_4] = 3.512 \text{ M} \qquad [NMNA]_o = 5.012 \times 10^{-4} \text{ M}$$

$$[\text{Nitrite trap}] = 2.00 \times 10^{-3} \text{ M}$$

Trap Species	Final Concentration of NMA/M
Sodium Azide	5.6×10^{-5}
Sulphamic Acid	4.9×10^{-5}
Hydroxylamine Sulphate	✳
Hydrazine Sulphate	5.1×10^{-5}
Urea	4.0×10^{-5}
Ferrous Ammonium Sulphate	3.0×10^{-5}
Ferrous Ammonium Sulphate under N_2	3.1×10^{-5}

✳ This species possesses a U.V. absorbtion peak which precludes our measurement of the yield of NMA.

As the yield of NMA will be maximised by the presence of an efficient nitrite trap we may use these data to produce the table of trap efficiencies depicted below

Sodium Azide

Hydrazine Sulphate

Sulphamic Acid

Urea

Fe^{2+} under N_2

Fe^{2+}

INCREASING

TRAP

EFFICIENCY

Whilst this approach takes no account of the varying degrees of protonation experienced by each species it does allow us to compare their bulk reactivities. It is encouraging to note that the top four elements of the series are arranged in an order which parallels that obtained from the more sophisticated kinetic study by Williams[59]. The indications are that the ferrous ion is a poor nitrite trap being less efficient than even the worst of the classical nitrite traps, urea.

So, to recap. We have evidence that the ferrous ion is capable of reacting with both HNO_2 and NOBr at such a rate as to permit its use as a nitrite trap in the denitrosation of NMNA. We have also shown that it is far from being efficient amongst the nitrite traps we have to hand. Whilst these two sentences really contain the complete story as it is now there are certain aspects which would benefit from further discussion before we move on. Consider the problem of side-reactions.

In discussing the experimental results it has proved necessary to contemplate the existence of a series of possible side-reactions.

We may summarise these possibilities as follows.

GROUP ONE

$$Fe^{2+}/Fe^{3+} + NMNA \longrightarrow A \text{ hydrazine}$$

$$Fe^{2+}/Fe^{3+} + NMA \longrightarrow Products^{*}$$

$$Fe^{2+}/Fe^{3+} + NMNA \longrightarrow ditto$$

$$Fe^{2+}/Fe^{3+} + Rearrangement\ Product \longrightarrow ditto$$

$$Fe^{2+}/Fe^{3+} + Yellow\ Product \longrightarrow ditto$$
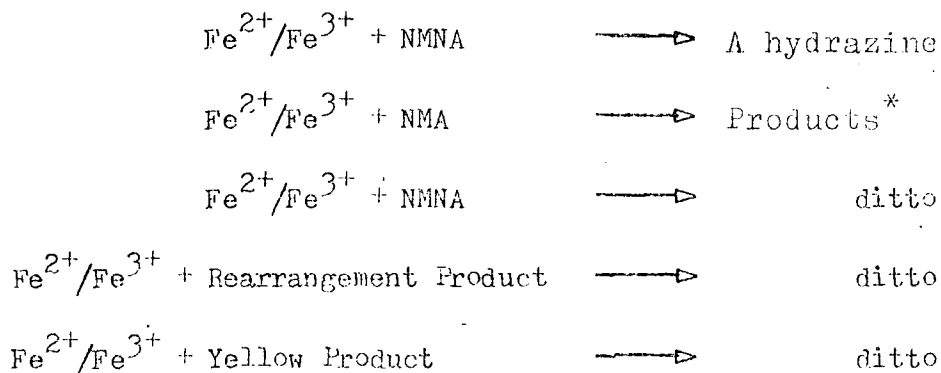
[*] Possibly of the form $FeSO_4 \cdot (Z)_2$?

GROUP TWO

$$Fe^{2+}/Fe^{3+} + NMNA \longrightarrow NMA$$
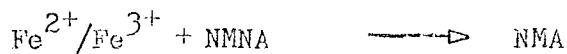
Consider the group one reactions. As we already have evidence concerning the percentage yields of NMA it is possible to place upper limits upon the percentage reaction via these group one schemes.

viz:

Nucleophile	% Reaction via Group One
H_2O	$\leq 15\%$
Br^-	$\leq 2\%$

I suspect that an increase in $[Fe^{2+}]$ would lower the figure of 15 considerably so that these group one reactions do not appear to be significant.

I do not believe that the same may be said of the Group Two reaction.

A series of investigations which were designed to quantify the kinetic

behaviour of the system met with little success but did show that

the kinetics were far more complicated than we might have expected

for a denitrosation in the absence of the ferrous ion. We may place

a number of interpretations upon this observation and it may be that

the reaction between NOY and Fe^{2+} proceeds so slowly that its kinetics

affect the rate of disappearance of the nitrosamine. However there is

an alternative explanation. It is conceivable that the ferrous ion

enters the rate expression for the denitrosation in a role additional

to that of a nitrite trap. Referring to Section 1.7 we might propose

that the reaction proceeds via the formation of a complex similar to

that invoked by Thompson[75] for the reaction of N-ethyl-N-nitroso urea.
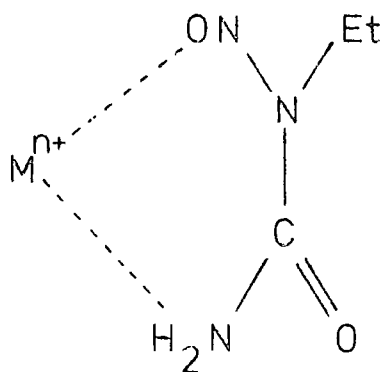
Such a reaction falls within Group Two. Whilst I believe that the

behaviour of the ferrous ion in the present system reflects its

ability to act as both a nitrite trap and an electrophile, proof

must derive from other sources.

In bringing this section to a close one final question springs to

mind. "Is the effect that has been observed limited to the ferrous

ion or common to a series of metal ions?" From the limited amount of

work that I have carried out on the cuprous ion it appears that the

latter may well be the case.

SECTION EIGHT

EXPERIMENTAL PROCEDURES

8.1 An Introduction

The following section contains details of the experimental techniques which were employed in obtaining the results of preceeding sections. Whilst reagent sources are described in a general way the supplier is identified only where a reagent is believed to be of limited availability.

The kinetic studies were generally carried out at low reagent concentrations, typically in the range $10^{-4} \longrightarrow 10^{-2}$ M. Where this proved possible reagents were made up into stock solutions in ethanol or water as appropriate. Varying proportions of these stock solutions were then used to prepare reaction mixtures of differing reagent concentrations the total volume being made up to a constant volume, typically 49ml, in each case by the addition of an appropriate quantity of the pure solvent. The reaction mixtures were then pre-warmed in a thermostat tank at 31°C for a period of not less than twenty minutes. One essential reagent was omitted from each reaction mixture; thus the addition of a fixed volume, typically 1ml, of a pre-warmed stock solution of this component to each reaction mixture served to initiate the reaction. The change in total volume of the stock solutions which occurs on their mixing is neglected; at such low reagent concentrations the magnitude of this volume change is expected to be small. The active reaction mixture was then transfered to a 1cm spectrophotometer cell. The progress of the reaction was monitored by observation of the change in magnitude of the u.v./visible absorption spectrum at fixed wavelength, λ , with respect to time. The wavelength, λ , was chosen such that the observed absorption was due to either a product or a reactant as appropriate. Reactions were thus followed via the agency of one of two available spectrophotometers. The Beckman 24 spectrophotometer is a

double-beam instrument capable of displaying values of the recorded absorbtion, abs (λ), (t), at wavelength λ and time t, in a digital form. The instrument may be operated in the fixed wavelength mode to monitor the change in abs (λ) with respect to time or may be used in the wavelength scan mode to monitor the change in abs (t) with respect to λ. Repeated scanning is the wavelength scan mode at known time intervals allows an alternative method of measuring the change in abs (λ) with time. Analogue representation of such trends is provided by an attached graph recorder. The recorder provides a wide range of chart speeds, 0.1 ipm to 10 ipm, and a fairly comprehensive range of absorbance scales from 0.1A to 2A full scale deflection. The spectro-photometer is fully operational in the range 190 to 700nm. The cell compartment is maintained at a temperature of $31^{\circ}C \pm 0.1^{\circ}C$ by an internal electrical heater/thermostat combination.

The Pye Unicam SP8000 spectrophotometer is in most respects similar to the Beckman instrument described above. The internal flat-bed recorder of the SP8000 provides only two choices of absorbance scale and a limited number of chart speeds. The instrument is fully operational in the range 190 to 700nm and temperature control is via the agency of an external thermostat tank. Attachment of the SP8005 accessory provides for automatic time programmed repeat scanning in the wavelength scan mode.

1cm pathlength "Far U.V." cells of fused silica construction were used exclusively.

In general the absorbance of a species, Z, at constant pathlength is noted to be directly proportional to its concentration. A consideration of the variation of abs (λ) with time thus leads directly to a value for k_o, the observed rate constant for the reaction. In the present work,

where the kinetic behaviour is predominantly first order, values of k_o for reactions in which the infinity value of the absorbance is known were calculated using the computer program RKISNA (Appendix 2). When the requisite infinity value was either unknown or was deemed to be unreliable a value for k_o was obtained via an application of either the guggenheim method or the initial slope method. Both methods are described in Appendix 1. Upon completion of the reaction the U.V. spectrum of the reaction mixture in the range 200nm to 700nm was usually recorded so as to provide evidence of product identities. A measurement of the acidity of the active reaction mixture was obtained by titration of a suitably sized aliquot with an aqueous solution of NaOH using phenol red as an indicator. In the case of ethanolic reaction mixtures the chosen aliquot was diluted with demineralised water before titration to improve the performance of the phenol red indicator.

Qualitative investigations which generally employed higher reagent concentrations are described in the relevent sections below.

Since the N-nitroso compounds are generally found to be potent carcinogens suitable gloves should be worn when spillage is considered to be even a remote possibility. A good deal of care should be exercised in choosing suitable gloves since their permeability to solvents has been found to differ widely. In the present work two pairs of "Kimguard" gloves (Kimberly-Clark Ltd.) were worn simultaneously.

9.2 The Denitrosation of N-methyl-N-nitrosourea

9.2.1 Materials

N-methyl-N-nitrosourea was obtained commercially (Cambrian Chemicals M44 C4) and was used without further purification. N-methylurea (Aldrich 11, 690-4) was similarly used without further purification.

Analar grades of the inorganic reagents, hydrazine sulphate, potassium bromide, potassium thiocyanate and sulphuric acid were used as supplied. Thiourea was obtained as the S.L.R. grade product and was purified by recrystallisation from ethanol. Solvent ethanol was of the "absolute grade".

8.2.2 Procedure

From the work of McCalla et al.[122] it is evident that the rate of decomposition of a solution of N-methyl-N-nitrosourea, MNU, is greatly enhanced by its being exposed to daylight, presumeably on account of the incursion of radical decomposition pathways. In the present work ethanolic stock solutions of MNU were therefore stored in complete darkness until required. Under such conditions the percentage decomposition of the stock solutions at 31°C was measured at approximately 10% over an eight hour period. Consequently fresh stock solutions of MNU were prepared at the start of each working day.

Aqueous stock solutions of the other reagents were used to prepare 49ml volumes of each of the required reaction mixtures. The reaction was initiated by the injection of a 1ml aliquot of a stock solution containing the nitrosourea.

In order that some estimate might be made concerning the extent of decomposition via the deamination pathway a number of runs were carried out at various acidities in the presence of added excess concentrations of p-chloroaniline. The presence of nitrous acid produced by the denitrosation pathway leads to the diazotisation of the added p-chloroaniline. 5ml aliquots removed from the reaction mixture at various times, t, were neutralised by the addition of a measured volume of aqueous sodium hydroxide. 1ml aliquots of these neutral solutions were then added to

10ml volumes of a solution containing borax together with an excess concentration of 2-napthol-3,6-disulphonic acid. The magnitude of the visible absorbance at 500nm due to the resultant diazo-dye was measured on a Pye Unicam SP500 spectrophotometer. Consideration of a run using sodium nitrite in place of the nitrosourea yielded a value of 19,710 for ε_{500} for the diazo dye. Thus a value for the percentage reaction via the denitrosation pathway could be calculated. A consideration of the variation of abs (500nm) with respect to time lead to approximate values of k_o, the observed first-order rate constant for the reaction. All other kinetic studies were performed via an observation of the variation in magnitude with respect to time of a U.V. peak at 245nm ascribed to MNU. These latter observations were carried out using a Beckman Model 25 Spectrophotometer operating in the fixed wavelength mode. Values of k_o for the reaction were calculated via an application of either the computer program RKISHA or the first-order Guggenheim treatment as appropriate.

This procedure was slightly modified in the case of reactions in the presence of either potassium thiocyanate or thiourea. Here the disappearance of MNU was monitored at 265nm to preclude interference by the adjacent low wavelength U.V. absorptions of the two sulphur compounds.

8.3 The N-nitrosation of N-methylurea

8.3.1 Materials

N-methylurea (Aldrich M3, 680-4) was used without further purification. Analar grades of the inorganic reagents potassium bromide, potassium thiocyanate, sodium nitrite and sulphuric acid were used as supplied.

8.3.2 Procedure

Aqueous stock solutions of reagents other than sodium nitrite were used to prepare 49ml volumes of each of the required reaction mixtures. Reaction was then initiated by the injection of a 1ml aliquot of an aqueous stock solution of sodium nitrite. The reaction was followed spectrophotometrically at 265nm on a Beckman Model 25 unit operating in the fixed wavelength mode. The observed absorbance at this wavelength, although almost entirely due to the reaction product N-methyl-N-nitrosourea, does contain a small contribution from N-methylurea. In the present case N-methylurea is present in an excess concentration and its contribution to abs (265nm) is effectively constant throughout the course of the reaction. Since the contribution is both small ($\sim 0.05A$) and constant it may be neglected. All values of k_o were calculated using the computer program RKISNA.

8.4 The Denitrosation of p-substituted NMNA Derivatives in Acidic Ethanolic Solution

8.4.1 Materials

NMNA was prepared by the reaction of N-methylaniline with sodium nitrite under the usual conditions[141] and was purified by fractional distillation under reduced pressure, (B.P. = 120 $\pm$ 1°C at 13mm Hg). The p-NO, p-Cl, p-Me and p-OMe substituted compounds were prepared via a two stage synthetic route commencing with the corresponding p-substituted aniline according to the scheme depicted over.
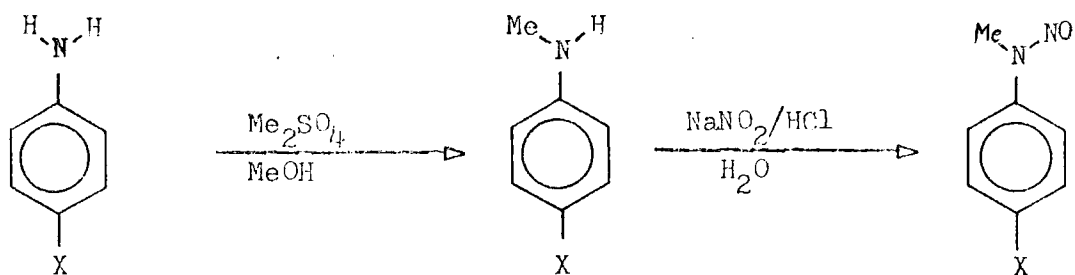
Thus an initial N-methylation by dimethyl sulphate is followed by an N-nitrosation under the usual conditions. The p-substituted NMNA derivatives were recrystalised from aqueous ethanol before use.

This preparative procedure was modified slightly for the production of the p-Me compound in that an additional purification stage was introduced immediately prior to the N-nitrosation. The crude N-methyl-p-methylaniline obtained from the N-methylation was purified by fractional distillation under reduced pressure using an automated Fischer Spaltrohr HMS 500 column. (BP $= 86 \pm 1^{O}$C, P $= 7.5$mm Hg). In the absence of this purification stage crude N-methyl-p-methylaniline employed in the subsequent N-nitrosation reaction gave a substantial yield of an azo dye due to the presence of unreacted primary amines. Analar grade ascorbic acid (Koch-Light Laboratories Ltd. - 0462h) was used without purification. Ethanolic solutions of HCl were made by passing dry HCl gas into ethanol of an "absolute" grade.

8.4.2 Procedure

Ethanolic stock solutions of HCl prepared as above were not found to be indefinately stable and were discarded some three days after preparation.

Fresh stock solutions of HCl were used in conjunction with an ethanolic solution of ascorbic acid and solvent respectively to produce 40ml volumes of each if the required reaction mixture. Reaction was then initiated

by the addition of a 1ml volume of an ethanolic stock solution containing the nitrosamine. Progress of the reaction at $31^{\circ}C$ was monitored spectrophotometrically by monitoring the magnitude of the U.V. absorption due to the nitrosamine. Values of λ chosen for the study were dependent on the precise nature of the nitrosamine.

NMNA	λ/nm
p-H	300
p-NO$_2$	310
p-Cl	305
p-Me	305
p-OMe	315

The decomposition of ascorbic acid in ethanolic HCl solution has been reported by Williams[142] to yield products which themselves absorb U.V. light at a wavelength of 300nm. Decomposition of the ascorbic acid can thus lead to a distortion of infinity values. In the present case all kinetic runs exhibited good first order behaviour throughout, the time scale of the decomposition relative to that of the denitrosation being such as to preclude interference. Values of the observed first-order rate constant were calculated by the computer program RKISRA.

8.5 The Denitrosation of N-nitrosodiphenylamine at High Nucleophile Concentrations

8.5.1 Materials

N-nitrosodiphenylamine is conveniently prepared by the action of sodium nitrite upon diphenylamine in acidic ethanolic solution[143]. The nitroso compound crystalises out and is purified by recrystallisation from methylated spirits. Analar grades of the inorganic reagents

potassium bromide, potassium thiocyanate, sodium azide and sulphuric acid were used as supplied. Solvent ethanol was of the absolute grade.

8.5.2 Procedure

Aqueous stock solutions of reagents other than NDPA were used to prepare 49ml volumes of the required reaction mixture and reaction was initiated by the addition of a 1ml aliquot of an ethanolic solution of NDPA. The reaction was monitored by following the decreasing magnitude of a U.V. absorption peak at 325nm due to the N-nitroso compound. The reaction at $31^{\circ}C$ was found to exhibit good first-order behaviour throughout its entire course and values of k_o the observed first-order rate constant were calculated by the computer program RKISNA.

8.6 The Denitrosation of p-substituted NMNA Derivatives at High Nucleophile Concentrations

8.6.1 Materials

The preparation of NMNA and the requisite derivatives has already been described in Section 8.4.1. Analar grades of hydrazine sulphate and sulphuric acid were used as supplied whilst thiourea was obtained in a G.P.R. grade and was purified by recrystalisation from ethanol.

8.6.2 Procedure

Aqueous stock solutions of hydrazine sulphate, sulphuric acid and thiourea were used to prepare 49ml volumes of the required reaction mixture. The reaction was thus initiated by the addition of a 1ml aliquot of an ethanolic solution of the requisite nitrosamine. The progress of the reaction at $31^{\circ}C$ was monitored by following the decreasing magnitude of the U.V. absorption due to the nitrosamine. Values of λ chosen were

higher than those employed for the reaction in ethanol since it was
necessary to avoid interference from an adjacent U.V. peak due to
thiourea

NMNA	λ /nm
p-H	310
p-NO$_2$	325
p-Cl	320
p-Me	320
p-OMe	325

All kinetic runs displayed good first-order behaviour and values
of k_o, the observed first-order rate constant were calculated by the
computer program RKISNA.

8.7 The Reaction of NMNA with Thiourea and Alkyl Thioureas

8.7.1 Materials

N-methyl-N-nitrosoaniline was prepared as detailed in Section 8.4.1.
The thioureas were all obtained in the form of the S.L.R. grade products
as follows: thiourea (B.D.H. 30423), N-methylthiourea (Koch-Light
Labs. Ltd. 3770h), N,N'-dimethylthiourea (Aldrich Chemical Co. D18,870-0),
trimethylthiourea (I.C.I. - gift), tetramethylthiourea (Aldrich Chemical
Co. 11536-9). All were purified by recrystalisation from aqueous
ethanol before use. The inorganic reagents hydrazine sulphate,
magnesium sulphate, sodium carbonate, sodium hydroxide and sulphuric acid
were obtained as the analar grade products and were used as supplied.
Solvent ether was of the analar grade.

8.7.2 Procedure

8.7.2.1 Qualitative

Two qualitative investigations were carried out, at low and high reagent concentrations respectively.

Thus for the reaction at high reagent concentrations an aqueous solution containing 0.636g of NMNA in 12ml of distilled water was added to 108ml of an aqueous solution containing sulphuric acid ($\sim$ 3 M) together with excess concentrations of both hydrazine sulphate and thiourea. The reaction was allowed to go to completion and the reaction mixture neutralised with aqueous sodium hydroxide solution. The resultant solution was ether extracted. The ether extracts were dried for 20 mins over anhydrous magnesium sulphate and the solvent removed on a rotary evaporater. The golden yellow residue was identified as pure N-methylaniline by comparing its I.R. spectrum with that of an authentic sample.

For the investigations at low reagent concentrations the following reaction mixtures were prepared to give a total volume of 250ml in each case.

A   thiourea   9.006 x 10^{-3} M

        sulphuric acid   0.85 M

        hydrazine sulphate   2.009 x 10^{-3} M

        NMNA   3.040 x 10^{-4} M

B   N-methylthiourea   3.004 x 10^{-3} M

        sulphuric acid   0.85 M

        hydrazine sulphate   2.009 x 10^{-3} M

        NMNA   2.579 x 10^{-4} M

<u>C</u> N,N'-dimethylthiourea 1.979×10^{-3} M

sulphuric acid 0.85 M

hydrazine sulphate 2.009×10^{-3} M

NMNA 2.579×10^{-4} M

<u>D</u> trimethylthiourea 2.016×10^{-3} M

sulphuric acid 0.85 M

hydrazine sulphate 2.009×10^{-3} M

NMNA 2.579×10^{-4} M

<u>E</u> tetramethylthiourea 2.010×10^{-3} M

sulphuric acid 0.85 M

hydrazine sulphate 2.009×10^{-3} M

NMNA 3.040×10^{-4} M

On completion of the reaction each reaction mixture was neutralised by the addition of a suitable quantity of solid sodium carbonate. The neutral solution so obtained was left overnight under continuous ether extraction using 250ml of solvent. The resultant ethereal fraction was dried over anhydrous magnesium sulphate and evaporated down to a final volume of about 5ml. The inorganic material precipitated by this action was filtered on a number 3 sinter. A sample of the ethereal function from each experiment was fed into a V.G. Micromass 12B gas chromatograph fitted with automatic temperature programming facilities and a mass spectrometer detection system. Samples were held on column O_{30} at 85°C until all ether was removed and the temperature was then raised at the rate of 4°C/min to a maximum of 220°C. All but one of the samples yielded only one detectable component other than ether. This component was identified as N-methyaniline by comparing its mass spectrum with that of an authentic sample. The sample derived from the N,N'-dimethyt-

thiourea experiment yielded two components. One was identified as
N-methylaniline whilst the other would appear on the basis of its mass
spectrum to be the thiourea itself.

8.7.2.2. Quantitative

Aqueous stock solutions of the various nucleophiles together with
aqueous stock solutions of hydrazine sulphate and sulphuric acid were
used to prepare 49ml volumes of the appropriate reaction mixtures. A
1ml volume of an aqueous solution containing NMNA was then added to
start the reaction. Progress of the reaction at 31°C was followed
spectrophotometrically by observing the decreasing magnitude of a U.V.
peak due to NMNA, generally at a wavelength of 275nm. In the case of
the unsubstituted thiourea the presence of a U.V. absorption peak due to
the nucleophile causes little in the way of complications since it lies
at low wavelength and makes only a small contribution at 275nm. As the
present work was carried out under conditions involving the use of a
twenty fold excess concentration of the thiourea over that of the
nitrosamine this small contribution is effectively constant and may be
discounted. An increase in the degree of N-methyl substitution is noted
to result in a bathochromic shift of λ for the low wavelength U.V.
absorption of the thioureas. As $\varepsilon(\lambda_{max})$, the extinction coefficient
for the absorption is substantially unchanged by the increasing degree
of substitution the bathochromic shift results in an increased per unit
concentration contribution by the thioureas towards the observed value
of λ (275nm). In practice this contribution was found to be small for
all except the tetramethyl substituted thiourea. For reactions involving
this latter compound the disappearance of the nitrosamine was monitored
at a wavelength of 300nm so that interference was reduced to manageable

proportions.

In general the kinetic runs were seen to exhibit good first-order behaviour throughout their extent. Values of k_0', the observed rate constant, were calculated by the computer program RKISNA or, in the absence of a reliable value for abs $(\lambda)(\infty)$, via an application of a first-order Guggenheim Method. Values of k_0' were corrected on the basis of the observed yield of the rearrangement product so as to give a value for k_0 the rate constant for the denitrosation. The yield of the rearrangement product was measured spectrophotometrically given that under the conditions employed $\lambda_{max} = 333nm$ and $\varepsilon_{333nm} = 20,050$.

In the case of reaction in the absence of added nucleophiles at low acidity observed rates of reaction are very slow and values of k_0 were obtained via a consideration of the initial rate.

8.8 The Reaction of NMNA with Cysteine, Glutathione, S-methylcysteine and Methionine

8.8.1 Materials

The sulphur nucleophiles L-glutathione (reduced) (Koch-Light Labs. Ltd. 2895h), L-methionine (Koch-Light Labs. Ltd. 3600h), L-cysteine (B.D.H. Chemicals Ltd. 37218) and S-methyl-L-cysteine (Aldrich Chemical Co. 85,547-2) were all obtained in a pure form and were used as supplied. L-alanine (Koch-Light Labs. Ltd. 0136h) was similarly obtained.

NMNA was prepared as detailed in Section 8.4.1. The inorganic reagents hydrazine sulphate and sulphuric acid were obtained in analar grades and used as supplied.

8.8.2 Procedure

Aqueous stock solutions of the nucleophiles were prepared in the

presence of small amounts of sulphuric acid, as an aid to solubility and these stock solutions were used in conjunction with solutions containing hydrazine sulphate and sulphuric acid to provide 49ml volumes of the requisite reaction mixtures. The reaction was then started by the addition of 1ml of an aqueous solution of NMNA. In each case the progress of the reaction, at $31^\circ C$, was monitored by observing the decreasing U.V. absorption peak of the nitrosamine at a wavelength of 285nm.

In general the kinetic runs exhibited good first-order behaviour throughout their extent and values of k_o', the observed first order rate constant, were obtained from the computer program RKISNA, application of the Guggenheim Method or via a consideration of the initial rate as appropriate. These values of k_o' were corrected for rearrangement, using the methods of Section 8.7.2 to yield a value for k_o the rate constant for the denitrosation. In the case of reactions involving L-cysteine some evidence for a deviation from first-order behaviour in the latter stages of the reaction is available. This deviation, noticeable only at values of percentage reaction greater than 75% is slight in its extent and values of k_o were obtained as above.

8.9 The Interaction of NMNA with Metal Ions

8.9.1 Materials

N-methyl-N-nitrosoaniline was prepared as detailed in Section 8.4.1. N-methylaniline was obtained as the S.L.R. grade product and was purified by fractional distillation. (B.P. = 193 - $1^\circ C$, P = 760mm Hg). p-Nitroso-N-methylaniline was prepared from N-methyl-N-nitrosoaniline in the usual manner[144] and was recrystallised from benzene.

Analar samples of hydrazine sulphate, phenylhydrazine, sulphamic

acid and urea were used without further purification. The solvents, acetone, benzene, diethyl ether, toluene and methanol were all obtained as the analar grade products and were used as supplied. In general the inorganic reagents were also readily obtainable in this form so that ammonium ferrous sulphate, magnesium sulphate, potassium bromide, sodium azide, sodium hydroxide and sulphuric acid were used as obtained.

8.9.2 Procedure

8.9.2.1 Qualitative

The following reaction mixture was prepared in aqueous solution to give a final volume of 250ml.

ammonium ferrous sulphate	0.746 M
N-methyl-N-nitrosoaniline	1.234×10^{-2} M
sulphuric acid	3.512 M

The reaction was allowed to proceed to completion and the resultant solution neutralised to pH8 by the addition of solid sodium hydroxide. The precipitate formed at this stage ($Na_4Fe(OH)_6 \longrightarrow Fe_2O_3 \cdot nH_2O$?) was removed by filtration under vaccuum using a number 4 sinter in conjunction with Whatman "Qualitative" papers. The collected residue was washed with 3 x 25ml portions of distilled water and the washings added to the filtrate. The resulting solution was then extracted with 3 x 200ml portions of diethyl ether and the extracts dried over anhydrous magnesium sulphate. The ethereal extracts were then reduced in volume to 5ml by the removal of solvent on a rotary evaporator. 2ml of this solution was further evaporated to about 0.2ml and subjected to a T.L.C. investigation by comparison with authentic samples of the expected products. The remaining 3ml portion was evaporated to dryness and yielded a minute quantity of a tarry substance deemed unsuitable for

further analysis by G.L.C. or mass spectrometry. The experiment was
repeated twice in an effort to obtain material suitable for analysis
by these latter two methods without success. It may be that the low
yield of organic material is due to its partial removal with
precipitated iron salts at the filtration stage because of complex
formation and/or adsorption.

The T.L.C. investigations were carried out using each of seven
different solvent systems in turn, their compositions being as given
below.

Solvent system	Composition by volume	Polarity
Toluene	1	LOW
Benzene-acetone	90:10	
Benzene-methanol	95:5	
Benzene-acetone	80:20	
Benzene-diethyl ether	40:60	
Diethyl ether-methanol	99:1	
Methanol	1	HIGH

The stationary phase in all cases consisted of Kieselgel GF254
(Fluka Chemicals).

The best separation was achieved with the benzene:acetone (80:20)
solvent system and the R_f values given in the text are for this system
at a temperature of 22°C. Samples of the concentrated ethereal solutions
were spotted onto the plate in the normal manner together with pure
samples of the expected products. When a solvent front movement of 15cm
relative to the base-line had been recorded the T.L.C. plate was removed
from the solvent tank, dried and examined under each of the three forms
of lighting described in the text. The plate was then exposed to

iodine vapour and re-examined in daylight. Values of R_f are defined as below.

$$R_f(a) = \frac{\text{distance travelled by component a in cm's}}{\text{distance travelled by solvent front in cm's}}$$

R_f values in Section 7.2 are given under four headings depending on the components visibility under each of the four methods of detection.

8.9.2.2. Quantitative

An estimation of the rate of air oxidation of an aqueous solution of ammonium ferrous sulphate under neutral and acidic conditions was carried out as follows. Aqueous solutions of the appropriate reagents were used to prepare 50ml aliquots of the requisite reaction mixtures. The U.V. spectrum of each reaction mixture between the limits 200nm and 600nm was recorded immediately after preparation and again after 8hrs. The value of ε_{375nm} for the U.V. absorption of Fe^{3+} had already been calculated as 177 using a standard solution of $FeCl_3 \cdot 6H_2O$ so that a direct measurement of the percentage decomposition of the ammonium ferrous sulphate over the 8hr period was easily obtained. The estimation was repeated using solutions deoxygenated by the passage of nitrogen gas for a period of 15 minutes.

The effect of the presence of ferrous ions upon the observed yield of NMA obtained from NMNA was studied as follows. A 50ml aliquot of each of the required reaction mixtures was prepared from aqueous stock solutions of the appropriate reagents. In each case the reaction was allowed to proceed to completion. A 5ml portion of the acidic reaction mixture was then removed and neutralised by the addition of 37.15ml of an N aqueous solution of sodium hydroxide. The resultant precipitate $(Na_4Fe(OH)_6 \longrightarrow Fe_2O_3 \cdot nH_2O?)$ was removed on a number 3 sinter and the

U.V. spectrum of the filtrate was recorded between the limits 200nm
and 600nm. A measure of the concentration of NMA present in the
sample was obtained by a comparison of the observed absorbance at
237nm with that of a reference solution of NMA. Several of the
investigations were repeated using solutions which had been deoxygenated
by the passage of nitrogen gas in an attempt to elucidate the role of
atmospheric oxygen in the reaction.

APPENDIX ONE

THE MATHEMATICAL TREATMENT

OF DATA.

The Mathematical Treatment of Data

In general the reactions of the present work have been carried out under conditions which produce first-order kinetic behaviour. Information concerning the progress of the reaction has normally been collected in the form of absorbance vs time data and these values have been employed directly to provide values of k_o the observed first-order rate constant by reference to the following equation.

$$k_o = \frac{1}{t} \ln \frac{(abs(\lambda)(\infty) - abs(\lambda)(0))}{(abs(\lambda)(\infty) - abs(\lambda)(t))}$$

where $abs(\lambda)(t)$ represents the recorded U.V. absorbance at wavelength λ and time t.

In the present work the actual arithmetic has been carried out by the computer program RKISNA, (see appendix 2), whose first action is to calculate a series of values for k_o using each of the available values of $abs(\lambda)(t)$. Ideally of course these "instantaneous" values of k_o should be identical but in practice this state of affairs is never realised. Variations of $\pm 10\%$ around the mean figure appear to be common but where this variation was exceeded the applicability of first-order kinetics was critically reassessed.

If the data satisfied this subjective test the program was allowed to continue. In its next stage the computer assesses the mean of the instantaneous rate constants and calculates the variance of the individual values. Values which differed from the mean by a margin which exceeded seven times the variance were discarded, the process being repeated until a set of statistically significant data remained. The value of k_o is thus presented as the mean of these values together with a value of the error derived from the square root of the variance of this mean. In the present work the error in k_o calculated by this method has been observed

to represent less than 1% of the total value of k_o.

In some experiments the value of abs(λ)(∞) was not known and in these instances the value of k_o has been derived from an application of the first-order Guggenheim Method[145]. Consider the equation given below upon which the approach is based.

$$\ln \left| \text{abs}(\lambda)(t) - \text{abs}(\lambda)(t+\Delta) \right| = -k_o t + \text{constant}$$

where Δ represents a fixed time interval.

A plot of $\ln$ abs(λ)(t) - abs(λ)($t+\Delta$) vs t should thus yield a straight line of slope $-k_o$. In the present work these plots were visually assessed for linearity, to test the applicability of the method. On the assumption that values of $\ln$ abs(λ)(t) - abs(λ)($t+\Delta$) are less accurate than values of t the data were then subjected to a simple "least squares" treatment to yield a value for k_o. Whilst this approach gives some degree of protection from mis-use the ability of this Guggenheim Method to produce straight lines from data which do not reflect first-order kinetics has been noted[123] and it is prudent to ensure that the reaction is first-order before applying it.

In the present work where values of Δ were chosen so as to represent between two and three half-lives the standard error in values of k_o calculated by this method was equal to around 2% of the total value of k_o.

For a few reactions which proved to be exceptionally slow values of k_o were calculated via the initial rates method. Calculations concerning the standard error in values of k_o derived by this method have not been carried out but it would seem that the greater part of any error here would be due to an error in drawing the tangent to the concentration vs time curve and determining its slope. In the present work the method was used only for very slow reactions where the slope of

the tangent does not change rapidly with time so that the standard error in k_o has been estimated as representing less than 5% of its total value.

Whilst mention has been made concerning the inherent errors present in values of k_o calculated by each of the above methods it is perhaps pertinent at this stage to say that these errors of $\pm$ 1%, $\pm$ 2% and $\pm$ 5% respectively represent the precision of the calculated value. Thus these errors merely describe the precision with which each value of k_o fits the data from which it was derived. If we were to prepare a series of "identical" reaction mixtures and carry out separate kinetic runs using each in turn we should not expect to obtain identical values for k_o. Indeed these values would vary around a mean value within bounds defined by the "repeatability error". The "repeatability error" is expected to be larger than the precision described above since it possesses as components both the precision and errors due to variations in reagent concentrations between the various runs etc.

In the present work this "repeatability error" is estimated by observation to represent approximately $\pm$ 5% of the value of k_o. Although the precision is of great practical use in allowing an appreciation of the consistency of data collected in a single run its importance is diminished in the light of the above discussion and it is omitted from the text.

In a number of instances a linear correlation between collected parameters has formed the basis for further calculations. An example occurs in Section 6.2.3 where values of k_3 are plotted against thiourea to provide a value of $k_1 K h_o$ from the relationship given below.

$$k_o = k_1 K h_o \, [\text{thiourea}]$$

In each case the expression is of the following form.

$$y = mx + c$$

If errors in m are assumed to arise mainly from errors in y we can calculate a value for m on the basis of the least squares method. Thus,

$$m = \frac{N \sum x_i y_i - \sum x_i \sum y_i}{N \sum x_i^2 - (\sum x_i)^2}$$

where x_i and y_i represent the individual values of x and y respectively and N is the number of x, y data pairs to be considered.

A value for c is similarly obtained using the expression given below.

$$c = \frac{\sum y_i \sum x_i^2 - \sum x_i \sum x_i y_i}{N \sum x_i^2 - (\sum x_i)^2}$$

In a large number of cases the value of r, the correlation coefficient was also calculated using the following formula.

$$r = \frac{N \sum x_i y_i - \sum x_i \sum y_i}{((N \sum x_i^2 - (\sum x_i)^2)(N \sum y_i^2 - (\sum y_i)^2))^{\frac{1}{2}}}$$

The correlation coefficient measures the degree of association between the two random variables x and y. Whilst a perfect correlation is reflected in a value for r of unity values of $r > 0.9$ are to be considered acceptable. In most cases a pictorial representation of the observed trend has also been provided.

APPENDIX TWO

THE COMPUTER PROGRAM

RKISNA

The Computer Program RKISNA
===========================

The complete listing of the PL/C computer program RKISNA as run
on the NUMAC IBM 360 Computer under the MTS operating system is given
below.

The program represents an amended form of the program RKISNA
developed at Durham University by Dr. G. Kohnstam.

```
RKISNA:PROC OPTIONS (MAIN);
            /* CALCULATION OF RATE CONSTANTS FOR FIRST-
            ORDER REACTIONS */
            ON ENDFILE (SCARDS) GOTO STOP;
            DCL NAME CHAR (80);
     DATA:GET LIST (NAME, NC, R00);
            /* NAME, IDENTIFYING DESCRIPTION OF EXPERIMENT */
            /* NC, NUMBER OF READINGS INCLUDING ZERO BUT
            EXCLUDING INFINITY */
            /* R00, INFINITY READING */
     ONE:BEGIN;
            DCL (TC, RC) (NC);
            /* TC, TIME */
            /* RC, READING AT TIME TC */
            DO I=1 TO NC;
            GET LIST (TC(I),RC(I));
            END;
            GET LIST (N0,N);
            /* N0, POSITION OF ZERO READING IN DATA LIST */
            /* N, NUMBER OF TC/RC DATA PAIRS TO BE
            READ INCLUDING ZERO PAIR */
```

```pli
TWO:BEGIN;
    DCL (T,R,RK,WW,WD)(N);
    A=RØØ-RC(NØ);
    TØ=TC(NØ);
    DO I=1 TO N;
    T(I)=TC(NØ+I)-TØ;
    R(I)=RØØ-RC(NØ+I);
    RK(I)=LOG(A/R(I))/T(I);
    /* RK(I), THE INSTANTANEOUS RATE CONSTANT
    AT TIME T */
    END;
    PUT PAGE LIST (NAME);
    PUT SKIP(2) EDIT ('TOTAL READINGS=', NC,
    'ZERO AT NO', NØ, 'ZERO READING=', RC(NØ),
    'NUMBER TAKEN=',N, 'INFINITY=', RØØ)
    (A,X(2), F(5), X(4), A, X(2), F(4), X(4), A, E(12,5), X(4),
    A, X(2), F(5), X(4), A, E(12,5));
    PUT SKIP(2) EDIT ('TIME', 'READING', 'K')
    (A, X(12), A, X(9), A);
    PUT SKIP;
    DO I=1 TO N;
    PUT SKIP EDIT (T(I), RC(NØ+I), RK(I))
    (E(12,5),X(4));
    END;
    WW=1;
    PUT SKIP(4) EDIT ('DISCARDS:I=')(A);
    M=N;
```

```
MEAN:L=M;
     SY=SUM(WW*RK);
     RKM=SY/M;
     /* RKM, THE MEAN VALE OF THE INSTANTANEOUS
     RATE CONSTANTS */
     WD=WW*(RK-RKM)**2;
     VAR=SUM(WD)/(M-L);
     /* VAR, THE VARIANCE OF INDIVIDUAL VALUES OF
     THE INSTANTANEOUS RATE CONSTANTS */
     PUT SKIP;
     DO I=1 TO N;
     IF WN(I) > 0.0000 THEN IF (RK(I)-RKM)**2
     > (7*VAR) THEN DO;
     WN(I)=0.0000;
     M=M-1;
     PUT EDIT (I) (X(2),F(5));
     END;
     END;
     /* VALUES OF RK FOR WHICH (RK-RKM)**2
     >7*VAR ARE DISCARDED. VALUES OF THE MEAN
     AND VARIANCE ARE THEN RECALCULATED*/
     IF L>M THEN GOTO MEAN;
     VARM=VAR/M;
     /* VARM, THE VARIANCE OF THE MEAN */
     PUT SKIP EDIT ('MEAN') (SKIP,A);
     PUT SKIP EDIT ('K',RKM,'ERROR=',SQRT(VARM))
     (A,E(12,5),X(4));
     END TWO;
     END ONE;
```

```
        GOTO DATA;

    STOP:END RKISNA;
```

Data input to the program takes the following form:

NAME

NC

R$\emptyset\emptyset$

TC(1)

RC(1)

TC(2)

RC(2)

$\vdots$

TC(I)

RC(I)

$\vdots$

TC(NC)

RC(NC)

N$\emptyset$

N

where

'NAME' IS AN IDENTIFYING DESCRIPTION OF THE EXPERIMENT, IT MUST NOT
        EXCEED 80 CHARACTERS IN LENGTH INCLUDING BLANKS.

'NC'   REPRESENTS THE NUMBER OF DATA PAIRS COLLECTED, EXCLUDING
        THE INFINITY READING BUT INCLUDING THE ZERO PAIR.

'R$\emptyset\emptyset$'   IS THE INFINITY READING

TC(I)   REPRESENTS A TIME

RC(I)   REPRESENTS THE READING AT TIME TC(I)

N$\emptyset$ REPRESENTS THE POSITION OF THE NOMINATED ZERO
        READING IN THE LIST OF TC/RC DATA PAIRS

N   REPRESENTS THE NUMBER OF TC/RC DATA PAIRS TO BE USED

        IN THE CALCULATION EXCLUDING THE ZERO PAIR AND THE

        INFINITY READING.

The order of the input data as described above must be maintained in all cases.

Data items should be separated by a space.

More than one set of data items may be submitted.

Input values of TC(I) in units of seconds yield a first-order rate constant with units of s^{-1}.

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Thursday 9th March

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Thursday 4th May

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Tuesday 9th May

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