The Absence of Nucleophilic Catalysis in the Nitrosation of Amides. Kinetics and Mechanism of the Nitrosation of Methylurea and the Reverse Reaction

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Rate constants have been determined for the nitrosation of methylurea (MU) in acid solution and also for the reverse reaction, the denitrosation of *N*-methyl-*N*-nitrosourea (MNU). The nitrosation reaction is essentially irreversible at the low acidities (0.01—0.4M-H_2SO_4) chosen for the experiments whereas the denitrosation reaction was examined at higher acidities (0.5—2.7M-H_2SO_4) in the presence of excess of hydrazine sulphate (a trap for free nitrous acid) when it is irreversible. For nitrosation the rate law, rate = $k[HNO_2][MU][H^+]$ was established and there was no catalysis by substantial concentrations of added potassium bromide or potassium thiocyanate. Similarly the rate law for denitrosation was found to be rate = $k[MNU]h_A$ (where h_A is the acidity function used for the protonation of amides), and again there was no catalysis by added potassium bromide, potassium thiocyanate, or thiourea. The absence of nucleophilic catalysis in the nitrosation of amides had previously been noted and is a puzzling feature when comparison is made with the well established catalysis for amines. This is explained, together with the other observed results, by a detailed consideration of the individual kinetic steps involved in both reactions, and in particular by application of a limiting condition to both forward and reverse reactions in which the actual rate constant for the denitrosation process in an amide is very large compared with the proton transfer to the solvent from the intermediate.

It is well known [1] 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. This is believed to be due to the rapid equilibrium formation of the corresponding nitrosyl halide, *etc.* (NOX), which acts as a more powerful nitrosating agent than does nitrous acid itself. For very reactive amines (*e.g.* some aniline derivatives [2]) the rate constants approach the values expected for diffusion-controlled reactions. There is one report [3] which claims that nitrosation of amides is also subject to halide ion catalysis but more recently a number of workers [4-6] have presented evidence that this is not so although the reason for the difference in behaviour when compared with amines has never been established. Similar patterns of behaviour have been observed for the reverse reactions *i.e.* for the denitrosation of *N*-nitroso-compounds. For the nitrosamines *N*-methyl-*N*-nitrosaniline [7] and *N*-nitrosodiphenylamine,[8,9] denitrosation is very sensitive to the nature and reactivity of the nucleophile (and follows the Pearson nucleophilicity parameter [10] quite well); thiocyanate ion has recently been shown [11] to be effective in bringing about the denitrosation of some alicyclic nitrosamines. However, in contrast, the denitrosation of *N*-n-butyl-*N*-nitrosoacetamide,[4] *N*-nitroso-2-pyrrolidone [12] and *N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide [13] proceed in aqueous acid without any indication of catalysis by halide ion, thiocyanate ion, or thiourea. In these cases it is believed that the initial *N*-protonation is rate determining, whereas for nitrosamines the rate-determining step is the attack by the nucleophile at the nitroso-nitrogen atom of the protonated form of the nitrosamine. It has also been possible to effect this change in rate-determining steps for nitrosamine substrates by (a) changing the solvent from water to ethanol [14] and (b) by working in the presence of high concentrations of the added nucleophile (bromide ion).[8,9] There is also evidence in the diazotisation of aniline derivatives that the kinetic dependence upon the concentration of the nucleophile is lost at high [nucleophile] (a) for reactions in methanol solvent,[15] and (b) when the aniline derivatives contain powerfully electron-attracting substituents.[2]

In order to confirm the absence of nucleophilic catalysis in the nitrosation of amides, and to attempt to explain the mechanistic differences from amine nitrosation reactions, we have undertaken kinetic studies of the nitrosation of methylurea (MU) and of the reverse reaction, the denitrosation of *N*-methyl-*N*-nitrosourea (MNU); the results are presented and discussed in this paper.

EXPERIMENTAL

Methylurea (MU), *N*-methyl-*N*-nitrosourea (MNU), thiourea, potassium bromide, potassium thiocyanate, and hydrazine sulphate were all commercial samples of the highest purity available, and were used without further purification. Fresh solutions of MNU (in ethanol) were prepared daily and were stored in the dark. For the nitrosation of MU the yield of MNU was measured spectrophotometrically at 265 nm after ten half-lives, using a measured extinction coefficient; the reaction was found to be quantitative. In the case of denitrosation of MNU, the product studies involved including *p*-chloroaniline in the reactants, removing samples periodically, and coupling with the sodium salt of 3-hydroxynaphthalene-2,7-disulphonic acid in borax after adjusting the pH. Over the whole acid range studied (0.5—2.7M-H_2SO_4) values of % HNO_2 formed fell in the range 96—102%. Denitrosation is thus quantitative here, with no competing pathway leading to deamination.

The kinetics of nitrosation of MU were followed spectrophotometrically at 31° by noting the appearance of an absorption at 265 nm due to MNU. A typical run is given in Table 1. Similarly denitrosation was followed by noting

the decreasing absorbance at 245 nm with time when excess of hydrazine sulphate was present. A typical run is given in Table 2. All rate measurements were carried out at 31° in the cell block of a Beckman model 25 recording spectrophotometer. Generally rate constants were reproducible to ±5%.

TABLE 1

Typical run for the nitrosation of MU (1.2×10^{-2}M) with $NaNO_2$ (6×10^{-4}M) and H_2SO_4 (0.02M)

t/s	0	30	60	90	120
OD	0.654	0.926	1.116	1.252	1.338
10^2k_0/s^{-1}		1.20	1.20	1.21	1.19
t/s	150	180	210	240	∞
OD	1.406	1.454	1.482	1.506	1.554
10^2k_0/s^{-1}	1.20	1.22	1.20	1.22	

TABLE 2

Typical run for the denitrosation of MNU (2.68×10^{-4}M) with H_2SO_4 (2.15M) containing hydrazine sulphate (1.0×10^{-3}M) and potassium thiocyanate (0.04M)

t/s	0	30	60	90	120	150
OD	0.793	0.741	0.696	0.657	0.623	0.593
10^3k_0/s^{-1}		4.86	4.87	4.87	4.89	4.92
t/s	180	210	240	270	300	∞
OD	0.570	0.547	0.527	0.511	0.497	0.410
10^3k_0/s^{-1}	4.85	4.90	4.94	4.94	4.94	

RESULTS AND DISCUSSION

(a) *Nitrosation of Methylurea* (MU).—These reactions were carried out in aqueous acid solution under the following experimental conditions; H_2SO_4, 0.01—0.04M, $NaNO_2$, 6×10^{-4}M, and MU, 0.012—0.048M. Under these conditions of acidity the reverse reaction is negligibly slow [see part (b)] so that we can neglect step k_{-2} in Scheme 1. This was confirmed in the product analysis study which showed quantitative formation of MNU as measured spectrophotometrically after ten half-lives. All the kinetic experiments gave good first-order plots, so that (not unexpectedly) nitrosation *via* N_2O_3 does not operate under these conditions. The reaction was also first order in [MU] as can be seen by inspection of the results presented in Table 3, where k_0 (defined by $d[MNU]/dt = k_0[HNO_2]$) is given as a function of the initial [MU]. The reaction is also acid catalysed (see Table 4) as expected if nitrosation occurs

TABLE 3

Variation of k_0 with [MU] in the nitrosation of MU at 0.02M-H_2SO_4

10^2 [MU]/M	10^2k_0/s^{-1}
1.20	1.21
2.40	2.38
3.59	3.53
4.78	4.61

by attack of the nitrous acidium ion ($H_2\overset{+}{N}O_2$), the nitrosonium ion (NO^+), or nitrosyl halide on the unprotonated form of MU. The extent of protonation of MU under these conditions is negligible. This establishes the rate law given in equation (1), with $k = 36$ l^2 mol^{-2} s^{-1} at 31°. The [H$^+$] values were obtained from [H_2SO_4] *via* ref. 16. This rate law was also established by Mirvish [17] in a study (by initial rate measurements) concerned with

$$\text{Rate} = k[HNO_2][MU][H^+] \quad (1)$$

the possible involvement of nitrosoureas in human gastric cancer.

We have, further, examined the effects of added potassium thiocyanate and bromide upon the rate constant for nitrosation of MU. Both of these salts give substantial rate increases in the nitrosation (or diazotisation) of amines when present in the concentrations used in this study. The results (Table 5) show

TABLE 4

Acid catalysis in the nitrosation of MU at [MU] 1.20×10^{-2}M

[H_2SO_4]/M	10^2k_0/s^{-1}
0.010	0.50
0.020	1.23
0.030	1.84
0.040	2.44

quite clearly that the rate constant for reaction of MU is virtually unaffected by the presence of these salts. The very slight increase of k_0 with [KBr] probably represents a small salt effect, whilst the small reduction in k_0 with [KSCN] is probably due to the reduction in the effective acidity of the solution by protonation of SCN^-, together with the significant reduction in [HNO_2] by partial conversion to NOSCN, which overcomes the salt effect. These results agree with the earlier observation of Berry and Challis [4] and of Stedman.[6]

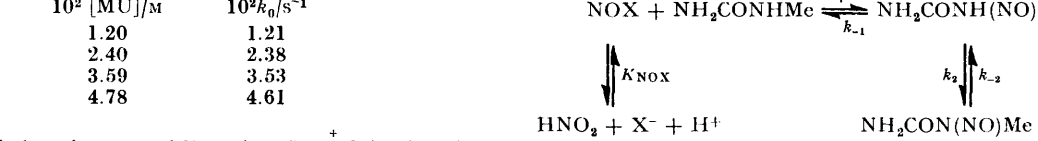
The absence of nucleophilic catalysis for amides can be explained by the full rate equation for nitrosation

TABLE 5

Effect of added KBr and KSCN on the rate constant for nitrosation of MU at 0.02M-H_2SO_4 and [MU] 1.20×10^{-2}M

Added salt	10^2k_0/s^{-1}
0	1.21
0.1M-KBr	1.14
0.2M-KBr	1.28
0.3M-KBr	1.30
0.1M-KSCN	1.13
0.2M-KSCN	1.10
0.3M-KSCN	1.11
0.4M-KSCN	0.98

(Scheme 1). The predicted rate equation is given by equation (2). Catalysis by X^- would disappear if $k_{-1}[X^-] \gg k_2$. This cannot be the full explanation,



SCHEME 1 Step k_{-2} is negligible at the acidities used

since we observe the same k_0 for all cases in Table 5. If however $k_{-1}[X^-]$ is large then the pathway for reaction

$$\text{Rate} = \frac{k_1k_2K_{NOX}[HNO_2][MU][H^+][X^-]}{k_{-1}[X^-] + k_2} \quad (2)$$

via NOX must be reduced in rate considerably. This then allows reaction to occur *via* HNO_2 (*i.e.* $H_2NO_2^+$ or NO^+) where no X^- catalysis is expected. The difference between this situation for amides and that of amines is that the introduction of the C=O group is expected to increase k_{-1} substantially. It will be seen in section (b) that application of the same inequality $k_{-1}[X^-] \gg k_2$ for the reverse reaction (denitrosation of MNU) also explains the observed absence of any nucleophilic catalysis for nitrosamides [4,12] (and a nitrososulphonamide [13]). It is possible that the situation is not quite as simple as we have made out, in that proton transfer to and from the amino-nitrogen atom in the nitrosamide may not in fact be a simple one-stage process. The most likely site of protonation in an *N*-nitroso-compound is the oxygen atom, so that it is conceivable that the *N*-protonated form arises by some sort of $O \longrightarrow N$ rearrangement. There is no definite evidence concerning this point, so we retain Scheme 1 as an outline mechanism.

(b) *Denitrosation of* N-*Methyl*-N-*nitrosourea* (MNU).—Some kinetic results have been reported on the denitrosation reactions of nitrosamides; Berry and Challis [4] examined the reaction of *N*-n-butyl-*N*-nitrosoacetamide and found two concurrent pathways, one leading to denitrosation and the other to deamination. The denitrosation pathway was not catalysed by added

TABLE 6

The yield of HNO_2 produced from MNU (5.5×10^{-4}M) in H_2SO_4 (0.539M) containing sodium bromide (0.025M)

t/s	% HNO_2	10^4k_0/s^{-1}
90	3	2.9
660	31	5.5
1 440	55	5.6
2 940	81	5.7
4 800	93	5.6
7 320	98	5.3
13 080	100	

sodium chloride. Similarly for the related *N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide [13] no chloride ion, bromide ion, or thiourea catalysis was found. We have now examined the denitrosation of MNU (a) to confirm the generality of absence of such catalysis for nitrosamides and (b) so that comparison can be made with the forward reaction, the nitrosation of MU discussed earlier in section (a).

The decomposition of MNU in aqueous solution has been studied by McCalla *et al.*[18] at various pH values. Decomposition is encouraged by light, so that in our work stock solutions (in ethanol) were stored in the dark. These solutions decomposed to the extent of *ca.* 9% over 8 h, but since our kinetic experiments typically were followed for 2—20 min, the decomposition rate was considered negligible; fresh stock solutions were however prepared daily.

Berry and Challis [4] and Challis and Jones [12] found that both *N*-n-butyl-*N*-nitrosoacetamide and *N*-nitroso-2-pyrrolidone gave substantial amounts of deamination as well as denitrosation. We followed the appearance of free nitrous acid from MNU by carrying out the denitrosation in the presence of excess *p*-chloroaniline and coupling portions with 3-hydroxynaphthalene-2,7-disulphonic acid. The results for one such experiment are given in Table 6. Clearly the yield of nitrous acid increases regularly towards 100%. Further the average value of the first-order rate constant (excluding the first point) for the few points taken is 5.6×10^{-4} s^{-1} which agrees exactly with the value obtained (at the same acidity) by following the disappearance of MNU directly (see Table 7). Similarly the yield of nitrous acid was

TABLE 7

Acid catalysis in the denitrosation of MNU

$[H_2SO_4]$/M	10^3k_0/s^{-1}
0.539	0.56
1.072	1.40
1.613	2.91
2.148	4.64
2.694	7.49

determined at each of the following acidities: 1.07, 1.61, 2.15, 2.69M-H_2SO_4. Final values were found to vary between 96 and 102% and in each case the rate constant agreed with that given in Table 7.

In order to avoid any complication due to reversibility of the denitrosation the main kinetic experiments were carried out in the presence of excess of hydrazine sulphate. This nitrite trap has the advantage over *p*-chloroaniline in that it does not absorb in the region of the spectrum where MNU does absorb. It was found that as long as $[N_2H_5^+] > [MNU]$ then the reaction was zero-order in $[N_2H_5^+]$. Good first-order plots were obtained in every case. Values of k_0 (defined by $-d[MNU]/dt = k_0[MNU]$) as a function of the acidity are given in Table 7. Clearly the reaction is acid-catalysed. The plot of log k_0 *versus* the H_A acidity function gives the best line with slope −0.93. For the reactions of nitrosamines the H_0 acidity function seems the more suitable whereas the H_A function correlation was also found [4] for the denitrosation of *N*-n-butyl-*N*-nitrosoacetamide in perchloric acid.

There was no catalysis by added KBr, KSCN, or $SC(NH_2)_2$ as shown by the data in Table 8, for reaction

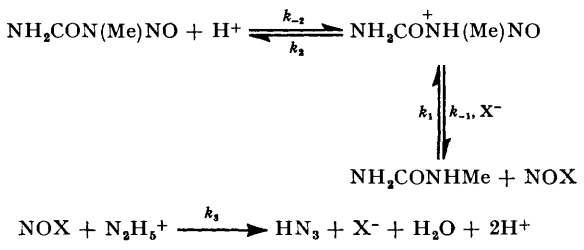
TABLE 8

Test for catalysis by Br^-, SCN^-, and $SC(NH_2)_2$ in the denitrosation of MNU at $[H_2SO_4]$ 2.148M

Added nucleophile	10^3k_0/s^{-1}
0	4.64
0.1M-KBr	4.73
1.0M-KBr	4.88
0.02M-KSCN	4.70
0.04M-KSCN	4.90
0.002M-$SC(NH_2)_2$	4.64
0.004M-$SC(NH_2)_2$	4.83

in 2.148M-H_2SO_4. The small changes, which in many cases are within the experimental error of measurement, are at best due to small salt effects. Similar concentration of KBr, *etc.* produced very large kinetic effects for denitrosation of *N*-methyl-*N*-nitrosoaniline [7] (NMNA) and *N*-nitrosodiphenylamine.[8] For example the addi-

tion of bromide ion (0.01M) increases k_0 by about ten-fold in the denitrosation of NMNA and similarly thiocyanate ion (0.001M) increases k_0 by *ca.* 100. Scheme 2 shows the probable outline mechanism for denitrosation which explains the observed facts. Again we have written the

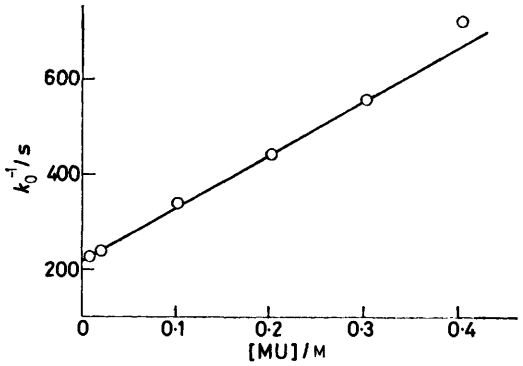


SCHEME 2 Step k_1 negligible in the presence of excess $N_2H_5^+$

initial *N*-protonation of MNU as a one-stage process for simplicity and have not considered *O*-protonation. In the presence of excess of hydrazine sulphate step k_1 can be ignored. The product analysis for free nitrous acid produced showed quantitative denitrosation at all acidities studied and so we have no competing path here of the deamination reaction. Under these conditions k_0 is given by equation (3). Catalysis by X^- disappears only if $k_{-1}[X^-] \gg k_2$, when equation (3) reduces to

$$k_0 = \frac{k_{-2}h_A k_{-1}[X^-]}{k_2 + k_{-1}[X^-]} \qquad (3)$$

$k_0 = k_{-2}h_A$, which is the form observed experimentally. Scheme 2 of course is the reverse sequence of steps shown in Scheme 1 with the added reaction of the decomposition of NOX by the nitrite trap added. We have retained the same rate constant symbols for identical reactions in



Plot of k_0^{-1} against [added MU] in the denitrosation of MNU

both Schemes. It can be seen that application of the same inequality, $k_{-1}[X^-] \gg k_2$, for both Schemes results in the same form of the rate equation as is observed in practice, the essential features of which are for amides and nitrosoamides, acid catalysis, and no nucleophilic catalysis.

The overall reversibility of denitrosation of MNU was investigated briefly by noting the variation in k_0 as a function of added excess MU. The results are in Table 9, where it is apparent that k_0 is decreased significantly by the addition of MU. Under these circumstances it is clear that step k_1 in Scheme 2 is not now insignificant and so equation (3) is not valid. The full rate expression for k_0 [equation (4)] can be deduced from steady state treatments on both $NH_2\overset{+}{C}ONH(Me)NO$ and NOX intermediates. This has been tested, at least in part, by using the data from Table 9, by plotting k_0^{-1} against

$$k_0 = \frac{k_{-2}h_A k_{-1}[X^-]k_3[N_2H_5^+]}{(k_2 + k_{-1}[X^-])k_3[N_2H_5^+] + k_1k_2[MU]} \qquad (4)$$

[MU]. The expected linear relationship is shown in the Figure 1, with a positive slope and intercept. A similar term arises for reaction *via* HNO_2. The presence of the $[X^-]$ term in equation (4) arises since the reaction of NOX with hydrazine will always be catalysed by X^-.

TABLE 9

Effect of added MU in the denitrosation of MNU

[MU]/M	10^3k_0/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
0.402	1.38

Reaction conditions, $[H_2SO_4]$ 2.148M, [MNU] 2.71×10^{-4}M, [Hydrazine sulphate] 1.013×10^{-3}M

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