The Chemistry of Nitroso Compounds. Part 13.[1] Decomposition of *N*-Nitroso-2-pyrrolidone under Basic Conditions, an Unusual Example of Nucleophilic Catalysed Hydrolysis of an Amide Derivative

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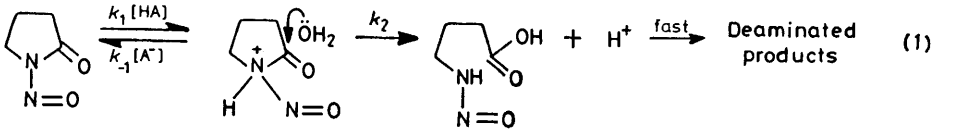
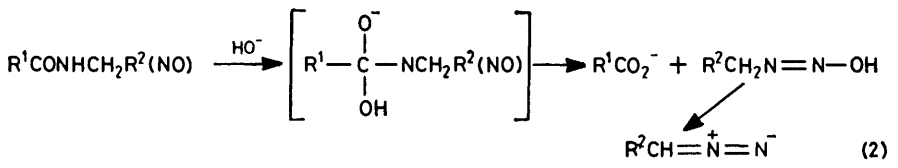
Kinetic studies are reported for the decomposition of *N*-nitroso-2-pyrrolidone in neutral and alkaline aqueous buffer solutions at 25 °C, where only products of deamination (hydrolysis) are obtained. This reaction is shown to be catalysed by basic entities and the Brønsted plot so generated has a slope $\beta = 0.66$. The absence of significant catalysis by sterically hindered bases (*e.g.* 2,6-lutidine), the strong catalysis by imidazole relative to HPO_4^{2-} ($k_{Imidazole}/k_{HPO_4^{2-}} = 83$) and by hydroxide ion relative to imidazole ($k_{HO^-}/k_{Imidazole} = 4\,300$), and the observation of a second-order catalytic term for imidazole {Rate = [*N*-nitroso-2-pyrrolidone] (k_A-[Imidazole] + k'_A-[Imidazole]^2} are interpreted in terms of an addition–elimination pathway involving *nucleophilic* attack by the catalyst at the carbonyl carbon atom. The preference for nucleophilic rather than general base-catalysed hydrolysis is related to the enhanced leaving properties of the *N*-nitrosamine fragment.

N-NITROSAMIDES, such as *N*-nitroso-2-pyrrolidone, are relatively unstable compounds of current interest as potential metabolities of carcinogenic *N*-nitrosamines.[2] They are known to decompose thermally *via* a diazo-ester intermediate,[3] but this reaction is unlikely to be of biological consequence at 37 °C. More recently, we have shown that *N*-nitroso-2-pyrrolidone readily undergoes concurrent denitrosation and deamination by acid-catalysed pathways in aqueous solutions at pH < 3 and 25 °C.[4] The deamination (or hydrolysis) reaction, which is relevant to the present investigation, involves hydrolytic cleavage by an S_N2 displacement on the *N*-conjugate acid [equation (1)], whose formation (step k_1) is rate limiting under most conditions.[4] This reaction is unusual in that acid-catalysed hydrolysis of amidic compounds is commonly supposed [5] to occur by an addition–elimination reaction of an *O*-conjugate acid formed in a rapid pre-equilibrium step. However, the strong electron withdrawing properties of the *N*-nitroso substituent would be expected to make both protonation of the substrate more difficult and cleavage of the C–N bond easier.



Hydrolysis of amides under neutral and alkaline conditions occurs by a base-catalysed pathway,[6] which should be particularly favourable for *N*-nitroso compounds because of the enhanced leaving ability of the amino fragment. Indication to this effect comes from the ready generation of diazo-alkanes from *N*-alkyl-nitrosamides in alkaline solution [equation (2)] [7] and from our earlier observation of base-catalysed decomposition of *N*-nitroso-2-pyrrolidone in carboxylic acid buffers.[4] The mechanism of this decomposition is not known, although by analogy with ordinary amides an addition–elimination pathway [equation (2)] involving a tetrahedral intermediate derived from the neutral substrate seems likely.[6a] We have therefore extended our kinetic measurements for the decomposition of *N*-nitroso-2-pyrrolidone to a wider range of alkaline and neutral buffers where the mechanism of the base catalysis can be examined in detail. In view of several findings [8] that *N*-nitrosamines effect alkylation of DNA components *in vivo*, these reactions may be of particular significance to their mechanism of carcinogenesis.



EXPERIMENTAL

N-Nitroso-2-pyrrolidone was prepared and purified as previously described.[4] Reaction solutions were prepared from AnalaR $ClCH_2CO_2H$, HCO_2H, $MeCO_2H$, NaH_2PO_4, $Na_2B_4O_7$, pyridine, $HClO_4$, and NaOH reagents without further purification other than vacuum drying where appropriate. Otherwise, reagent grade buffer components, purified by conventional methods, were used. Ionic strengths were adjusted with dried AnalaR $NaClO_4$ and the pH of the reaction solutions was checked with a Radiometer model 26 meter using glass–calomel electrodes.

Decomposition rates were measured spectrophotometrically from the decrease in substrate absorbance at either 253 or 426 nm using a Unicam SP 1800 spectrophotometer with cells thermostatted to 25 ± 0.1 °C. The details have been described previously.[4] The reaction solutions were checked quantitatively for nitrous acid (indicative of denitrosation concurrent with hydrolytic decomposition) by adding

sulphanilamide or *p*-chloroaniline and estimating the amount of diazo product produced by coupling. As noted earlier,[4] significant denitrosation was observed only for reaction in Cl_2CHCO_2H and $ClCH_2CO_2H$ buffers.

RESULTS AND DISCUSSION

Our earlier studies showed the base (or nucleophilic) catalysed deamination made a small, but significant, contribution to the overall decomposition rate of *N*-nitroso-2-pyrrolidone in both Cl_2CHCO_2H and $ClCH_2CO_2H$ buffers, and was the sole decomposition pathway in aqueous HCO_2H and $MeCO_2H$ buffers at 25 °C.[4] Decomposition catalysed by bases stronger than formate ion (pK_a 3.75) should therefore follow relatively uncomplicated kinetics and result in the formation of a single product. With the exception of imidazole-catalysed decomposition, both expectations are met for catalysts ranging from pyridine (pK_a 5.17) to n-butylamine (pK_a 10.59).

For all the catalysts examined, good first order plots [equation (3)] in excess of 90% reaction were obtained from the experimental results and no HNO_2 was found to be released. Generally, rates were measured at 25 °C for several different catalyst concentrations at a fixed pH

$$\text{Rate} = k_0[N\text{-Nitroso-2-pyrrolidone}] \quad (3)$$

(*i.e.*, constant buffer ratio [HA]/[A⁻]) with the ionic strength μ 1. These results are summarised in Table 1. Except for decomposition in imidazole buffers where a more complex kinetic relationship prevails, it is apparent that k_0 has a first-order dependence on catalyst concentration [A⁻], so the full kinetic expression is given by equation (4). Values of k_{A^-} obtained from plots of k_0

$$\text{Rate} = k_{A^-}[N\text{-Nitroso-2-pyrrolidone}][A^-] \quad (4)$$

versus [A⁻] are summarised in Table 2 together with those obtained previously[4] for $CF_3CO_2^-$, $ClCH_2CO_2^-$, $Cl_2CHCO_2^-$, HCO_2^-, and $MeCO_2^-$.

No catalysis was observed for decomposition in 2,6-lutidine buffers (Table 1). The plot of k_0 *versus* [2,6-Lutidine] has, in fact, a negative slope, probably owing to a specific retarding salt effect by the 2,6-lutidinium ion. The ineffectiveness of the 2,6-lutidine has important mechanistic implications which are discussed further below. Catalysis by hydroxide ion could not be accurately ascertained from measurements in dilute NaOH because of the rapidity of these reactions, but this datum (Figure 1) was obtained from experiments in tetraborate buffers at pH 9.0, 9.18, 9.30, and 9.40 (Table 1). These results show further that the tetraborate base

TABLE 1

Decomposition rates of *N*-nitroso-2-pyrrolidone in aqueous buffers at 25 °C and μ 1; initial [Substrate] 1—2 × 10^{-4}M

[Buffer base]/M	Buffer ratio ([HA]/[A⁻])	$10^5k_0/s^{-1}$
Pyridine [a]		
0.05	1.0	3.91
0.07	1.0	4.53
0.10	1.0	5.20
0.15	1.0	6.67
2,6-Lutidine [a]		
0.1	1.0	11.9
0.2	1.0	10.4
0.3	1.0	9.44
0.4	1.0	8.8
Phosphate (HPO_4^{2-})		
0.025	1.0	11.0
0.050	1.0	16.2
0.100	1.0	30.9
0.150	1.0	42.1
Imidazole		
(a) In imidazole buffer at pH 7.05		
0.01	1.0	85.0
0.02	1.0	214
0.04	1.0	568
0.05	1.0	722
0.06	1.0	950
0.08	1.0	1 300
0.10	1.0	1 890
(b) In phosphate buffer at pH 7.04		
0		20
0.0052		61.4
0.0084		87.9
0.0105		106
Morpholine		
0.005	1.0	257
0.007	1.0	307
0.010	1.0	395
0.015	1.0	576
Sodium tetraborate ($B_4O_7^{2-}$)		
0.019	1.0	1 010
0.025	1.0	950
0.031	1.0	1 070
0.050	1.0	1 200
0.0066	0.661	1 340
0.073	0.661	1 410
0.032	0.661	1 430
0.0602	0.661	1 710
0.040	0.501	1 960
0.053	0.501	2 230
0.083	0.501	2 510
0.100	0.501	2 650
0.043	0.398	2 370
0.057	0.398	2 380
0.072	0.398	2 660
0.089	0.398	2 680
0.107	0.398	2 990
n-Butylamine		
0.0138	13.5	10 530
0.0207	13.5	14 510

[a] Initial [Substrate] = 1—2 × 10^{-3}M.

TABLE 2

Catalytic rate coefficients (k_{A^-}) for the decomposition of *N*-nitroso-2-pyrrolidone at 25 °C

Catalyst (A⁻)	pK_a	$10^3k_{A^-}$/l mol^{-1} s^{-1}
$CF_3CO_2^-$	0.22	0.171 [a]
$Cl_2CHCO_2^-$	1.37	0.031 [a]
$ClCH_2CO_2^-$	2.87	0.049 [a]
HCO_2^-	3.75	0.121 [a]
$MeCO_2^-$	4.75	0.157 [a]
Pyridine	5.25	0.305
2,6-Lutidine	6.77	
Imidazole	7.05	204
HPO_4^{2-}	7.21	2.45
Morpholine	8.39	348
$B_4O_7^{2-}$	9.00	60
n-Butylamine	10.59	5 717
HO^-	15.75	880 000 [b]

[a] From ref. 4. [b] From reaction in tetraborate buffers.

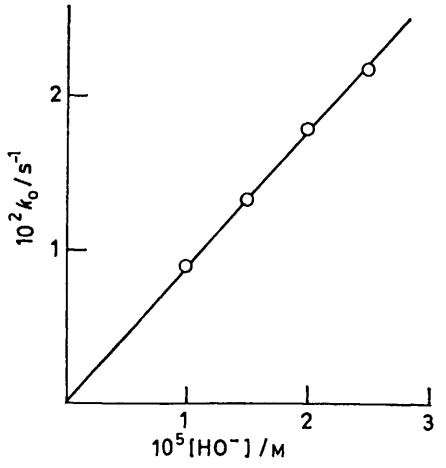


FIGURE 1 HO$^-$-Catalysed decomposition of *N*-nitroso-2-pyrrolidone in tetraborate buffers at 25 °C

also mildly catalyses the decomposition reaction. As noted above, decomposition of *N*-nitroso-2-pyrrolidone in imidazole buffers was exceptional in that k_0 increased more rapidly than expected for a simple first-order dependence on [Imidazole] (Figure 2). The same trend was apparent also from experiments (Table 1) where imidazole was added to phosphate buffer at pH 7.04. From related observations for both ester and amide hydrolyses,[6a] it seemed likely that the imidazole catalysed decomposition followed equation (5) containing both a first- and a second-order imidazole term. This possibility was tested by means of equation (6), derived

$$\text{Rate} = [N\text{-Nitroso-2-pyrrolidone}]\text{-}\{k_{A^-}\text{-}[\text{Imidazole}] + k'_{A^-}\text{-}[\text{Imidazole}]^2\} \quad (5)$$

$$k_0/[\text{Imidazole}] = k_{A^-} + k'_{A^-}\text{-}[\text{Imidazole}] \quad (6)$$

directly from equations (3) and (5). The relevant plot of k_0/[Imidazole] *versus* [Imidazole] also shown in Figure 2 is sharply curved, which requires that the k'_{A^-}-term be unimportant at high buffer base concentrations.

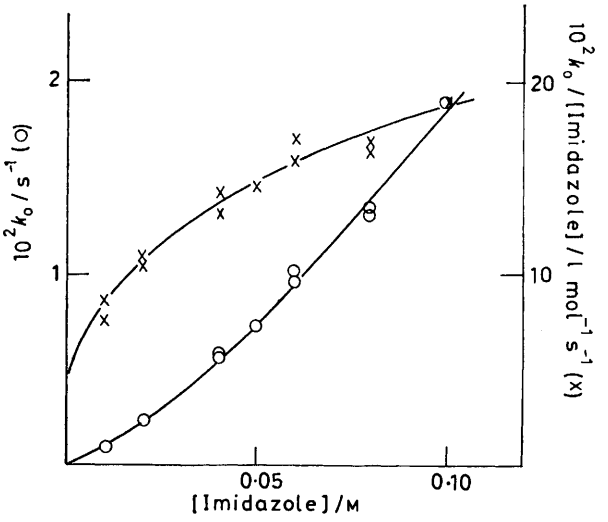
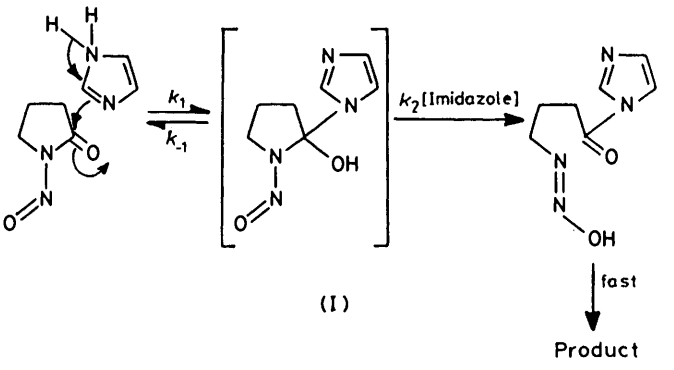


FIGURE 2 Decomposition of *N*-nitroso-2-pyrrolidone in imidazole buffers at 25 °C

Mechanism.—These new results confirm that the decomposition of *N*-nitroso-2-pyrrolidone is catalysed by basic entities and they also throw some light on the mechanism by which the catalysis occurs. From related studies of the hydrolyses of many other carboxylic acid derivatives,[9] it seems probable that an addition–elimination pathway involving a tetrahedral intermediate is followed, and the results in imidazole buffers support this conclusion strongly. The curvature of Figure 2 rules out decomposition *via* concurrent first- and second-order imidazole catalysed pathways, but it is consistent with a stepwise mechanism (such as the Scheme) where a second imidazole molecule assists in decomposing the tetrahedral intermediate (I) to products. The change in the kinetic dependence on [Imidazole] implied by the curvature of Figure 2 then relates to a shift in the rate-limiting step from k_2 at low [Imidazole] to k_1 at high [Imidazole]. This kinetic phenomenon has been widely reported for the hydrolysis of simple amides.[6a] Further, the observation of a second-order imidazole dependence



SCHEME Addition–elimination pathway for the nucleophilic catalysed decomposition of *N*-nitroso-2-pyrrolidone

requires that formation of the tetrahedral intermediate (step k_1) involves participation of imidazole as a nucleophilic catalyst (rather than as a general base) as shown in the Scheme. It follows that the dependence of k_0 on the rate coefficients for the individual steps of the Scheme is given by equation (7) which on taking reciprocals leads to equation (8). Significantly, the plot of [Imidazole]/k_0 *versus* 1/[Imidazole] is linear from which a value of k_1 = 0.204 l mol^{-1} s^{-1} is obtained from the intercept. This

$$k_0 = k_1k_2[\text{Imidazole}]^2/(k_{-1} + k_2[\text{Imidazole}]) \quad (7)$$

figure is cited in Table 3 as k_{A^-} for imidazole catalysis. From the slope of [Imidazole]/k_0 *versus* 1/[Imidazole] a value of k_2/k_{-1} = 57.4 mol l^{-1} is obtained, which shows that collapse of the tetrahedral intermediate to products is highly favoured as expected for the good nitrosamino leaving group.

$$[\text{Imidazole}]/k_0 = k_{-1}/k_1k_2[\text{Imidazole}] + 1/k_1 \quad (8)$$

The above conclusion as to the mechanism of catalysis is nicely corroborated by the relative magnitudes of several catalytic rate coefficients listed in Table 3. In particular, the ineffectiveness of 2,6-lutidine (pK_a 6.77) relative to pyridine (pK_a 5.17) and the very small

catalysis by tetraborate ion should be noted. Both observations suggest that steric hindrance strongly attenuates catalytic efficiency as would be expected for a nucleophilic catalysed pathway. The relative magnitudes of the catalytic coefficients for imidazole, HPO_4^{2-} and HO^- have been cited [9] as good indices of the type of catalysis associated with the hydrolysis of carboxylic acid derivatives, and here too, the values for

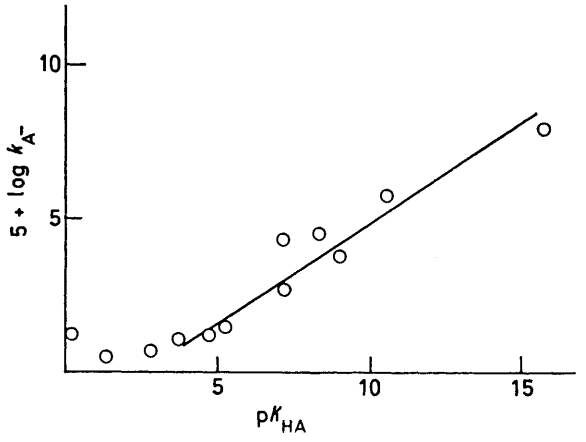


FIGURE 3 Brønsted plot for the decomposition of *N*-nitroso-2-pyrrolidone at 25 °C

N-nitroso-2-pyrrolidone [$k_{Imidazole}/k_{HPO_4^{2-}} = 83$ and $k_{HO^-}/k_{Imidazole} = 4\ 300$] are indicative of a nucleophilic catalysed reaction.

The hydrolyses of simple amides under alkaline conditions usually proceed by a general base catalysed reaction path,[6a] so the observation of nucleophilic catalysis in the present instance is exceptional. This difference of mechanism is undoubtedly related to the presence of an *N*-nitroso substituent which must increase the leaving ability of the amino fragment substantially. An increased susceptibility towards nucleophilic catalysis for compounds bearing good leaving groups is well established [9] and, significantly, other examples of nucleophilic catalysed amidic hydrolyses usually involve compounds such as the acetylimidazolium ion [10] where the fragment expelled carries a positive charge.

With the exception of sterically hindered bases (*e.g.*, 2,6-lutidine, tetraborate ion), the catalytic rate coefficients (k_{A^-}) increase with the base strength of A^- (Table 3). An interesting observation is that the Brønsted plot (Figure 3) obtained from these coefficients may well be curved as the slope $\beta = 0.66$ obtained for the more basic catalysts is substantially higher than that deduced previously (β *ca.* 0.2) from limited data for weakly basic carboxylate ions.[4] This is tentative evidence for a change of mechanism with catalytic reactivity, with general base catalysis for the weaker bases in contrast to nucleophilic catalysis for the stronger ones. Precedents for this effect with other substrates are well documented [9] and, significantly, the changeover of mechanism often occurs with catalyst pK_a 4—5 as in the present case.

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REFERENCES

[1] Part 12, B. C. Challis and S. A. Kyrtopoulos, *J.C.S. Perkin II*, 1978, 1296.
[2] R. Schoental, *Brit. J. Cancer*, 1973, **28**, 436.
[3] E. H. White and D. J. Woodcock, ' Chemistry of the Amino Group,' ed. S. Patai, Wiley, London, 1968, p. 407; J. I. G Cadogan, *Accounts Chem. Res.*, 1971, **4**, 186.
[4] B. C. Challis and S. P. Jones, *J.C.S. Perkin II*, 1975, 153.
[5] For a recent resumé see A. Williams, *J. Amer. Chem. Soc.*, 1976, **98**, 5645.
[6] (*a*) B. C. Challis and J. A. Challis, ' Chemistry of the Amides,' ed. J. Zabicky, Wiley, London, 1971, p. 731; (*b*) C. J. O'Connor, *Quart. Rev.*, 1971, **24**, 553.
[7] T. J. Lobl, *J. Chem. Educ.*, 1972, **49**, 730.
[8] P. D. Lawley, ' Chemical Carcinogens,' ed. C. E. Searle, A.C.S. Monograph 173, 1976, ch. 4.
[9] S. L. Johnson, *Adv. Phys. Org. Chem.*, 1967, **5**, 237.
[10] J. Gerstein and W. P. Jencks, *J. Amer. Chem. Soc.*, 1964, **86**, 4655; W. P. Jencks, F. Barley, R. Barnett, and M. Gilchrist, *ibid.*, 1966, **88**, 4464.