Nitrosation under Alkaline Conditions

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Summary Both gaseous N_2O_3 and N_2O_4, but not NO, are shown to effect the nitrosation of primary and secondary amines in neutral and alkaline aqueous solutions; the reaction rates are rapid, insensitive to amine basicity and are not inhibited by HO^-, which is consistent with radical pathways involving NO_2 and NO, and therefore carcinogenic *N*-nitrosamines may form under a much wider range of experimental conditions than hitherto suspected.

GASEOUS oxides of nitrogen are often used to effect either nitrosation or nitration of organic substrates,[1] but little is known about the mechanism of their reactions in solution. Generally organic solvents are preferred for synthesis,[1] possibly because of expectations that nitrogen oxides rapidly hydrolyse in neutral or alkaline aqueous solution. In turn, this has led to a widespread belief that *N*-nitrosamine formation (or deamination and diazotisation) will only occur rapidly in acidic aqueous solutions[2] where, for example, N_2O_3 is in equilibrium with HNO_2 [equation (1)].[3] Although both NO and NO_2 bear unpaired electrons the realisation of rapid nitrosation and nitration of amines by free radical processes that are largely independent of solvent has not been reported hitherto.

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O \quad (1)$$

Reaction of NO with secondary amines was examined in carefully degassed MeCN and EtOH at 25 °C. A study of the variation of % reaction with time for piperidine, morpholine, and diphenylamine in MeCN shows that the reaction to form the corresponding *N*-nitrosamine is very slow ($t_{\frac{1}{2}}$ *ca.* 8 days), is independent of amine reactivity, and follows zeroth order kinetics. The introduction of a small amount of air or oxygen into the reaction vessel, however, results in 100% *N*-nitrosamine formation in less than 4 min, which suggests that the reactions observed stem from slow leakage of air into the reaction vessel resulting in the oxidation of NO to a more effective reagent. Thus NO, itself, appears to be a very poor nitrosating agent, presumably because it is unable to abstract amino-hydrogen atoms. Previous observations to the contrary[4] must arise from the presence of oxygen in the reaction solutions.

Oxidation of NO leads initially to NO_2 and, in turn, to the formation of N_2O_3 and N_2O_4. We have confirmed that *N*-nitrosamine formation from gaseous N_2O_3 and N_2O_4 is

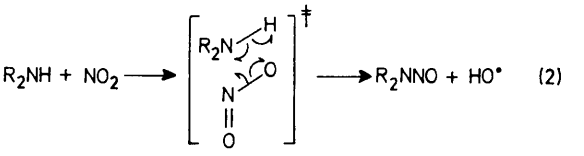
rapid and quantitative in organic solvents at 25 °C, but their reaction in aqueous alkali is both more significant and mechanistically informative. Amines of widely different basicity compete effectively with both H_2O and HO^- for the nitrogen oxides to yield substantial amounts (8—70%) of *N*-nitrosamine or diazonium ion in < 5 min at 25 °C. A competitive product, particularly with N_2O_4, is the corresponding *N*-nitramine, whose concentration is increased with more dilute nitrogen oxides. No reaction is found with piperidine (pK_a 11·12)[5] in phosphate buffer (pH 6·85), but nitrosation of the less basic *N*-methylpiperazine (pK_a 5·11)[5] and *p*-nitroaniline (pK_a 0·99)[5] is not inhibited by reducing the pH. These pH dependencies show that only the unprotonated amine is reactive.

TABLE. Nitrosation of various amines by gaseous N_2O_4 and N_2O_3 in 0·1M-NaOH at 25°. Initial [amine] = 20—5 × 10^{-4} M, $P(N_2O_4) = P(N_2O_3) = 0·083$ atm.

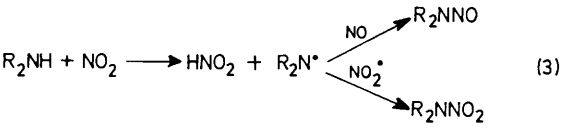
Amine	pK_a	% Nitrosation after 5 min.[a] N_2O_4	N_2O_3
Piperidine	11·12	39	64
Morpholine	8·33	19	—
N-Methylpiperazine ..	5·11	33(44)[b]	51(59)[b]
Aniline	4·6	27	47
p-Nitroaniline ..	0·99	25(38)[b]	37(40)[b]
Diphenylamine ..	0·79	6[c]	—

[a] Reactions were carried out by injecting 5 ml of gaseous nitrogen oxide at 1 atm into sealed 60 ml conical flasks containing 5 ml of reaction solution. The flask contents were shaken for 5 min, after which samples were extracted for g.l.c. or colorimetric analysis. [b] Figures in parentheses for reaction in phosphate buffer, pH 6·85. [c] Isolated from 8 products by t.l.c. prior to analysis.

The most striking feature of the reactions is their insensitivity to amine basicity; for example, similar amounts of nitrosation are observed for piperidine and *p*-nitroaniline.[5] This behaviour contrasts markedly with the diazotisation of aromatic amines in acidified nitrite, where the reaction is believed to involve N_2O_3, and aniline (pK_a 4·6)[5] is *ca.* 10^3 times more reactive than *p*-nitroaniline.[6] This difference, together with the inability of HO^- to compete with the amino substrates (in particular, compare the reactions of *p*-nitroaniline at pH 6·85 and in 0·1M-NaOH), seems to exclude any nitrosation mechanism involving nucleophilic attack on undissociated N_2O_3 or N_2O_4. As both oxides are largely dissociated in the gaseous phase,[7] their constituent radicals (NO and NO_2) are probably responsible for the reactions observed in solution. Further, the inability of NO, itself, to effect amine nitrosation, suggests that either NO_2 reacts directly *via* a four-centre transition state [equation (2)] or it generates an amino radical that combines with both NO and NO_2 [equation (3)]. The observation of *N*-nitramines as competitive products is consistent with equation (3), but the mechanism requires further investigation.



(2)



(3)

Our results show that nitrogen oxide nitrosation is feasible in neutral and alkaline solutions and, indeed, *N*-nitrosodialkylamine formation is very much faster here than under conventional acidic conditions.[8] A very important consequence, bearing in mind that nitrogen oxides are common pollutants arising from most combustion processes, is that carcinogenic *N*-nitrosamines may readily form under a much wider range of experimental conditions (including cellular pH) than hitherto suspected. Further, decomposition of HNO_2 results in the formation of NO and NO_2,[9] so it is possible that free-radical pathways also contribute to nitrosation (*e.g.* diazotisation) under acidic conditions.

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