

The Chemistry of Nitroso-compounds. Part 11.¹ Nitrosation of Amines by the Two-phase Interaction of Amines in Solution with Gaseous Oxides of Nitrogen

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The formation of secondary *N*-nitrosoamines when MeCN solutions of amines are brought into contact with gaseous NO, N₂O₃, and N₂O₄ at 25 °C is reported. With NO, *N*-nitrosoamine formation from piperidine, morpholine, and diphenylamine occurs very slowly (*t*_{1/2} ca. 8 days). Reaction rates are largely independent of the amine, suggesting that oxidation of NO by adventitious oxygen is the slow step. Very much faster reactions are observed, however, with N₂O₃ and N₂O₄. With a ca. 10-fold excess of N₂O₃ or N₂O₄, quantitative yields of both *N*-nitrosopiperidine and *N*-nitrosodiphenylamine are found in less than 3 min. With a 27-fold excess of piperidine and N₂O₄ only, ca. 8% *N*-nitropiperidine and 92% *N*-nitrosopiperidine are obtained. Rapid reactions also ensue when solutions of either primary aromatic or secondary amines dissolved in 0.1M aqueous NaOH are brought into contact with gaseous N₂O₃ and N₂O₄. With a ca. 2–400-fold excess of these nitrogen oxides, 12–65% of the amine is converted either to *N*-nitrosoamine or diazonium ion in less than 3 min. Competitive hydrolysis of the nitrogen oxide by the solvent is not HO⁻-catalysed and the amine : H₂O reactivities are in ca. 1 000 : 1. The extent of *N*-nitrosation varies insignificantly over a wide range of basicities (p*K*_a 11.12 to -1.0), but no reaction occurs with either 2,4-dinitroaniline or *N*-butyl-acetamide. With N₂O₄, smaller amounts of *N*-nitroamines form concurrently and increase with decreasing amine basicity. The results are discussed in relation to amine nitrosation by N₂O₃ and N₂O₄ in aqueous acidic solutions. It is suggested that the lower selectivity for the dissolved gaseous reagents may relate to the presence of more reactive N₂O₃ and N₂O₄ isomers. The results also show that carcinogenic *N*-nitrosoamines form under a much wider range of experimental conditions than previously known, some of which are relevant to atmospheric pollution.

MUCH recent interest in the occurrence and formation of *N*-nitrosoamines has originated from their possible implication in human cancer.² Their formation in aqueous solutions is generally presumed³ to require acidic conditions (pH < 5) where nitrite is converted to nitrous acid and H₂ONO⁺ (nitrous acidium ion) exists, albeit at low concentration. These reactions, together with the related deamination and diazotisation of primary amines, have been extensively investigated and reviewed.^{3,4} With one or two exceptions, nitrosation appears to involve the unprotonated amine and a reagent such as N₂O₃, NOCl, H₂ONO⁺, or NO⁺ (nitrosonium ion) existing in equilibrium with both HNO₂ and NO₂⁻, neither of which is a reactive entity. For the more basic amines (p*K*_a ≥ ca. 5) only reaction by N₂O₃ seems to be significant and several investigations^{3,5} have found maximum rates in the region pH 3–4. The other nitrosating agents are effective at lower pH with less basic amines (particularly aromatic amines) and some of these reactions appear to be encounter-controlled and therefore very rapid.⁴ Under strongly acidic conditions, there is evidence that the conjugate acids of aromatic amines also react with the more powerful nitrosating agents such as H₂ONO⁺ and NO⁺.⁶ *N*-Nitrosoamine formation (or deamination and diazotisation) under neutral or alkaline aqueous conditions is exceptional because all the effective reagents cited above derive from the nitrous acidium ion (H₂ONO⁺), e.g. NO₂⁻ + H₂ONO⁺ ⇌ N₂O₃ + H₂O. Nonetheless, *N*-nitrosoamine formation above pH 5 has been reported in the presence of either formaldehyde or chloral, but these reactions are very slow (typically, ca. 1% *N*-nitrosodiethylamine after 17 h at pH 6.4 and 24 °C) and their mechanism is uncertain.⁷

Gaseous oxides of nitrogen are known to effect both nitrosation and nitration of organic materials under mild

conditions,⁸ but their reactions with amines have not been exhaustively examined. White and Feldman⁹ found rapid *N*-nitrosoamine formation from N₂O₄ in organic solvents, with competitive *N*-nitroamine formation increasing as the reaction temperature was lowered to -70 °C. These reactions have been exploited recently in the direct synthesis of alkyl nitrates from primary amines.¹⁰ In the synthesis of *N*-nitrosoamines, however, more use has been made of nitric oxide (NO),¹¹ particularly in conjunction with metal catalysts under patented procedures.¹² From kinetic studies of the reaction between diethylamine and NO in the presence of Cu^{II} salts, Brackman and Smit¹³ deduced that nitrosation was brought about by a copper-nitrosyl salt in which NO was effectively oxidised to NO⁺. This accords with several observations that metal nitrosyl complexes (e.g. nitroprusside)¹⁴ are powerful nitrosating agents under a wide range of conditions.¹⁵ Most of this previous work has concerned reactions with amines in organic solvents, presumably because of expectations that N₂O₃, N₂O₄, and metal nitrosyls will rapidly hydrolyse in neutral or alkaline aqueous solutions. We have shown¹⁶ that this assumption is incorrect for N₂O₃ and N₂O₄, and now provide further evidence for facile *N*-nitrosoamine formation and diazotisation in aqueous alkaline media and organic solvents.

EXPERIMENTAL

Reagents, Substrates, and Products.—N₂O₄ (Matheson, 99.5%) was purified by fractional distillation, but on finding that both purified gas and that direct from the cylinder gave identical results, this procedure was not always carried out. N₂O₃ (Matheson, 99%) required careful purification particularly with regard to the removal of excess of NO₂. It was found that the NO₂ content steadily increased both in the cylinder after each removal of gas, and in purified liquid N₂O₃ on standing, presumably because NO

is more volatile than NO_2 . Thus, N_2O_3 from the cylinder was condensed in a trap immersed in an acetone–solid CO_2 cooling mixture and purified NO was then bubbled through the condensed blue liquid in the trap for *ca.* 5 min. After stopping the flow of NO , the contents of the trap were allowed to warm up to their b.p. (*ca.* 3.5 °C) and samples of the vapour standing above the liquid were extracted with a hypodermic syringe through a Subaseal stopper for immediate use in reactions. These samples were certainly devoid of excess NO_2 , whose presence could be detected by the formation of *N*-nitroamines on reaction with secondary amines. NO (B.O.C., 99%) was purified by passing through aqueous 10N NaOH , followed by drying over solid KOH and anhydrous CaCl_2 .

Amines and *N*-butylacetamide, obtained as commercial products, were purified either by distillation or by fractional recrystallisation from ethanol–water mixtures. *N*-Methyl-4-nitroaniline was prepared by reacting *N*-trifluoroacetyl-4-nitroaniline with MeI .¹⁷ Recrystallisation from ethanol–water gave orange prisms, m.p. 148–150 °C (lit.,¹⁸ 152 °C).

Authentic samples of *N*-nitrosoamines and *N*-nitroamines were prepared by literature procedures¹⁹ and characterised by physical and spectral properties. *N*-Methyl-*N*-nitroso-4-nitroaniline was a green crystalline solid which gave a positive test for nitrite following the addition of acid; m.p. 98 °C; λ_{max} (EtOH) 220 and 309 nm; ν_{max} (Nujol) 1 344, 1 304, 1 070, and 856 cm^{-1} , δ (CDCl_3) 3.5 (3 H, s) and 7.8–8.4 (4 H, AB quartet).

Analytical Procedures.—Dialkyl and heterocyclic *N*-nitrosoamines and *N*-nitroamines, as well as aniline, were assayed by g.l.c. against standard solutions of authentic materials, prepared in the same solvent as that under investigation. Typically, 1–5 μl of the reaction solution was injected directly onto the column of a Perkin-Elmer F.11 gas chromatograph at 180 °C using N_2 carrier gas. The columns were either Antarox CO-990 on Chromosorb W AW-DMCS, 80–100 mesh or 15% Carbowax 20M on Chromosorb W, 80–100 mesh. Concentrations were linearly related to peak heights, which were reproducible for any given sample to better than $\pm 8\%$.

Where necessary, *N*-nitrosodiphenylamine was separated from the reaction mixture by extraction with benzene followed by t.l.c. on 1-mm silica plates using benzene as eluant (R_F 0.77). After manual separation of the relevant band and extraction into ether, the concentration of *N*-nitrosodiphenylamine was estimated by its u.v. absorbance at 296 nm ($\log \epsilon$ 3.75). *N*-Methyl-*N*-nitroso-4-nitroaniline was separated similarly (R_F 0.15). For quantitative estimation, it was extracted with CHCl_3 after t.l.c., the extract was washed with water, dried, and the solvent removed on a rotary evaporator. The residue was dissolved in a few drops of EtOH, *ca.* 5 ml 2N HCl added and the mixture then analysed for inorganic nitrite produced by decomposition of the *N*-methyl-*N*-nitroso-4-nitroaniline.

For the reaction of aniline with N_2O_4 in which the material balance was checked, the amount of unreacted aniline was determined by g.l.c. (see above) and the yield of phenyldiazonium ion (equal to the amount of *N*-nitrosation) by coupling with R-salt (see below). Separation of the *N*-nitroaniline co-product was effected by ether extraction after cooling and adjusting the pH of the reaction solution to 3–4. The dried ether extract was concentrated to 0.5 ml before t.l.c. development on 1-mm silica plates with CHCl_3 –EtOH (9 : 1). The band corresponding to *N*-nitroaniline was separated, extracted with EtOH, and the amount

of *N*-nitroaniline present in the solution was determined by the absorbance at 278 nm ($\log \epsilon$ 2.7).

For the reactions of primary aromatic amines, yields of diazonium compounds were determined by coupling an aliquot of the reaction solution with R-salt (2-hydroxynaphthalene-3,6-disulphonic acid) in aqueous borax buffer at pH 9 and measuring the absorbance of the resultant azo-dye solution at 491 nm. In the case of 4-nitroaniline and 2-chloro-4-nitroaniline the coupling was carried out at pH 7–8 to minimise the loss of diazonium ion by HO^- -catalysed hydrolysis. Absorbances at 491 nm were converted to azo-dye concentrations (and hence to amounts of *N*-nitrosation) with extinction coefficients obtained by exhaustive diazotisation of a known concentration of the relevant aromatic amines followed by coupling with R-salt. These standard solutions obeyed the Beer–Lambert law. Although reproducible to $\pm 4\%$, the procedure may underestimate the extent of *N*-nitrosation because of the instability of diazonium compounds in the alkaline reaction solutions.

Concentrations of NO in solution were ascertained by two methods. The first involved aerial oxidation under alkaline conditions followed by analysis (see below) of the inorganic nitrite produced. The second, based on the procedure of Walters and Taylor,²⁰ involved coupling with alkaline sulphite. Here, a small quantity (usually 2 μl) of the NO solution was injected through a Subaseal stopper into a degassed solution of 10^{-2}M Na_2SO_3 in 0.025% KOH contained in a 10-mm cuvette. The absorbance at λ_{max} 258 nm ($\log \epsilon$ 3.81) was read against a sulphite solution reference.

Inorganic nitrite was estimated by the modified Shinn colourimetric procedure described by Kershaw and Chamberlin.²¹

Reaction Methods.—With NO , reaction solutions were prepared on a vacuum line to obtain anaerobic conditions. A solution (10–25 ml) of the amine was placed in a two-necked 50-ml round-bottomed flask. One neck of the flask was closed by a Subaseal stopper, the other by a stopcock. After attachment to the vacuum manifold, the contents of the flask were carefully degassed by several freeze–thaw cycles. Purified NO gas was introduced into the flask *via* the manifold. The flask was agitated to assist absorption of NO into solution, and before separation of the flask from the vacuum line, the NO pressure above the solution was adjusted to atmospheric. The flask was immersed in a water–bath at 25 °C and aliquots of the reaction solution (usually 1–2 μl) were withdrawn with a syringe through the Subaseal stopper at timed intervals for immediate g.l.c. analysis.

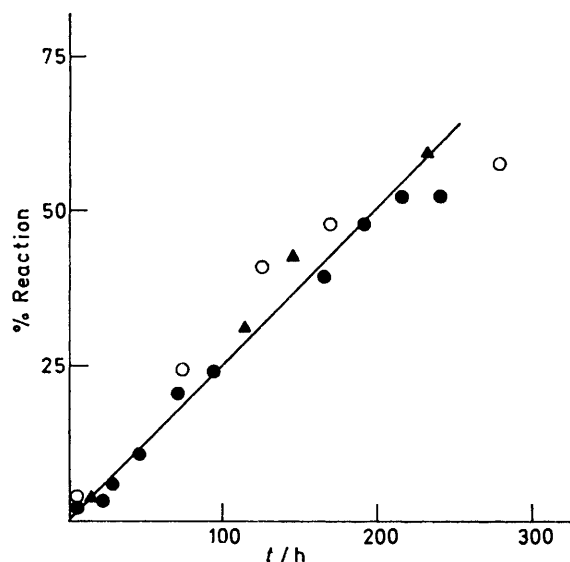
For the reactions with N_2O_3 and N_2O_4 , 5 ml of the amine solution was placed in either a 50-ml round-bottomed or conical flask. For N_2O_3 , the contents of the flask were degassed and left standing under N_2 before closure with a Subaseal stopper. With a syringe, 1–5 ml quantities of purified gaseous N_2O_3 or N_2O_4 (measured at 1 atm and 25 °C) were injected into the flask through the Subaseal stopper. After shaking the contents for 2–3 min a small sample (1–5 μl) was withdrawn for immediate g.l.c. analysis. The dead volume above the solution was usually 60 ml; thus, when 5 ml of gas was used, the partial pressure of the nitrogen oxide was 0.083 atm.

In some experiments, with N_2O_3 and N_2O_4 , 5 ml of the gas was injected into a 5 l round bottom flask filled with oxygen-free N_2 , giving a partial pressure of 10^{-3} atm for the nitrogen oxide. The gas mixture was allowed to equilibrate before

being displaced by means of a peristaltic pump through 5-ml amine solution (degassed if necessary) at a rate of 2.3 l h^{-1} for 12–24 h. An aliquot of the reaction was then analysed for products by g.l.c.

RESULTS AND DISCUSSION

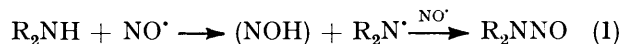
Preliminary experiments confirmed that both air (oxygen) and certain metal salts strongly catalysed amine nitrosation by nitrogen oxides, particularly by nitric oxide (NO). Our studies were therefore carried out in carefully purified solvents and, where necessary, the reaction solutions were degassed and prepared on a vacuum line (see Experimental section). Contact with stainless steel could not be entirely avoided, however, because syringes were necessary for the g.l.c. assay, but independent tests established that no significant catalysis arose from this contact under our conditions.



Formation of *N*-nitrosoamines from nitric oxide (NO) in MeCN at 25 °C. Initial [NO] = $1.4 \times 10^{-2} \text{ M}$, [amine] = $6.5\text{--}2.03 \times 10^{-3} \text{ M}$; (●) piperidine; (▲) morpholine; (○) diphenylamine

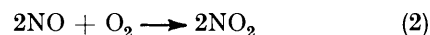
Nitrosation by Nitrogen Oxides in Solvent MeCN.—Reaction of piperidine, morpholine, and diphenylamine in solvent MeCN saturated with NO was examined at 25 °C. The concentration of dissolved NO was found to be $1.4 \times 10^{-2} \text{ M}$ and, since the reactions were carried out under an atmosphere of NO with *ca.* 10-fold less amine, it should be maintained throughout by absorption of NO from the gaseous phase. For all three amines, *N*-nitrosoamine formed at a similar, slow rate with a half-life of *ca.* 8 days. The percentage reaction *versus* time plots (Figure) for 60% reaction are approximately linear, indicating a zero-order dependence on [amine]. This observation, coupled with similar rates despite the expected difference in amine reactivity ($\text{p}K_{\text{a}}$ 11.2–0.78), suggests that some process independent of the amine is rate-limiting. The most likely process is oxidation of NO to a more reactive entity brought about by slow diffusion of air into the reaction vessel. Indeed, when air was deliberately injected into the reaction solution,

N-nitrosoamine formation was complete within 5 min, the time required for sampling. We believe that NO, itself, is a poor nitrosating agent for molecular amines because it is unable to abstract an amino-hydrogen atom and generate a dialkylamino-radical ($\text{R}_2\text{N}^\bullet$) which might be expected to combine with further NO [equation (1)]. Certainly, the relative stabilities of $\text{R}_2\text{N}^\bullet$ and NO^\bullet would make the first step of equation (1) very endothermic.

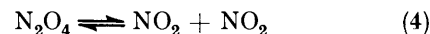
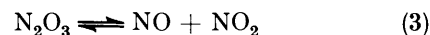


Various examples¹¹ of facile nitrosation by NO may result from reaction in the presence of either air or metal catalysts, or under circumstances where $\text{R}_2\text{N}^\bullet$ is generated independently.

The aerial catalysis referred to above arises from the facile oxidation of NO to NO_2 [equation (2)] and we observed an instantaneous brown colouration associated with NO_2 formation on injection of air. NO_2 , itself, is unlikely to be an effective nitrosating agent, but it may either combine with NO to form N_2O_3 [equation (3)] or



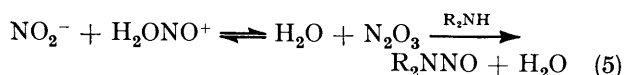
self-associate to form N_2O_4 [equation (4)]. Both equilibria [(3) and (4)] are known to be well to the left in



MeCN at 25 °C ($\{K_{\text{N}_2\text{O}_4} = 3 \times 10^{-3} \text{ mol l}^{-1};^{22} K_{\text{N}_2\text{O}_3} = 8.2 \times 10^{-5} \text{ mol l}^{-1}^{23}\}$) and both N_2O_3 and N_2O_4 are effective nitrosating agents.^{8–10} This was demonstrated further by studying the reaction of N_2O_3 and N_2O_4 with piperidine and diphenylamine in MeCN at 25 °C. The reaction flask and contents were carefully degassed beforehand for N_2O_3 , but not usually for N_2O_4 . Separate experiments, however, showed that the presence of air had no effect on the N_2O_4 reactions. For either $2.3 \times 10^{-2} \text{ M}$ N_2O_3 or N_2O_4 with $2.03 \times 10^{-3} \text{ M}$ piperidine, and for $2.9 \times 10^{-2} \text{ M}$ N_2O_3 with $1.4 \times 10^{-2} \text{ M}$ diphenylamine, formation of the corresponding *N*-nitrosoamine was quantitative on first sampling of the reaction solution after *ca.* 3 min. For N_2O_4 with piperidine, the reaction solutions were also assayed for the formation of *N*-nitropiperidine. None could be detected in reactions carried out (as above) with excess N_2O_4 , but when 1.01M piperidine was reacted with *ca.* $3.7 \times 10^{-2} \text{ M}$ N_2O_4 , the yield of *N*-nitroso- and *N*-nitro-products after 3 min was 3.33×10^{-2} and $3.6 \times 10^{-3} \text{ M}$, respectively. Thus under these conditions, *ca.* 92% of the N_2O_4 acts as a nitrosating agent.

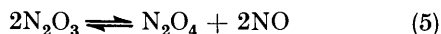
Nitrosation in Aqueous Solution by Contact with N_2O_3 and N_2O_4 in the Gas Phase.—The nitrosation of both primary and secondary amines (as well as other compounds) in aqueous acidic solutions (pH 2–4) has long been considered to involve reaction by N_2O_3 in order to explain the observed second-order dependence on nitrous acid concentration.^{3,4} From extensive studies conditions have been realised where either the formation of N_2O_3 (from reaction of NO_2^- with H_2ONO^+) or its

interaction with the neutral amine is rate-limiting [equation (5)].²⁴ Evidence for comparable reactions of



N_2O_4 is much weaker. In particular, rate enhancements induced by added NO_3^- are lower than those from added NO_2^- , and they have usually been interpreted as salt effects rather than nitrosation *via* N_2O_4 .²⁵

In the experiments reported below, 3 ml of gaseous N_2O_3 or 5 ml of gaseous N_2O_4 diluted with *ca.* 60 ml of nitrogen or air,* respectively, at atmospheric pressure and 25 °C were reacted as described in the Experimental section with 5 ml aqueous amine solution to which either excess NaOH or phosphate buffer (pH 6.85) had been added. The base or buffer prevented the formation of acidic conditions following hydrolysis of the nitrogen oxides and therefore any reaction by the usual acid-catalysed nitrosation pathways. The relatively low partial pressure ($p_{\text{N}_2\text{O}_4} = 0.083$ atm; $p_{\text{N}_2\text{O}_3} = 0.05$ atm) produced extensive dissociation of both nitrogen oxides in the gas phase prior to mixing, calculated from the relevant dissociation constants ($K_{\text{N}_2\text{O}_4} = 0.15$ atm;²² $K_{\text{N}_2\text{O}_3} = 2.42$ atm²⁶) as 55% for N_2O_4 and >98% for N_2O_3 . On dissolving into the aqueous phase, however, the dissociation should diminish sharply ($K_{\text{N}_2\text{O}_3} = 7.3 \times 10^{-5}$ mol l⁻¹;²⁷ $K_{\text{N}_2\text{O}_4} = 1.53 \times 10^{-5}$ mol l⁻¹²⁶) and N_2O_3 may exist in equilibrium with N_2O_4 and NO [equation (5)]. Analysis of the aqueous phase showed reaction was



extremely rapid to give nitrosation (*i.e.* *N*-nitrosoamine or diazonium ions), nitration (*N*-nitroamine), and hydrolysis (*i.e.* NO_2^- and NO_3^-) products. Samples taken immediately after mixing and up to 60 min later gave identical product compositions within experimental error. Because of their rapidity, no attempts were made to measure the rate of reaction and the yields referred to below were generally measured 3–5 min after adding the nitrogen oxide.

The amounts of nitrosation observed for several representative secondary amines, primary aromatic amines, and *N*-butylacetamide at 25 °C are summarised in Table 1. The percentage reaction is based on initial amine concentration and is the average of at least two determinations reproducible to $\pm 10\%$ for g.l.c. assay and to $\pm 5\%$ for diazo-coupling. The initial amine concentration was in the range 10^{-3} – 10^{-4} M with the nitrogen oxide in *ca.* 2–400-fold excess. The maximum proportion of nitrogen oxide reaction with amine is *ca.* 12%, the remainder reacting concurrently with the solvent to give hydrolysis products (*e.g.* NO_2^-). For example, from 2×10^{-3} M piperidine and 10.6×10^{-3} M N_2O_3 (3 ml) the yield of *N*-nitrosopiperidine is 1.3×10^{-3} M (*i.e.* 12% N_2O_3 and 65% piperidine). One interesting feature of the results in Table 1 is an insensitivity in the amount of

* Independent tests established that dilution of N_2O_4 with N_2 instead of air had no significant effect on either the type or yield of products. This rules out catalysis or inhibition by oxygen.

nitrosation to wide differences of substrate basicity. This particularly applies to both primary and secondary

TABLE 1

Nitrosation of various amino-compounds by gaseous N_2O_3 and N_2O_4 in aqueous 0.1M NaOH at 25 °C. Initial [amine] = 10^{-3} – 10^{-4} M; $p_{\text{N}_2\text{O}_4} = 0.083$ atm; $p_{\text{N}_2\text{O}_3} = 0.05$ atm

Amine	pK_a	% Nitrosation ^a	
		N_2O_4 (5 ml) ^c	N_2O_3 (3 ml) ^b
Piperidine	11.12	39 (0) ^c	65 (0) ^c
Morpholine	8.33	19	52
<i>N</i> -Methylpiperazine	9.8, 5.11	33 (44) ^c	39 (45) ^c
Aniline	4.65	27	45
<i>N</i> -Methyl-4-nitroaniline	(1.19) ^d	16	27
4-Nitroaniline	0.99	24 (38) ^c	29 (31) ^c
Diphenylamine	0.78	6	
3,5-Dinitroaniline	(0.35) ^d	14	
2-Nitroaniline	–0.3	11	
2-Chloro-4-nitroaniline	(–1.0) ^d	13	13
2,4-Dinitroaniline	–4.53	0	
<i>N</i> -Butylacetamide	–0.29	0	

^a Based on [amine]. ^b Generally percentage nitrosation here is higher than reported previously¹⁶ because original measurements were made with N_2O_3 containing significant amounts of N_2O_4 . ^c Figures in parentheses refer to reaction in 0.2M phosphate buffer, pH = 6.85. ^d Estimated from substituent constants.

amines from piperidine (pK_a 11.12) to *p*-nitroaniline (pK_a 0.99) where percentage variations are random rather than systematic. Thereafter the product yield drops by a factor of *ca.* 2 for compounds less basic than *p*-nitroaniline and neither 2,4-dinitroaniline (pK_a –4.53) nor *N*-butylacetamide (pK_a –0.29) appeared to react at all. Apparently, the weakest bases are unable to compete with hydrolysis by the solvent, but factors other than basicity must also be relevant to explain the inertness of *N*-butylacetamide. Another clear indication from Table 1 is that N_2O_3 generally gives more *N*-nitrosation than N_2O_4 and the difference is probably mechanistically significant.

Several reactions were examined with phosphate buffers (pH 6.85) in place of the usual 0.1M NaOH and these results are given in parentheses in Table 1. No detectable reaction other than hydrolysis was found for piperidine (pK_a 11.12), but the extent of *N*-nitrosation was substantially unaltered for *N*-methylpiperazine (pK_a 9.8 and 5.11) and *p*-nitroaniline (pK_a 0.99). These findings show that only the unprotonated amine bases are reactive. The dibasic *N*-methylpiperazine is extensively monoprotonated at pH 6.85. Thus the neutral *N*-methylpiperazine and its conjugate acid must also have very similar reactivities towards the nitrogen oxide reagents.

In view of the earlier findings⁹ for N_2O_4 in organic solvents, several of the above reactions with this reagent were examined for the formation of *N*-nitroamine. The findings are summarised in Table 2, where the percentage reaction again relates to initial amine concentration and is the average of at least two reproducible determinations. Independent checks showed that the *N*-nitroamines did not arise from oxidation of an *N*-nitroso-precursor either in the reaction flask or during the assay procedure. Comparison of Tables 1 and 2 shows that nitration is

more dependent than nitrosation on the amine structure and the extent, surprisingly, increases with decreasing

TABLE 2

Nitration of amino-compounds by gaseous N_2O_4 in aqueous 0.1M NaOH at 25 °C. Initial [amine] = 10^{-3} – 10^{-4} M; $p_{N_2O_4} = 0.083$ atm

Amine	pK_a	% Nitration *
Piperidine	11.12	1
<i>N</i> -Methylpiperazine	9.8, 5.1	8
Aniline	4.6	30

* Based on [amine].

basicity (and hence nucleophilic reactivity) of the substrate. For aniline, *N*-nitration by N_2O_4 proceeded as readily as diazonium ion formation, but, significantly, very little ring nitration was observed. After acidification of the reaction solution to pH 2, an ether extract showed eight bands, mostly highly coloured, on silica t.l.c. A careful materials balance of the unreacted aniline, benzenediazonium ion (by independent azo-coupling), and *N*-nitroaniline showed that no more than 2% of other products could be formed. None of these observations are inconsistent with a free radical pathway for *N*-nitration or a mechanism in which the stability of the relevant amino-radical is important. The yield of *N*-nitropiperidine (*ca.* 1%) obtained under the standard reaction conditions was very close to the limit of detection by the g.l.c. procedure employed. This product increased substantially when more dilute gaseous N_2O_4 was employed and was also detectable at low level with diluted N_2O_3 (see Table 3). These experiments involved

TABLE 3

Concurrent nitrosation and nitration of piperidine by dilute gaseous N_2O_3 and N_2O_4 in aqueous 0.1M NaOH at 25 °C. Initial [piperidine] = 2×10^{-3} M; $p_{N_2O_3} = p_{N_2O_4} = 10^{-3}$ atm

Reagents	% <i>N</i> -Nitrosopiperidine *	% <i>N</i> -Nitropiperidine *
N_2O_4	22	28
N_2O_3	68	6

* Based on [amine].

a different procedure whereby the 5 ml of nitrogen oxide gas was first diluted with 5 l of inert gas (*i.e.* to give 1 000 p.p.m. nitrogen oxide, equivalent to $p_{N_2O_4} = p_{N_2O_3}$, *ca.* 10^{-3} atm). The resultant mixture was then bubbled through 5 ml 0.1M aqueous NaOH containing 2×10^{-3} M piperidine at a rate of 2.3 l h⁻¹ for 24 h, followed by product analysis in the usual way. Independent checks again eliminated *N*-nitropiperidine formation by oxidation of the *N*-nitroso-compound, so the increased yield of *N*-nitropiperidine must relate to the enhanced dissociation of the diluted nitrogen oxides. Dilution also had an interesting effect on the *N*-nitrosoamine yield (see Table 3), producing a 50% reduction with N_2O_4 but a slight increase with N_2O_3 .

Nature of the Reactive Species.—The interaction of gaseous N_2O_3 and N_2O_4 with aqueous solutions of many amines is characterised by a very low selectivity and an ability to effect *N*-nitrosation in the presence of HO⁻.

The latter might also be construed to relate to their low selectivity (*i.e.* little discrimination between HO⁻ and amine) but the negligible effect of pH on the amount of *N*-nitrosation of *N*-methylpiperazine and 4-nitroaniline (substantially the same in 0.1M NaOH and pH 6.85) shows that the competitive hydrolysis reaction cannot be strongly HO⁻-catalysed. The hydrolysis observed must result overwhelmingly from reaction by H₂O itself. Applied to the results for 2×10^{-3} M piperidine, where *ca.* 4% of the available N_2O_4 reacts to form the *N*-nitroso-derivative, it follows that piperidine must react with N_2O_4 about 10³ times faster than H₂O, whose effective concentration is 55.5M. Similar relative reactivities of amine to H₂O must apply to all the substrates down to 4-nitroaniline for both N_2O_3 and N_2O_4 , because the proportion of amine reacting in 0.1M NaOH is not substantially different from that observed with piperidine.

Certain aspects of this low selectivity have also been observed for the conventional nitrosation of secondary amines using HNO₂ in dilute acid (pH 2–4) where rates follow equation (6) and N_2O_3 is believed to be the

$$\text{Rate} = k_3[\text{amine}][\text{HNO}_2]^2 \quad (6)$$

reagent. Mirvish³ has shown that k_3 varies by a factor of only 34 (*i.e.* from 21 to 0.62×10^5 l² mol⁻² s⁻¹ at 25 °C) for 15 secondary amines with pK_a 11.2–4.85, and in a way that is clearly unrelated to basicity. There is no evidence in the literature, however, of significant nitrosation following equation (6) for weakly basic amines ($pK_a \leq 2$)²⁸ in contrast to our findings. The difference is exemplified by 4-nitroaniline which reacts readily with N_2O_3 of gaseous origin, but requires the more reactive nitrous acidium ion (H₂ONO⁺) for diazotisation in dilute acid.²⁹ Further, there is very little evidence for nitrosation *via* N_2O_4 in dilute aqueous acids (*cf.* H₂ONO⁺ + NO₃⁻ \rightleftharpoons N_2O_4 + H₂O) where relatively weak catalysis by added NO₃⁻ has been interpreted principally as a salt effect.²⁵

The low selectivities of N_2O_3 and N_2O_4 towards amines is characteristic of reactions that occur on encounter. However, molecular rate coefficients calculated from the data³ for reaction of N_2O_3 in dilute aqueous acid (*ca.* 4×10^6 l mol⁻¹ s⁻¹) and from our data for gaseous N_2O_3 and N_2O_4 in 0.1M NaOH (*ca.* 4×10^7 l mol⁻¹ s⁻¹) are all substantially lower than the expected * encounter rate of two neutral reagents ($k_{\text{encounter}}$ *ca.* 7×10^9 l mol⁻¹ s⁻¹ at 25 °C) as has been noted previously for the N_2O_3 diazotisation of aniline.⁴ At first sight this appears to rule out the possibility of encounter-controlled reactions. We regard this conclusion with circumspection, however, because there are good grounds for expecting N_2O_4 to be as reactive as NOCl, for example, whose molecular rate coefficients for the diazotisation of anilines are close to the encounter limit.⁴ One possible explanation which has not been seriously considered previously is that both N_2O_4 and N_2O_3 exist in isomeric forms, of which the reactive nitrosating agents are only a proportion depen-

* Calculated from $k_{\text{encounter}} = 8RT/3\eta$.

dent on the source of the reagent. This would also explain the different reactivities of weakly basic amines (e.g. 4-nitroaniline) towards N_2O_3 obtained either as a gaseous reagent or from aqueous HNO_2 . Further, the different pattern of reactivity with respect to amine basicity for the concurrent formation of *N*-nitrosoamine and *N*-nitroamine from N_2O_4 is more consistent with reaction of two N_2O_4 isomers than of one isomer *via* two independent pathways.

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