The Chemistry of Nitroso-compounds. Part 15.[1] Formation of *N*-Nitrosamines in Solution from Gaseous Nitric Oxide in the Presence of Iodine

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Quantitative results are reported for the *N*-nitrosation of piperidine, morpholine, and *N*-methylpiperazine by NO (saturated) and iodine under anaerobic conditions in solvent EtOH, EtOH–water, and MeCN at 25 °C. The reactions are very much faster than those with NO in the absence of iodine, with maximum yields of *N*-nitrosamine being obtained in *ca.* 5—30 min depending on the solvent. The rates, however, are largely independent of the initial concentrations of either amine or iodine, and are similar for all three amines despite their different basicities. With excess of amine, 2 moles of *N*-nitrosamine are obtained per mole of iodine. Otherwise, the limiting yields of *N*-nitrosamine depend on both the concentration and basicity of the amine, with *ca.* 50, 66, and 100% reacting for piperidine, morpholine, and *N*-methylpiperazine, respectively. Addition of NaOH to the reaction solution, however, increases the yield to 100% throughout. Both added NaN_3 and thiourea inhibit the formation of *N*-nitrosamines. The mechanism of these reactions is considered to involve rate-limiting formation of NOI (principally from NO and I_2) followed by rapid reaction with the amine base. The amine cation is unreactive; NaN_3 and thiourea inhibit reaction by competing for the NOI reagent. The results are also discussed in relation to human exposure to carcinogenic *N*-nitrosamines.

WE reported earlier [2] that primary and secondary amines are subject to very rapid *N*-nitrosation by gaseous N_2O_3 and N_2O_4 in both neutral and alkaline aqueous solutions and in organic solvents. In contrast, nitric oxide (NO) is relatively inert unless air or oxygen is present to effect oxidation to the higher nitrogen oxides.[2,3] Other investigators have shown, however, that *N*-nitrosamines will form in organic solvents from secondary amines and nitric oxide if metal catalysts are also present. Much of this work has been reported only in the patent literature,[4] but Brackman and Smit [5] have presented fuller details of the reaction with diethylamine in the presence of Cu^{II} salts. From kinetic studies, they concluded [5] that *N*-nitrosation of the amine in both EtOH and MeCN was brought about by a Cu-nitrosyl salt in which NO was effectively oxidised to NO^+. This mechanism is consistent with several other observations that metal-nitrosyls (*e.g.* nitroprussides) are effective nitrosating agents under a wide range of conditions.[6] Very recently, however, we have shown that *N*-nitrosamine formation from secondary amines and nitric oxide in the presence of Ag^I-salts probably involves oxidation of the amine (to a radical cation) rather than NO itself.[7]

Nitric oxide is a common pollutant produced by most combustion processes [8] and its ability to interact with amino-compounds to form carcinogenic *N*-nitrosamines is of interest to human health.[9] In the course of investigating metal-catalysed reactions, we observed that elemental iodine is a powerful catalyst for the formation of *N*-nitrosamines from nitric oxide.[10] The scope and mechanisms of these reactions is now reported in more detail.

EXPERIMENTAL

Reagents, Substrates, and Products.—Reagent grade piperidine, morpholine, and *N*-methylpiperazine were dried over solid KOH and then vacuum-distilled. Iodine was recrystallised from benzene and then sublimed. n-Butyl alcohol and cyclohexane were dried over anhydrous K_2CO_3 and sodium wire, respectively, and then fractionally distilled; MeCN was heated under reflux over P_2O_5 prior to fractional distillation and storage over 4A molecular sieves. AnalaR grade KI, EtOH, and HCl were used without further purification. Nitric oxide (B.O.C. 99%) was purified by passage through two wash bottles, one of which contained 30% w/v KOH–water and the other acidic saturated aqueous sulphanilamide. It was then passed through a drying tower (30 cm) containing both NaOH pellets and anhydrous $CaCl_2$. n-Butyl nitrite [11] and *N*-nitrosamines [12] were prepared and purified by literature procedures.

Reaction Methods.—The nitrosation reactions were carried out in 50-ml three-necked flasks fitted with a Subaseal stopper, a gas inlet, and an aspirator. The solvent (40 ml) containing the catalyst was placed in the flask, which was then carefully degassed either by several freeze–thaw cycles under vacuum or by passing purified nitrogen for *ca.* 100 min. The latter procedure did not cause significant loss of solvent. The flask was then placed in a water-bath at 25 °C and purified NO passed into the reaction vessel for *ca.* 20 min. The reaction was initiated by injecting amine into the reaction flask *via* the Subaseal stopper with a microlitre syringe. At timed intervals, 1—5 μl aliquots of the reaction solution were withdrawn *via* the Subaseal stopper and analysed by g.l.c. immediately.

The g.l.c. analysis for the *N*-nitrosamines was carried out mainly on a Perkin-Elmer F11 instrument (f.i.d.) using a 2-m × $\frac{1}{8}$-in stainless-steel column packed with either Antarox CO-990 on Chromosorb WAW-DMCS (80—100 mesh) or 8% Carbowax 20M + 2% KOH on Chromosorb W (80—100 mesh), both at 180 °C using N_2 carrier gas. The instrument was calibrated with standard solutions of the *N*-nitrosamines dissolved in the appropriate solvent. Concentrations were linearly related to peak heights which were reproducible for any given sample to better than ±8%.

Equilibrium between Bu^nONO *and* NO.—The dissociation of Bu^nONO was determined by adding equimolar amounts of KI and HCl to 10^{-4}M-Bu^nONO in solvent EtOH under anerobic conditions in a glove box. This immediately produced a highly-coloured solution indicative of the formation of I_3^- [$\lambda_{max.}$ (H_2O) 288 and 353 nm, log ε 4.60 and 4.42].[13] The solutions were transferred to a tightly-stoppered cuvette, and the absorption was measured at 290 nm using a Unicam SP 1800 spectrophotometer.

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Independent checks established that the absorptions at this wavelength of Bu^nONO (log ε 1.7) and I_2 (log ε 1.98) were insignificant.

The related equilibrium between I_2 and I_3^- was examined similarly by preparing solutions of I_2 in EtOH and adding equimolar concentrations of KI and HCl in an anaerobic glove box. The concentration of I_3^- was determined spectrophotometrically as above.

The formation of Bu^nONO from saturated solutions of NO in Bu^nOH and I_2 was examined by a procedure analogous to that employed for the formation of *N*-nitrosamines. The amount of Bu^nONO was estimated by h.p.l.c. assay using a Waters Associates 600 pump fitted with a 70-cm × 2.1-mm i.d. s/s column packed with C_{18} porasil–microbondapack-CN attached to a Cecil CE212 spectrophotometer. The eluant was 1% v/v MeOH–water, analytical samples were injected directly on to the column *via* a septum, and the absorption of the eluant was monitored at 230 nm. The instrument was calibrated with standard solutions of Bu^nONO in Bu^nOH and peak heights gave concentrations reproducible to ±5%. Analyses of NO in Bu^nOH showed that Bu^nONO formed in the absence of I_2. This was attributed to oxidation of NO to NO_2 by adventitious oxygen followed by rapid reaction with the solvent. NO_2 has been shown to be a powerful nitrosating agent.[2] The concentration of Bu^nONO produced in the absence of added I_2 was subtracted from the assay of the reaction solutions.

Concentration of Dissolved NO.—These were ascertained by two methods for pure EtOH and MeCN. The first, based on the procedure of Walters and Taylor,[14] 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% w/v KOH–water contained in a 10-mm cuvette. The absorbance at $\lambda_{max.}$ 258 nm (log ε 3.81) was measured against a sulphite solution reference. The second procedure involved direct measurement of the u.v. absorption of the solution containing NO at $\lambda_{max.}$ 325 nm (log ε 1.83).

TABLE 1

Concentrations of dissolved NO

Solvent	10^2[NO]/M
EtOH	2.1 (2.18)
10% v/v H_2O–EtOH	(1.85)
25% v/v H_2O–EtOH	(1.2)
50% v/v H_2O–EtOH	(0.92)
MeCN	1.4 (1.13)

Only the second procedure was used for the aqueous EtOH solutions. The concentrations found are summarised in Table 1, where the figures in parentheses refer to the direct u.v. estimation.

RESULTS AND DISCUSSION

Under anaerobic conditions, and in the absence of I_2, formation of *N*-nitrosamine from NO in EtOH and other solvents at 25 °C was exceedingly slow. Generally, very small amounts of *N*-nitrosamine were found immediately after injection of the amine substrate into the reaction solution, but thereafter its concentration increased only slowly or not at all over the next 2—3 days. We believe that NO, itself, is a very poor nitrosating agent for molecular amines because it is unable to extract the amino-H atom to generate the dialkyl amino-radical ($R_2N^{\cdot}$) which might then be expected to combine with further NO [equation (1)]. The relative stabilities of $R_2N^{\cdot}$ and $NO^{\cdot}$ make the first step of equation (1) very

$$R_2NH + NO^{\cdot} \longrightarrow (NOH) + R_2N^{\cdot} \xrightarrow{NO^{\cdot}} R_2NNO \quad (1)$$

endothermic. Any reaction in the absence of catalysts probably results from the adventitious leakage of air into the reaction vessel. This would produce either N_2O_3 or N_2O_4, both of which have been shown recently [2] to be powerful nitrosating agents.

N-Nitrosamine formation was very much faster in the presence of elemental iodine. Reaction was usually complete in *ca.* 20 min, and, oxygen apart, iodine appears to be one of the best promoters of nitrosation by NO. Unfortunately, these reactions did not lend themselves

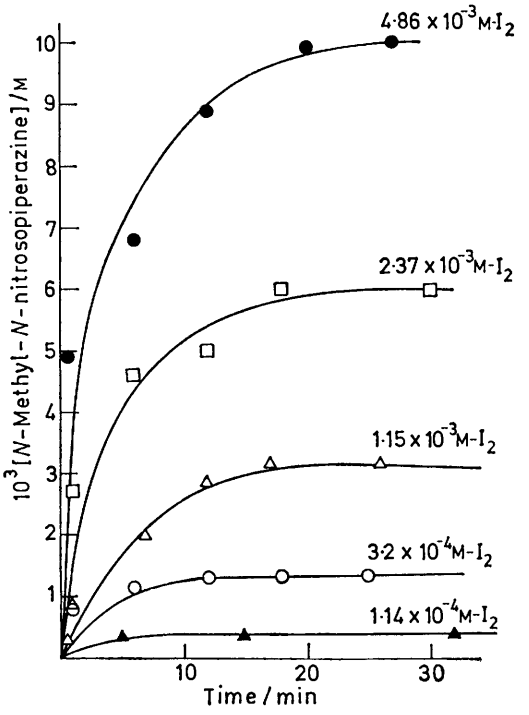


FIGURE 1 Effects of iodine on the formation of *N*-methyl-*N*-nitrosopiperazine from 10^{-2}M-*N*-methylpiperazine and saturated (*ca.* 2.1 × 10^{-2}M) NO in EtOH at 25 °C

to precise kinetic measurements because of difficulties both in controlling the NO concentrations in solution and in devising an artifact-free quenching procedure. Mechanistic deductions can be drawn, however, from the variation of percentage reaction with respect to time of g.l.c. injection typified by Figure 1 for 0.01M-*N*-methylpiperazine in EtOH saturated with NO and containing various deficient concentrations of iodine. The initial concentration of dissolved NO was found to be *ca.* 2.1 × 10^{-2}M and since the reactions were carried out under an atmosphere of NO, it may be maintained throughout reaction by absorption from the gaseous phase. Similar plots were obtained for the nitrosation of morpholine and piperidine and variations in the maximum yield of the corresponding *N*-nitrosamine, and its time of formation with initial concentrations of both iodine and

TABLE 2

Limiting yields and time of *N*-nitrosamine formation from secondary amines, NO, and I_2 in EtOH at 25 °C. Initial [NO] *ca.* 2.1×10^{-2}M

Amine	10^2[Amine]/ M	10^3[I_2]/ M	Limiting yield (%) [a]	Time/ min [b]
Piperidine	1.0	0	4	1 200
Piperidine	1.0	0.53	15	20
Piperidine	1.0	1.05	20	20
Piperidine	1.0	2.46	44	20
		(2.64) [c]	(48)	(20)
Piperidine	1.0	2.45 [d]	45	20
Piperidine	1.0	4.70	57	25
N-Methylpiperazine	1.0	0	4	80
N-Methylpiperazine	1.0	0.32	14	18
N-Methylpiperazine	1.0	1.14	34	17
N-Methylpiperazine	1.0	2.37	63	18
N-Methylpiperazine	1.0	4.86	100	20
Morpholine	1.0	0	4.3	450
Morpholine	1.0	1.15	22	20
Morpholine	1.0	2.60	54	22
		(2.63) [c]	(48)	(20)
Morpholine	1.0	5.15	58	25
Morpholine	0.50	2.51	46	22
Morpholine	2.0	2.46	25	20

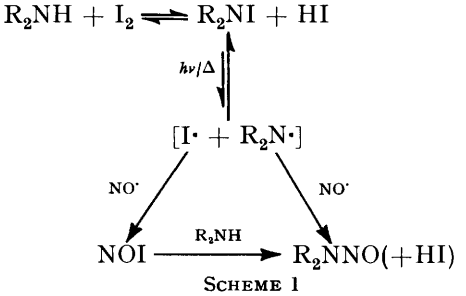
[a] Based on initial [Amine]. [b] Time to reach limiting yields. [c] Duplicate experiments in parentheses. [d] I_2 and NO in EtOH left standing for 40 h before addition of piperidine.

amine (in the case of morpholine) are summarised in Table 2. These results show several important characteristics. Thus, the maximum yield of *N*-nitrosamine is dependent on the initial iodine concentration, but is virtually independent of both the basicity (reactivity) and initial concentration of the amine substrate. Further, the time required to reach this maximum is about the same for all the reactions examined. The second observation implies that the amine is not involved in the rate-limiting step. The relationship between the maximum yield and initial iodine concentration is quantified by Figure 2 which shows that two moles of *N*-nitrosamine are produced by each mole of I_2. Thus, the reaction rate probably follows equation (2), although proof of the kinetic order for NO requires

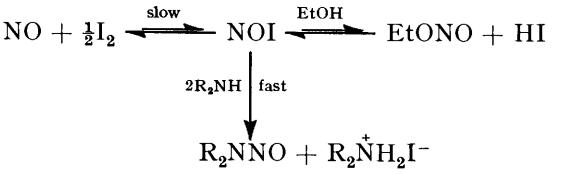
$$\text{Rate} = k[I_2]^{\frac{1}{2}}[NO] \qquad (2)$$

further measurements without a constant excess of this reagent.

Although *N*-iodoamines may be expected to react with NO under thermal [15] conditions, these pathways (*e.g.* Scheme 1) cannot apply to our reactions because both rates and yields are independent of the basicity and concentration of the secondary amines. These observations also rule out reaction *via* charge-transfer complexes, which are known to form readily from secondary



SCHEME 1



SCHEME 2 Mechanism for the formation of *N*-nitrosamines from NO and secondary amines in the presence of iodine

amines and iodine in organic solvents.[16] Further, no products typical of dialkylamino-radical intermediates, such as imines and tetra-alkyl hydrazines, could be detected in the reaction solutions. Thus, the preferred mechanism (Scheme 2) is one involving rate-limiting formation of nitrosyl iodide (NOI), principally from NO and iodine, followed by rapid reaction of this reagent with the amine. Two aspects of Scheme 2 require further comment. The first concerns the release of HI as a by-product which, as

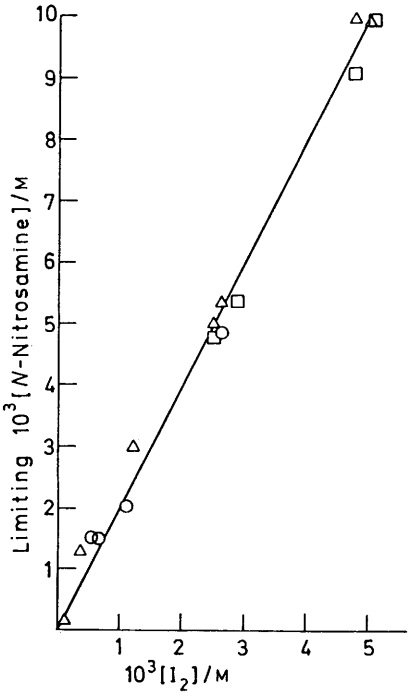


FIGURE 2 Dependence of limiting *N*-nitrosamine yields on the initial iodine concentration for the reactions of piperdine (○), morpholine (□), and *N*-methylpiperazine (△) with saturated NO (*ca.* 2.1×10^{-2}M) in EtOH at 25 °C

reaction proceeds, will both acidify the solution and lead to the formation of tri-iodide ion [$I^- + I_2 \rightleftharpoons I_3^-$]. The second concerns the formation of ethyl nitrite (EtONO) which could be the effective nitrosating agent in the presence of HI.[17] The production of HI must also lead to amine cation [$R_2\overset{+}{N}H_2I^-$] formation, and *inter alia*, a reduced yield of *N*-nitrosamine. This consequence is not clearly evident in Table 2 because an excess of amine was employed, but it may explain the slight reduction in the maximum yields of *N*-nitrosamine with increasing basicity of the amine [*i.e.* piperidine (pK_A 11.12) < morpholine (8.33) < *N*-methylpiperazine (9.8, 5.11)]. The effect is apparent, however, for reactions carried out with an excess of I_2 over amine. These additional

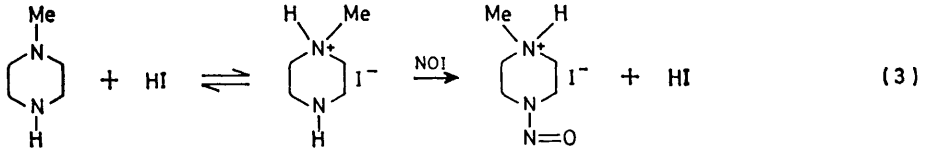
TABLE 3

Limiting yields for *N*-nitrosamine formation from secondary amines and NO with excess of I_2 in EtOH at 25 °C. Initial $[I_2]$ 0.99—1.04 × 10^{-2}M; [NO] *ca.* 2.1 × 10^{-2}M

Amine	10^3[Amine]/ M	Initial limiting yield (%) [a]	Added base [b] (μl of 4M-NaOH)	Final yield [c] (%)
Piperidine	1.25	16	100 (0.01)	100
Piperidine	2.5	14	100 (0.01)	99
Piperidine	3.75	21		
Piperidine	5.0	29	50 (0.005)	94
Piperidine	10.0	42	50 (0.005)	95
Piperidine	15.0	47		
Morpholine	5.0	65	100 [d] (0.01)	89
Morpholine	10.0	64	100 [d] (0.01)	93
Morpholine	10.0	58 [e]	100 [d] (0.01)	87
N-Methylpiperazine	10.0	100		
N-Methylpiperazine	10.0	100 [e]		

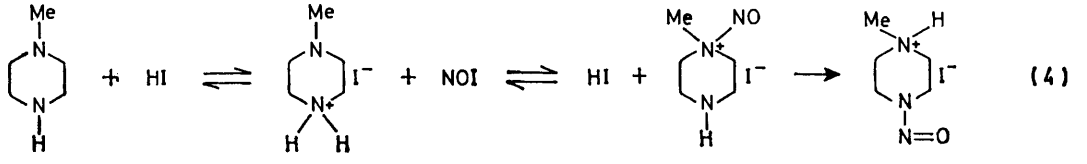
[a] Based on initial [Amine]. [b] Added after *ca.* 15 min. Molar concentration in solution in parentheses. [c] After addition of base, based on initial [Amine]. [d] μl 4.04M-NaOEt. [e] With 5 × 10^{-3}M-iodine.

results are summarised in Table 3. Significantly, the maximum yield of *N*-nitrosopiperidine does not normally exceed 50% of the initial piperidine concentration as required for the mechanism in Scheme 2. Further, the addition of a small amount of NaOH or NaOEt to the solution allows the reaction to proceed almost to completion. For *N*-methylpiperazine, however, formation of *N*-nitrosamine is virtually complete without the addition of NaOH. This amine is dibasic (pK_A 9.8, 5.11) so it may either protonate at the tertiary N-atom, but still react with NOI at the secondary position [equation (3)], or form a quaternary nitrosonium salt which then



(3)

undergoes an intramolecular transnitrosation [equation (4)]. In any event, under equivalent reaction conditions, free base concentrations will be higher for *N*-methylpiperazine than either piperidine or morpholine.



(4)

Examination of the reaction solutions by h.p.l.c. showed that low concentrations of ethyl nitrite were present. Attempts to quantify the amount were largely unsuccessful probably because this compound is very volatile (b.p. 17 °C [18]). The equilibrium between n-butyl nitrite and NO [equation (5)] gave more reproducible results; however significant errors in estimating the Bu^nONO by h.p.l.c. assay combined with complications arising from the additional equilibrium between iodine and tri-iodide ion $[I_2 + I^- \rightleftharpoons I_3^-]$, included in equation (5), prevented calculation of equilibrium constants. This process was examined, however, to give some idea of the extent of alkyl nitrite formation under kinetic conditions. Measurements were taken both of the dissociation of Bu^nONO in the presence of HI (by u.v. spectroscopy) and for the formation of Bu^nONO from NO plus iodine in Bu^nOH (using h.p.l.c.). Addition of HI (actually KI plus HCl) to equimolar amounts of Bu^nONO in EtOH as solvent immediately produced the characteristically strong absorptions attributed to I_3^- [$\lambda_{max.}$ (H_2O) 288 and 353 nm, log ε 4.60 and 4.42 [13]]. The I_3^- concentrations, assuming $\lambda_{max.}$ is independent of solvent and HI_3 is completely dissociated

$$4Bu^nONO + 5HI \rightleftharpoons 4Bu^nOH + 4NO + I_2 + HI_3 \quad (5)$$

TABLE 4

Equilibrium between HI plus Bu^nONO and I_3^- in EtOH at 25 °C

10^5[Bu^nONO]/ M	10^5[HI]/ M [a]	O.D. (λ 290 nm) [b]	$10^6[I_3^-]$/ M
1	1	0.01	0.25
2	2	0.02	0.50
3	3	0.085	2.2
6	6	0.255	6.38
8	8	0.465	11.6
10	10	0.625	15.6

[a] From equimolar KI and HCl. [b] 10-mm cell.

(Table 4), are not quantitative (*i.e.* $[I_3^-] < [HI]/3$). Independent measurements, however, showed that not all the iodine would be converted to I_3^- even in the presence of HI (Table 5). When adjusted for this factor, the results in Table 4 indicate that *ca.* 16% Bu^nONO remains in equilibrium with NO, I_2, and I_3^-. This figure agrees qualitatively (±5%) with direct h.p.l.c. estimates of the Bu^nONO formed in NO-saturated Bu^nOH containing 10^{-2}M-I_2, although these measurements were also uncertain owing to extraneous Bu^nONO formation on the h.p.l.c. column.* The most reliable

* This was apparent from the analyses of NO in Bu^nOH containing no added I_2. It probably arises from the oxidation of NO to NO_2 by adventitious oxygen. Data were corrected for this effect.

TABLE 5

Equilibrium between I_3^- and I_2 in EtOH at 25 °C and influence of added HI

$10^5[I_2]$/M	10^5[HI]/M	O.D (λ 290 nm) [a]	$10^5[I_3^-]$/M
2		0.16	0.4
5		0.64	1.6
10		1.00	2.5
15		1.18	2.95
20		1.27	3.18
10	2.5	1.46 [b]	3.65
10	5.0	1.80 [b]	4.5
10	7.5	2.04 [b]	5.1
10	10.0	2.32 [b]	5.8
10	12.5	2.56 [b]	6.4

[a] In 10 mm cell. [b] Reaction solutions diluted volumetrically by 2 for on-scale reading.

estimate of EtONO formation therefore comes indirectly from the limiting yields of *N*-nitrosamine. As noted above, these reflect the extent of amine protonation and, *inter alia*, the acidity of the reaction solutions. For the data in Table 3, the limiting yields of *N*-nitrosopiperidine before the addition of base decrease from *ca.* 42 to 14.5% with decreasing initial piperidine concentration, and the deficiency below 50% should equal the amount of HI produced by reaction of NOI with the solvent. This corresponds to 8 ± 2% of the initial iodine concentration.* Since 2 moles of HI arise from each mole of I_2, this agrees well with the estimate based on the dissociation of Bu^{n}ONO. Our conclusion is that under kinetic conditions, less than 10% of the initial iodine has reacted with the solvent to form EtONO.

Ethyl nitrite presumably arises from nucleophilic attack by the solvent on NOI, in competition with the secondary amine. Most alkyl nitrites, however, are ineffectual nitrosating agents under non-acidic conditions,[17,19] so the likelihood of *N*-nitrosamines arising from the ethyl nitrite in the presence of either an excess of amine (Table 2) or added NaOH (Table 3) seems remote. This conclusion was confirmed by observing that 0.011M-piperidinium hydrochloride with 0.0063M-Bu^{n}ONO and 0.0078M-KI in EtOH at 25 °C gave only 5×10^{-4}M-*N*-nitrosopiperidine (*ca.* 5% reaction) over a period of 24 h.

Reactions in Acetonitrile.—Examination of the reactions in solvent MeCN confirmed that ethyl nitrite is incidental to the formation of *N*-nitrosamines from the NO–iodine reagent. The change of solvent gave much faster reactions with limiting yields of *N*-nitrosamine being reached in less than 5 min. Data summarised in Table 6 show that with excess of iodine over amine, this limiting yield is *ca.* 50% for piperidine, 66% for morpholine, and 100% for *N*-methylpiperazine. These differences are probably related to protonation of the amine substrate by the HI generated as a by-product. As in EtOH, addition of NaOH to the reaction mixture increased the yields of *N*-nitrosomorpholine and *N*-nitrosopiperidine to *ca.* 100%.

* For example, with 10^{-2}M-iodine plus 10^{-2}M-piperidine, the limiting yield of *N*-nitrosopiperidine (Table 3) = 4.2×10^{-3}M. Thus the amount of I_2 reacting to produce HI is given by $(0.01 - 2 \times 0.0042)/2 = 0.0008$M (*i.e.* 8%).

Reactions in EtOH–H_2O.—The results summarised in Table 7 show that the addition of up to 50% (v/v) H_2O to solvent EtOH progressively reduces the limiting yield of *N*-nitrosamine. This reduction is more marked for *N*-nitrosopiperidine than for *N*-methyl-*N*-nitrosopiperazine. The presence of H_2O must result in competitive hydrolysis of NOI [equation (6)] to produce 2 moles of relatively strong acids (*i.e.* HNO_2 and HI).

$$NO + \tfrac{1}{2}I_2 \rightleftharpoons NOI \xrightleftharpoons{H_2O} HNO_2 + HI \qquad (6)$$

Thus protonation of the amine substrate will be more extensive than in pure EtOH which will lower the limiting yield of *N*-nitrosamine. The effect should be stronger for the more basic amine (*e.g.* piperidine) as observed experimentally. Further, equation (6) will be largely irreversible under the reaction conditions because of neutralisation by the amine substrate [*e.g.* $HNO_2 + HI + 2R_2NH \rightleftharpoons R_2NH_2^+I^- + R_2NH_2^+NO_2^-$]. This exacerbates the drop in the amount of product by consuming the iodine promoter, and is probably the

TABLE 6

Formation of *N*-nitrosamines from secondary amines, NO, and I_2 in MeCN at 25 °C. Initial [NO] *ca.* 1.4×10^{-2}M; $[I_2]$ 10^{-2}M

Amine	10^3[Amine]/M	Initial limiting yield [a] (%)	Added base [b] (μl of 4M-NaOH)	Final yield [c] (%)
Piperidine	10	75	50 (0.005)	95
Piperidine	5	50	100 (0.01)	94
Piperidine	2.5	56		
Piperidine	1.25	50		
Morpholine	5	66	100 (0.01)	100
N-Methylpiperazine	5	100		

[a] Based on initial [Amine] and reached after *ca.* 5 min. [b] Added after *ca.* 20 min. Molar concentration in reaction solution in parentheses. [c] After addition of base, based on initial [Amine].

TABLE 7

Effect of added H_2O on the nitrosation of piperidine and *N*-methylpiperazine by NO and I_2 in EtOH at 25 °C. Initial [Amine] 10^{-2}M

$10^3[I_2]$/M	H_2O : EtOH (v/v) (%)	Maximum yield [a] (%)	Time [b]/min
Piperidine			
2.46 (2.64 [d])	0	48 (44)	20 (20)
2.52 (2.40)	2.5	48 (40)	20 (23)
2.45	10	32	33
2.44 (2.45)	25	25 (26)	28 (25)
2.66 (2.66)	25	23 (11) [c]	25 (27)
2.44 (2.45)	50	16 (13)	25 (20)
N-Methylpiperazine			
2.37	0	63	18
2.44	2.5	52	20
2.39	10	46	20
2.45 (2.50)	25	45 (45)	25 (21)
2.49 (2.40)	50	36 (33)	25 (22)

[a] Based on initial [Amine]. [b] Time required to reach maximum yield. [c] With 2×10^{-2}M-piperidine. [d] Duplicate experiments in parentheses.

prime factor influencing the yield of *N*-methyl-*N*-nitrosopiperazine.

Addition of H_2O produces only a slight reduction in the rate of *N*-nitrosamine formation (Table 7) and, as in pure EtOH, these rates are virtually the same for all amines. Both observations suggest that formation of the NOI reagent from NO and iodine remains rate-limiting as in Scheme 2. Independent measurements (see Table 1) showed that H_2O progressively decreased the concentration of NO dissolved in the solvent and this would account for the slight reduction in reaction rates.

In view of much recent interest in the formation of *N*-nitrosamines under gastric conditions,[20] the influence of added mineral acid (either HCl or $HClO_4$) was also examined for the reactions in 25% (v/v) H_2O–EtOH. For piperidine or morpholine, a severe inhibitory effect was apparent: for example, reaction of 10^{-2}M-amine with 2.45×10^{-3}M-I_2 and 1.2×10^{-2}M-NO (saturated) gave insignificant amounts of the corresponding *N*-nitrosamine over 60 min in the presence of 0.024M-$HClO_4$, compared to at least 25% reaction over 20 min in its absence. Under the acidic conditions, both morpholine and piperidine should be extensively protonated and therefore unreactive. In contrast, addition of acid facilitated the nitrosation of *N*-methyl piperazine and the findings summarised in Table 8 show the optimum to be 0.01—0.02M HCl where the experimental pH * = 2.42—2.15. Closer scrutiny of Table 8 shows that HCl has a dual effect: it makes the yield of *N*-methyl-*N*-nitrosopiperazine quantitative and therefore independent of the initial iodine concentration, yet progressively reduces the rate of nitrosation. Thus, reactions were relatively slow at the highest acidities, but proceeded to completion (100% *N*-methyl-*N*-nitrosopiperazine) overnight. The kinetic effect is clearly linked to protonation of the *N*-methylpiperazine. The quantitative yields, however, show that in the presence of HCl iodine is a catalyst, rather than promoter, and therefore imply that NOI is formed *via* additional pathways to those shown in Scheme 2. These may be the reaction of HI with either HNO_2 [*i.e.* the reverse of equation (6)] or NO [equation (7)]. We favour the latter because independent experiments

$$2NO + HI \rightleftharpoons (NOH) + NOI \qquad (7)$$

(to be reported later [21]) show that NO is a powerful nitrosating agent when used in conjunction with HI or metal iodides. Further, the catalysis by HCl cannot be explained simply in terms of either the regular acid-catalysed nitrosation by HNO_2, itself, or by NOCl, because much slower reactions prevail in the absence of iodine (see Table 8).

Inhibition by NaN_3 *and Thiourea.*—Both these compounds are known to inhibit conventional nitrosation reactions by competing for the reagent and undergoing rapid deamination.[22] Results summarised in Table 9 show that they have a similar effect on the nitrosation of

* This refers to the pH measured in 25% (v/v) H_2O–EtOH using electrodes calibrated in wholly aqueous standard buffers.

TABLE 8

Effect of added HCl on the nitrosation of *N*-methylpiperazine in 25% (v/v) H_2O–EtOH at 25 °C. Initial [*N*-methylpiperazine] 10^{-2}M; [NO] *ca.* 1.2×10^{-2}M; [I_2] $2.4—2.5 \times 10^{-3}$M

	Experimental	
10^2[HCl]/M	pH [a]	Yield (%) [b]
0	8.10	46
0.25		46
0.50	2.70	68
0.75		70 [d]
1.0	2.42	76 [d]
2.0	2.15	80 [d]
2.0	2.15	5 [c]
3.0	2.03	63 [d]
4.0	1.86	19 [d]
5.0	1.77	5.5 [d]
7.0	1.65	0.5 [d]

[a] See footnote on this page. [b] After 25 min and based on initial [*N*-methylpiperazine]. [c] In absence of added I_2. [d] 100% overnight.

TABLE 9

Inhibition of *N*-nitrosopiperidine formation in EtOH from NO and iodine at 25 °C by added NaN_3 and thiourea. Initial [Piperidine] 10^{-2}M; [NO] *ca.* 2.2×10^{-2}M

10^3[Inhibitor]/M	10^3[I_2]/M	Limiting yield [a] (%)	Time [b]/min
Thiourea			
0	2.46	44	20
1.06	2.44	30	20
2.50	2.40	6	80
NaN_3			
0 [c]	3.10	48	23
2.50 [c]	3.16	40	20
5.63 [c]	3.23	22	20
0 [d]	2.45	26	25
3.23 [d]	2.55	8	80

[a] Based on initial [Piperidine]. [b] Time to reach limiting yield. [c] In 2.5% H_2O–EtOH. [d] In 25% H_2O–EtOH.

piperidine by NO and iodine. This supports our conclusion that neither N-iodoamines nor amine–iodine charge-transfer complexes are involved in *N*-nitrosamine formation.

Conclusions.—Nitric oxide appears to be an ineffectual nitrosating agent in the absence of catalysts. Its deamination of NH_3 is well known, however, particularly at elevated temperatures or pressures,[23] and similar transformations have been reported for both hydrazine [24] and hydroxylamine [25] (the latter under alkaline conditions only). Two other applications of NO to the diazotisation of aromatic amines may be influenced by the presence of oxygen.[26] In our hands, NO at atmospheric pressure under anaerobic conditions reacted only slowly with secondary aliphatic amines, but Drago and his colleagues [27] have shown that ammonium salts ($R_2NH_2^+$ $R_2NN_2O_2^-$) readily precipitate at much higher pressures.

NOI is a much more powerful nitrosating agent and its reactions have previously been investigated in connection with the diazotisation of aromatic amines by HNO_2 under acidic conditions in the presence of iodide salts.[28] Further, there is much evidence to suggest that NOI forms transiently in the gaseous phase from NO and iodine atoms.[29] It is therefore surprising that the combination of NO and I_2 has not previously been used

to effect nitrosation in solution. Our results demonstrate its efficacy under mild conditions and current work is directed towards various synthetic applications with substrates other than secondary amines.

All the experimental findings are consistent with the mechanism for *N*-nitrosamine formation outlined in Scheme 2. The conclusion that the generation of NOI is rate-limiting is consistent with the known properties of this entity. Thus, its reaction with aromatic amines in acidic aqueous solutions is believed to occur on encounter,[30] and the same conclusion should apply to the more basic (and therefore more reactive) aliphatic amines. Further, the formation of NOI from NO and iodine is thermodynamically unfavourable. This is evident from our own examination of the equilibrium concentration of alkyl nitrites in the reaction solutions, studies of the formation of NOI in aqueous media,[31] and the standard oxidation potentials for both $NO^+ + e^- \rightleftharpoons NO\uparrow$ [$E_o = 1.46$ eV in H_2O at 25 °C] and $I_2 + 2e^- \rightleftharpoons 2I^-$ [$E_o = 0.621$ eV in H_2O at 25 °C].[32] Further, in the gaseous phase, ΔG^o_{298} is *ca.* 8.7 kcal mol^{-1} for the formation of NOI from NO and iodine atoms.[29b]

Both the yield of *N*-nitrosamine and its rate of formation are dependent on the solvent. No reaction was evident in pure H_2O, probably because iodine is insoluble. Also, NO is less soluble in H_2O than in EtOH, and, as noted previously, this contributes to the inhibitory effect with mixed solvents. The faster rates in solvent MeCN compared to EtOH are more difficult to explain. It must relate, however, to the iodine, because the concentration of NO in MeCN is about half that in EtOH (see Table 1). Tri-iodide ion formation ($I_2 + I^- \rightleftharpoons I_3^-$) is bound to be less extensive in the aprotic MeCN and the free iodine concentration therefore larger. Further, iodine is known to interact by charge transfer with both MeCN [33] and EtOH,[34] which may further influence the above equilibrium and also alter the oxidation potential of iodine. The kinetic solvent effect is therefore consistent with rate-limiting NOI formation, where either the free iodine concentration is higher in MeCN, or iodine is more readily reduced by NO.

Our findings are also relevant to assessments of human exposure to carcinogenic *N*-nitrosamines [35] and, *inter alia*, to the aetiology of human cancer. *N*-Nitrosamine formation in the presence of iodine is very much faster than from dissolved NO alone ($t_{\frac{1}{2}}$ *ca.* 8 days). For the more basic amines, our reactions are also faster than the maximum rates of *N*-nitrosamine formation in aqueous HNO_2 {at pH 3—3.4 with [HNO_2] 2×10^{-2}M, $t_{\frac{1}{2}}$ (piperidine) equals *ca.* 34 days and $t_{\frac{1}{2}}$ (morpholine) *ca.* 67 min}.[36] NO is a common pollutant from most combustion processes,[8] and is produced from HNO_2 by reduction with antioxidants such as ascorbic acid.[37] Addition of ascorbic acid to the human diet has been recommended [38] to reduce *N*-nitrosamine exposure on the presumption that NO is an ineffectual nitrosating agent. This recommendation may need revision where the dietary intake of iodine is significant.

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