Oxidation of *N*-Nitrosodibenzylamine and Related Compounds by Metalloporphyrin-catalysed Model Systems for the Cytochrome P450 Dependent Mono-oxygenases

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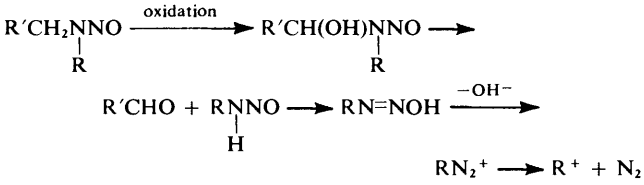
N-Nitrosodibenzylamine has been oxidised to benzaldehyde and benzyl alcohol by iodosylbenzene, 3-chloroperoxybenzoic acid and t-butyl hydroperoxide catalysed by tetraphenylporphyrinato-iron(III) chloride or -manganese(III) chloride. The influence of reaction conditions on the product yields and distribution have been studied. Kinetic isotope effects have been measured with deuteriated *N*-nitrosodibenzylamines for inter- and intra-molecular competition for the oxidants. The evidence presented is in favour of the iodosylbenzene and t-butyl hydroperoxide oxidations being initiated by hydrogen-atom abstraction by the oxidant from the α-hydrogen of the benzyl group. However, oxidations by the peroxy acid systems may proceed by an initial electron transfer. The reactions of *N*-nitrosodimethylamine and *N*-nitrosopiperidine with the metalloporphyrin-catalysed systems show that these substrates are surprisingly unreactive towards oxidation.

The proven toxicity and carcinogenicity of *N*-nitroso-amines to experimental animals,[1] coupled with the observation that *N*-nitroso-amines can be formed in the gastrointestinal tract from amines and nitrite present in food [2] or formed indirectly by bacterial reduction of nitrate,[3] has led to studies of the chemistry [4] and metabolism of these compounds.[4,5] For a typical *N*-nitrosodialkylamine biological activity arises through metabolic oxidation, although whether the oxidation is brought about by a cytochrome P450 dependent mono-oxygenase [6] or by a monoamine oxidase [5b,7] remains to be resolved.[8] The generally accepted pathway [1a,9] involves an initial α-hydroxylation followed by heterolysis to release a diazohydroxide, which can subsequently form an alkanediazonium ion and a carbocation (Scheme 1). Recent work points to the diazohydroxide as being the ultimate carcinogen that alkylates the cellular macromolecules.[10] An alternative alkylating species, the diazoalkane, has been rejected on the basis of deuterium-labelling experiments.[11]

Evidence for α-hydroxylation being the key step in the oxidative metabolism and biological activation comes from structure–activity relationships [12] and from the influence of α-deuteriation [13] or α-alkylation [12,14] on biological activity. Although the instability of α-hydroxy-*N*-nitroso-amines has prevented direct studies on these compounds, stabilised acetylated derivatives have been prepared and tested.[15] Thus *N*-nitroso(acetoxymethyl)methylamine, which is hydrolysed *in situ* by rat liver microsomes with the quantitative evolution of nitrogen, is a more active carcinogen than the parent *N*-nitrosodimethylamine.[16]

Studies on the oxidation of *N*-nitroso-amines with model systems for the cytochrome P450 dependent mono-oxygenases have been confined to Udenfriend's system (Fe^{II}/EDTA/ascorbic acid/dioxygen [17]). This research has included comparative studies of the products from the model and biological systems,[18] and the use of the model system to generate oxidation products from *N*-nitroso-amines in short-term mutagenicity tests.[18b,19] The results from these studies, although far from conclusive, are in agreement with the general mechanism for biological activation shown in Scheme 1.

Our interest in the mechanisms of oxidations brought about by cytochrome P450 dependent mono-oxygenases has led us to examine the reaction of *N*-nitroso-amines with some model oxidants. We report here on our studies with a selection of iron(III) and manganese(III) porphyrin-catalysed systems.



Scheme 1.

Results

Following the observation that the yields of cyclohexanol and cyclohexanone from the oxidation of cyclohexane by the Fe^{III}TPPCl ‡ /iodosylbenzene and Mn^{III}TPPCl/iodosylbenzene systems are higher in benzene than in dichloromethane (Table 1), all the *N*-nitroso-amine oxidations were carried out with benzene as solvent.

Oxidation of N-*Nitrosodibenzylamine*.—Benzaldehyde and benzyl alcohol are obtained when a solution of *N*-nitrosodibenzylamine in benzene is oxidised, under nitrogen, by iodosylbenzene, 3-chloroperoxybenzoic acid, or t-butyl hydroperoxide in the presence of catalytic amounts of Fe^{III}TPPCl or Mn^{III}TPPCl (Table 2). These oxidations are complete within 3 h and after this time the product yields remain constant for several days. The reaction of *N*-nitrosodibenzylamine with Fe^{III}TPPCl and 4-cyano-*N*,*N*-dimethylaniline *N*-oxide, however, is slow, owing to the insolubility of the *N*-oxide in the reaction mixture. At the time of analysis (after 20 h) the reaction mixture still contained unchanged *N*-oxide. The yields of the products from both the Fe^{III}TPPCl- and the Mn^{III}TPPCl-catalysed oxidations are unaffected by carrying out the reactions under air in place of nitrogen (Table 2). Decreasing the ratio of *N*-nitrosodibenzylamine to oxidant from 100 : 1 to 10 : 1 decreases the yield of oxidation

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‡ The following abbreviations are used in this paper: Fe^{III}TPPCl for tetraphenylporphyrinatoiron(III) chloride and Mn^{III}TPPCl for tetraphenylporphyrinatomanganese(III) chloride.

Table 1. Yields of cyclohexanol and cyclohexanone from oxidation of cyclohexane catalysed by $Fe^{III}TPPCl$ or $Mn^{III}TPPCl$ in air

Oxidant	Catalyst	Solvent	Yield (%) Cyclohexanol	Yield (%) Cyclohexanone
PhIO	$Fe^{III}TPPCl$	CH_2Cl_2	5	0.3
PhIO	$Fe^{III}TPPCl$	PhH	30	3
PhIO	$Mn^{III}TPPCl$	CH_2Cl_2	20	12
PhIO	$Mn^{III}TPPCl$	PhH	37	32
$3\text{-}ClC_6H_4CO_3H$	$Fe^{III}TPPCl$	PhH	1	0.2
Bu^tOOH	$Fe^{III}TPPCl$	PhH	20	15

Table 2. Yields of benzaldehyde and benzyl alcohol from oxidation of *N*-nitrosodibenzylamine in benzene catalysed by $Fe^{III}TPPCl$ or $Mn^{III}TPPCl$

Oxidant	Catalyst	Reaction conditions [a]	Yield (%) [b] PhCHO	Yield (%) [b] $PhCH_2OH$
PhIO	$Fe^{III}TPPCl$	N_2	16	4
PhIO	$Fe^{III}TPPCl$	Air	14	n.m.
$3\text{-}ClC_6H_4CO_3H$	$Fe^{III}TPPCl$	N_2	3	3
$3\text{-}ClC_6H_4CO_3H$	$Fe^{III}TPPCl$	Air	4	n.m.
Bu^tOOH	$Fe^{III}TPPCl$	N_2	17	1
Bu^tOOH	$Fe^{III}TPPCl$	Air	15	n.m.
$4\text{-}CNC_6H_4N(O)Me_2$	$Fe^{III}TPPCl$	N_2	8	0.6
PhIO	$Mn^{III}TPPCl$	N_2	36	5
PhIO	$Mn^{III}TPPCl$	Air	39	5
$3\text{-}ClC_6H_4CO_3H$	$Mn^{III}TPPCl$	N_2	7	1
$3\text{-}ClC_6H_4CO_3H$	$Mn^{III}TPPCl$	Air	9	1
Bu^tOOH	$Mn^{III}TPPCl$	N_2	13	0.2
Bu^tOOH	$Mn^{III}TPPCl$	Air	11	0.5

[a] Molar proportions of reactants, *N*-nitrosodibenzylamine : oxidant : catalyst 1 000 : 10 : 1. [b] n.m. = not measured.

Table 3. Influence of substrate–oxidant ratio on yields of benzaldehyde and benzyl alcohol from oxidation of *N*-nitrosodibenzylamine in benzene catalysed by $Fe^{III}TPPCl$ under nitrogen

Oxidant	Substrate : oxidant ratio	Yield (%) PhCHO	Yield (%) $PhCH_2OH$
PhIO	10 : 1	4	2
PhIO	40 : 1	9	n.m.
PhIO	100 : 1	16	4
$3\text{-}ClC_6H_4CO_3H$	10 : 1	1	0.3
$3\text{-}ClC_6H_4CO_3H$	40 : 1	3	n.m.
$3\text{-}ClC_6H_4CO_3H$	100 : 1	3	3
Bu^tOOH	10 : 1	3	0
Bu^tOOH	40 : 1	7	n.m.
Bu^tOOH	100 : 1	17	1

with all the model systems by 3—6-fold (Table 3). In none of these oxidations are detectable amounts (<0.1% yield) of phenol (from oxidation of benzene) or of diphenylmethane or benzyl chloride (from reactions of benzyl cations) formed.

Oxidation of Benzaldehyde.—When benzaldehyde, at a concentration equivalent to a 20% yield from an *N*-nitrosodibenzylamine reaction, was oxidised by the $Fe^{III}TPPCl$/iodosylbenzene system, 87% was recovered unchanged.

Oxidation of N-*Nitrosodimethylamine.*—*N*-Nitrosodimethylamine is surprisingly resistant to oxidation by iodosylbenzene, 3-chloroperoxybenzoic acid, or t-butyl hydroperoxide in the presence of $Fe^{III}TPPCl$. The extent of oxidation was monitored by measuring the yield of formaldehyde and *N*-nitrodimethylamine (Table 4). For the former analysis Nash's spectrophotometric method was used;[20] the alternative analysis with chromotropic acid [21] is unsuitable because

Table 4. Yields of formaldehyde and *N*-nitrodimethylamine from oxidation of *N*-nitrosodimethylamine catalysed by $Fe^{III}TPPCl$ in air

Oxidant	Yield (%) HCHO	Yield (%) Me_2NNO_2
PhIO	>1	*ca.* 1
$3\text{-}ClC_6H_4CO_3H$	1	2
Bu^tOOH	5	*ca.* 1

the method employs strongly acidic conditions which generate highly coloured, protonated porphyrin species that interfere with the spectrophotometric analysis. The presence of *N*-nitrodimethylamine in the oxidation mixtures was examined qualitatively by t.l.c. and quantitatively by g.l.c. No other products from these oxidations were detected.

Attempted Oxidations of N-*Nitrodimethylamine.*—*N*-Nitrodimethylamine at a concentration equivalent to 100% yield from an oxidation of *N*-nitrosodimethylamine was shown to be stable towards oxidation by the $Fe^{III}TPPCl$/iodosylbenzene system.

Oxidation of N-*Nitrosopiperidine.*—The reaction of *N*-nitrosopiperidine with the $Fe^{III}TPPCl$-catalysed model systems gave no 3-hydroxy- or 4-hydroxy-*N*-nitrosopiperidine or *N*-nitroso-4-piperidone (<0.5% yield of each). It was not possible to analyse for products from hydroxylation at the 2-position. With the $Mn^{III}TPPCl$-catalysed systems the only oxidation product detected was 4-hydroxy-*N*-nitrosopiperidine (*ca.* 1% yield) from oxidations with iodosylbenzene.

In the reactions of *N*-nitrosodimethylamine and of *N*-nitrosopiperidine with the iodosylbenzene and the t-butyl hydroperoxide systems the disappearance of the oxidant was

Table 5. Kinetic isotope effects obtained from competitive oxidations of *N*-nitrosodibenzylamine and *N*-nitroso[α,α,α′,α′-$^2H_4$]-dibenzylamine catalysed by Fe^{III}TPPCl or Mn^{III}TPPCl under nitrogen

Oxidant	Catalyst	k_H/k_D [a]
PhIO	Fe^{III}TPPCl	3.8 ± 0.6
PhIO	Mn^{III}TPPCl	5.0 ± 1.0
3-$ClC_6H_4CO_3H$	Fe^{III}TPPCl	1.5 ± 0.2
3-$ClC_6H_4CO_3H$	Mn^{III}TPPCl	1.7 ± 0.3
Bu^{t}OOH	Fe^{III}TPPCl	11.1 ± 3.0
Bu^{t}OOH	Mn^{III}TPPCl	10.0 ± 3.0

[a] Measured by g.l.c.–mass spectrometry from yields of benzaldehyde and [α-^{2}H]benzaldehyde.

Table 6. Kinetic isotope effects obtained from oxidation of *N*-nitroso-*N*-([α,α,4-$^2H_3$]benzyl)benzylamine catalysed by Fe^{III}TPPCl or Mn^{III}TPPCl under nitrogen

Oxidant	Catalyst	k_H/k_D [a]
PhIO	Fe^{III}TPPCl	1.9 ± 0.3
PhIO	Mn^{III}TPPCl	3.5 ± 0.5
3-$ClC_6H_4CO_3H$	Fe^{III}TPPCl	3.3 ± 0.5
3-$ClC_6H_4CO_3H$	Mn^{III}TPPCl	4.0 ± 0.6
Bu^{t}OOH	Fe^{III}TPPCl	6.8 ± 1.2
Bu^{t}OOH	Mn^{III}TPPCl	10.0 ± 3.0

[a] Measured by g.l.c.–mass spectrometry from yields of benzaldehyde and [α,4-$^2H_2$]benzaldehyde.

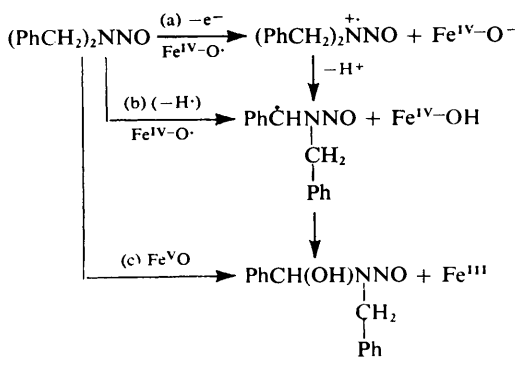
confirmed by the formation of iodobenzene and t-butyl alcohol respectively (g.l.c. analysis).

Measurement of Kinetic Isotope Effects in the Oxidations of N-*Nitrosodibenzylamine,* N-*Nitroso*-N-([α,α,4-$^2H_3$]*benzyl*)-*benzylamine and* N-*Nitroso*-[α,α,α′,α′-$^2H_4$]*dibenzylamine*.—(a) *Intermolecular kinetic isotope effects*. The relative reactivities of *N*-nitrosodibenzylamine and *N*-nitroso[α,α,α′,α′-$^2H_4$]dibenzylamine towards the oxidants in the model systems were obtained from the relative yields of the products benzaldehyde and [α-^{2}H]benzaldehyde, respectively (g.l.c.–mass spectrometric analysis) from competition experiments. The g.l.c.–mass spectrometric analysis was calibrated with standard mixtures of benzaldehyde and [α-^{2}H]benzaldehyde. These data (Table 5) show that the reactions supported by the three oxidants have very distinct kinetic isotope effects and that these values are not strongly dependent on the catalyst. The t-butyl hydroperoxide system has the largest isotope effect and that using 3-chloroperoxybenzoic acid the smallest. Similar, but less precise values for these isotope effects were also obtained for the Fe^{III}TPPCl systems by comparing the yields of benzaldehyde and [α-^{2}H]benzaldehyde when the two substrates were oxidised separately in equivalent reactions.

(b) *Intramolecular kinetic isotope effects*. The yields of benzaldehyde relative to [α,4-$^2H_2$]benzaldehyde (g.l.c.–mass spectrometric analysis) from the oxidation of *N*-nitroso-*N*-([α,α,4-$^2H_3$]benzyl)benzylamine by the model systems gave the intramolecular kinetic isotope effects listed in Table 6.

Discussion

Although it is generally accepted that the oxidative metabolism of *N*-nitroso-amines is initiated by α-hydroxylation, the mechanism of this step remains uncertain. The aim of this research was to study the mechanism of oxidation of *N*-nitroso-amines by the Fe^{III}TPPCl- and Mn^{III}TPPCl-catalysed models for cytochrome P450 dependent mono-oxygenases in the hope that the results would throw light on the initial step of the biological oxidation.



Scheme 2.

Three possible routes to an α-hydroxy-*N*-nitroso-amine are illustrated with *N*-nitrosodibenzylamine and an oxo-iron oxidant in Scheme 2. Path (a) involves an initial electron transfer. This is directly analogous to mechanisms proposed for chemical,[22] electrochemical,[23] and biological [24] oxidative dealkylations of amines although the large ionisation [25] and oxidation potentials [26] of *N*-nitroso-amines indicate that they are less likely to be oxidised by this mechanism than amines. In path (b) the active oxidant removes an α-hydrogen atom and the α-nitroso-amino radical is hydroxylated in the second step. This mechanism is based on that proposed by Groves and his co-workers for the hydroxylation of alkanes by cytochrome P450 mono-oxygenases.[27] The active oxidant in path (c) inserts an oxygen directly into the α-C–H bond of the *N*-nitroso-amine in an oxenoid mechanism.[28]

The large intermolecular and comparable intramolecular kinetic isotope effects from oxidations with the t-butyl hydroperoxide and iodosylbenzene systems suggest that α-C–H bond breakage is both rate-limiting and product-determining in these oxidations. These values are similar to the isotope effects obtained for the oxidative demethylation of anisole and [Me-$^2H_3$]anisole with Fe^{III}TPPCl and iodosylbenzene,[29] and for aliphatic hydroxylation by cytochrome P450 dependent mono-oxygenases,[27b,30] and are consistent with hydrogen-atom abstraction [path (b)] *via* a symmetric, linear transition state.[31] Path (c) would show a smaller isotope effect.[32] The absence of *N*-nitro-amines in the *N*-nitroso-amine oxidation mixtures also suggests that the radical mechanism is more likely since an oxenoid species would have been expected to *N*-oxidise an *N*-nitroso-amine (*N*-nitro-amines are prepared by peroxy acid [33] or molybdenum-catalysed hydroperoxide [34] oxidation of *N*-nitroso-amines). With path (a), assuming that electron transfer is irreversible and rate determining, there would be a small secondary intermolecular isotope effect for the electron transfer step and a larger intramolecular one for the proton transfer analogous to those observed in the one-electron oxidation of tertiary amines.[35] In agreement with the foregoing conclusions the active oxidants in the iodosylbenzene and alkyl hydroperoxide systems are generally thought to be a metal–oxo intermediate and an alkoxyl radical,[36] respectively, both of which are thought to react with C–H bonds by hydrogen-atom abstraction.

The product yields from the oxidations with the peroxy acid systems are small and mechanistic deductions are more ambiguous. However, the intramolecular isotope effects are significantly larger than the corresponding intermolecular values, suggesting that with this oxidant, unlike iodosylbenzene or t-butyl hydroperoxide, there may be separate

rate- and product-determining steps. This can be rationalised by path (a); the intermolecular values would be the secondary isotope effects for the rate-determining electron transfer and the intramolecular values the primary isotope effects for the product-determining proton loss (the rate- and product-determining isotope effects are similar to those obtained from the one-electron oxidation of amines [35a,b]). Alternatively, in the peroxy acid systems if the formation of a complex between the nitroso-amine and the metalloporphyrin-derived oxidising species were rate-determining the intermolecular isotope effect would be small. The kinetic isotope effect for the oxidation of the nitroso-amine would then only show up in the intramolecular measurement.

From Scheme 1 the breakdown of *N*-nitroso-(α-hydroxybenzyl)benzylamine should give equivalent yields of benzaldehyde and products from benzenediazohydroxide; however, in this study we were unable to account for the majority of the latter. The missing material was shown not to be diphenylmethane (from benzylation of the solvent) or benzyl chloride.

There are two factors that give rise to the low reactivity of *N*-nitrosodimethylamine in the iodosylbenzene and t-butyl hydroperoxide oxidations. First, hydrogen atoms are not readily abstracted from primary C–H bonds by these porphyrin-catalysed model systems.[36b] Thus Mansuy and his coworkers have shown that heptane is oxidised to a mixture of secondary alcohols and ketones with iodosylbenzene or cumene hydroperoxide-promoted systems but negligible reaction occurs at C-1.[36b] Secondly, there will be a strong polar effect between the electrophilic attacking species [37] and the nitroso-amine that will inhibit hydrogen abstraction from C–H bonds close to the strongly electron-withdrawing nitroso-amine group. The influence of polar effects on the regioselectivity of oxidation is well documented.[38] For these reasons *N*-nitrosodimethylamine is unreactive (it resembles acetonitrile and acetic acid, which are used as solvents or cosolvents for radical oxidations) and is an inefficient trap for the active oxidant, which is destroyed by self-oxidation.[39] An alternative explanation where initial hydrogen abstraction is followed by fragmentation of the α-nitroso-amino radical [reaction (i)] [40] cannot account for the absence of formaldehyde

$$\cdot CH_2N(CH_3)NO \longrightarrow CH_2{=}NCH_3 + NO \qquad (i)$$

because the imine product would be hydrolysed and estimated by Nash's reagent. Furthermore it seems unlikely, in the light of the low reactivity of benzaldehyde towards oxidation by the porphyrin model systems, that the absence of formaldehyde can be explained by further reaction with the oxidising species.

It is surprising that these model systems will oxidise cyclohexane but are unreactive towards *N*-nitrosopiperidine, particularly since the latter is oxidised at the 3- and 4-positions by Udenfriend's system.[18a] The origin of this effect is probably the polar influence of the nitroso-amine group described above, and is directly analogous to the greater reactivity of cyclohexane over cyclohexanol towards oxidation by peroxytrifluoroacetic acid.[38d] The regioselectivity of oxidation by these model systems is currently under investigation.

Experimental

Materials.—All the materials were of commercial reagent grade unless otherwise stated.

N-Benzyl-[4-^{2}H]benzamide was prepared by reaction of [4-^{2}H]benzoyl chloride with benzylamine. [4-^{2}H]Benzoic acid [41] (4.92 g) and oxalyl chloride (12 g) were dissolved in toluene (35 cm^3). The reaction was initiated with one drop of dimethylformamide and the mixture stirred at room temperature for 3 h, then heated on a steam-bath for 10 min. Evaporation *in vacuo* gave a yellow oil which was then dissolved in anhydrous diethyl ether (40 cm^3). Benzylamine (10.7 g) dissolved in anhydrous diethyl ether (25 cm^3) was added dropwise to the acid chloride solution with stirring and cooling in ice. The mixture was shaken with hydrochloric acid and the solid amide collected by filtration. Additional amide was recovered from the ether layer. The combined solids were recrystallised (ethyl acetate–hexane) to give *N*-benzyl-[4-^{2}H]benzamide (6.6 g, 78%) as colourless plates, m.p. 100—104 °C (lit.,[42] 104—106 °C for protio analogue), δ ($CDCl_3$) 7.8—7.3 (m, 9 H), 6.6 (br s, 1 H), 4.6 (s, 2 H), and 4.5 (s, 2 H).

N-([α,α,4-$^2H_3$]Benzyl)benzylamine was prepared in 73% yield by the method of Axenrod and Milne,[40] by reduction with lithium aluminium deuteride (Aldrich, 98% D) of *N*-benzyl-[4-^{2}H]benzamide, b.p. 110 °C at 0.3 mmHg (lit.,[40] 136—138 °C at 2 mmHg).

N-Nitrosodibenzylamine, and *N*-nitroso-*N*-([α,α,4-$^2H_3$]-benzyl)benzylamine were obtained in 63—90% yield by nitrosation of dibenzylamine and *N*-([α,α,4-$^2H_3$]benzyl)-benzylamine, respectively, using the method of Pensabene *et al.*[43] All the *N*-nitrosodibenzylamines were recrystallised from ethanol–water, then from ethyl acetate–hexane; m.p. 58—59 °C (lit.,[43] 58—59 °C). *N*-Nitrosodibenzylamine had δ (CD_3CN) 7.4—6.9 (m, 10 H), 5.3 (s, 2 H), and 4.65 (s, 2 H); *m/z* 226 (M^+, 5), 181 (7), 92 (8), 91 (100), 90 (8), 77 (6), 65 (27), and 51 (9). *N*-Nitroso-*N*-([α,α,4-$^2H_3$]benzyl)benzylamine had *m/z* 229 (M^+, 14), 184 (6), 94 (100), 93 (9), 92 (15), 91 (93), 78 (2), 77 (3), 68 (3), 67 (6), 66 (4), 65 (9), and 51 (4).

N-Nitroso[α,α,α′,α′-$^2H_4$]dibenzylamine was obtained by dissolving *N*-nitrosodibenzylamine (1.8 g) in a mixture of dry dioxane (15 cm^3), [*O*-^{2}H]methanol (15 cm^3), and sodium deuterioxide (0.37 g sodium in 16 cm^3 deuteriated water) and heating at 120 °C. After 100 h the mixture was concentrated under vacuum and extracted with diethyl ether. The extract was dried ($MgSO_4$) and evaporated under vacuum and the residue was recrystallised (aqueous methanol) to give *N*-nitroso[α,α,α′,α′-$^2H_4$]dibenzylamine (1.33 g, 73%), m.p. 58—59 °C. No benzylic hydrogens were detected in the ^{1}H n.m.r. spectrum (>99% tetradeuteriated); *m/z* 230 (M^+, 7), 184 (4.5), 94 (8), 93 (100), 91 (6), 67 (13), 66 (11), and 51 (6).

N-Nitroso-4-piperidone, *N*-nitroso-4-hydroxypiperidine, and *N*-nitroso-3-hydroxypiperidine were prepared by nitrosation of the parent amine or its hydrochloride by the method of Pensabene *et al.*[43] The *N*-nitroso-4-piperidone, purified by sublimation, had m.p. 63—64.5 °C (lit.,[18a] 63.5—64.5 °C). *N*-Nitroso-4-hydroxypiperidine was purified by distillation; b.p. 115—118 °C at 0.15 mmHg (lit.,[18a] 135 °C at 0.2 mmHg); *N*-nitroso-3-hydroxypiperidine had b.p. 105—108 °C at 0.15 mmHg (lit.,[18a] 120 °C at 0.2 mmHg). The three *N*-nitrosopiperidine derivatives each gave one spot on t.l.c. (silica with $CHCl_3$–MeOH, 9 : 1) and could be separated by g.l.c. on OV-17 (5% w/w) at 180 °C.

N-Nitrodimethylamine was prepared following the method of Emmons,[33] and had m.p. 54—55 °C (lit.,[33] 55—56 °C), pure by t.l.c. (silica with $CHCl_3$) and g.l.c. [OV-17 (5%, w/w) at 120 °C].

[α-^{2}H]Benzaldehyde was prepared from benzil following the method of Burgstahler *et al.*; [44] ^{1}H n.m.r. spectroscopy showed this to be ⩾99.5% monodeuteriated.

[α,4-$^2H_2$]Benzaldehyde was prepared from [4-^{2}H]benzoic acid. [4-^{2}H]Benzoic acid [41] was converted into methyl [4-^{2}H]-benzoate by refluxing in methanol and sulphuric acid. The ester was then reduced with lithium aluminium deuteride (Aldrich, 98% D) to give [α,α,4-$^2H_3$]benzyl alcohol, δ ($CDCl_3$) 7.25 (s, 4 H) and 2.65 (s, 1 H). The [α,α,4-$^2H_3$]benzyl alcohol was oxidised to [α,4-$^2H_2$]benzaldehyde with silver carbonate

on Celite by the method of Fetizon *et al.*;[45] δ ($CDCl_3$) 7.5 and 7.9 (ABq, *J* 8 Hz, 4 H); *m/z* 108 (M^+, 84), 107 (13), 106 (97), and 78 (100).

Tetraphenylporphyrinatoiron(III) chloride and iodosylbenzene were prepared as described previously.[37] Tetraphenylporphyrinatomanganese(III) chloride was prepared by the method of Adler *et al.*[46] from *meso*-tetraphenylporphyrin and manganese(II) chloride and was purified by chromatography on neutral alumina ($CHCl_3$).

Methods.—A Varian 3 700 gas chromatograph equipped with a flame ionisation detector and a column packed with OV-17 (5% w/w) on 80—100 mesh Chromosorb W-HP was used for g.l.c. analyses. Combined g.l.c.-mass spectrometry was carried out on a Hewlett-Packard 5992A system with a J and W DB-1 methylsilicone bonded phase fused silica WCOT capillary column (15 m × 0.3 mm i.d.). For t.l.c. E.M. Reagents silica gel 60 F_{254} and Whatman KC18 reverse-phase plates were used.

^1H N.m.r. spectra were recorded on a Varian T-60 spectrometer. Visible and u.v. spectra were obtained with a Cary 118 spectrophotometer.

Oxidation Procedure.—In a typical oxidation the oxidant (2.2×10^{-2} mmol) was added to a stirred solution of the catalyst (2.2×10^{-3} mmol) and substrate (2.2 mmol) in benzene (1 cm^3) under nitrogen or air. After 3 h, when the reaction was complete, the products were analysed. Qualitative analysis was performed by t.l.c. and quantitative analysis of all the products, except formaldehyde, by g.l.c.

Measurement of Kinetic Isotope Effects.—For oxidations of deuteriated substrates the procedure above was used on a half-scale, except that the quantity of substrate, *N*-nitroso-*N*-([α,α,4-$^2H_3$]benzyl)benzylamine, or combined substrates, *N*-nitrosodibenzylamine and [α,α,α′,α′-$^2H_4$]-*N*-nitrosodibenzylamine was reduced by 80%. The reactions were analysed by g.l.c. and combined g.l.c.–mass spectrometry. Kinetic isotope effects were calculated from the relative yields of benzaldehyde and either [α-^{2}H]benzaldehyde or [α,4-$^2H_3$]benzaldehyde.

Measurement of Formaldehyde Yield.—Samples from *N*-nitrosodimethylamine mixtures were analysed for formaldehyde by Nash's colorimetric method.[20] The magenta colouration that developed was monitored at 412 nm and compared with background absorptions from blank oxidation mixtures.

Standardisation of the G.l.c.–Mass Spectrometric Analysis for Measuring the Relative Yields of Benzaldehyde and [α-^{2}H]-*Benzaldehyde.*—Synthetic mixtures of benzaldehyde and [α-^{2}H]benzaldehyde were analysed by g.l.c.–mass spectrometry. The ratio of *m/z* 107 to *m/z* 106 ion in the benzaldehyde eluted from the chromatograph, obtained by selective ion monitoring, gave a good linear calibration when plotted against the molar ratio of [α-^{2}H]benzaldehyde to benzaldehyde.

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