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OXIDATION OF THE METHYL GROUPS OF *N, N*-DIMETHYLBENZAMIDES BY A CYTOCHROME P450 MONO-OXYGENASE MODEL SYSTEM

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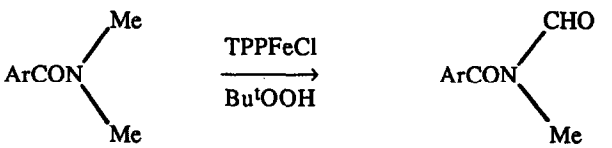
Summary Oxidation of *N, N*-dimethylbenzamides to the corresponding *N*-formyl-*N*-methylbenzamides using tetraphenylporphyrinato-iron(III)-Bu^{t}OOH is independent of the substituent in the aryl ring of the benzamide group and subject to a kinetic deuterium isotope effect of 5.6. These results are consistent with a mechanism involving direct hydrogen atom abstraction from the substrate.

Certain *N,N*-dialkylamides have important bioactivity. For example, *N,N*-dimethylformamide (DMF) is an industrially important solvent that is toxic on occupational exposure[1] and *N, N*-diethyl-3-toluamide (DEET) is the most effective topical insect repellant in worldwide use.[2] DMF undergoes hydroxylation of the *N*-methyl group by cytochrome P450 as a major metabolic pathway.[1] DEET is rapidly absorbed through the skin and metabolised *via* a cytochrome P450 *N*-deethylation reaction.[3] Thus, the mechanism of cytochrome P450 metabolism of *N*-alkylamides is of cental importance to their cytotoxicity. We have therefore undertaken studies of *N,N*-dimethylbenzamides with a cytochrome P450 model system, tetraphenylporphyrinatoiron(III) chloride (TPPFeCl) using *t*-butyl hydroperoxide as co-oxidant, and report that a combination of substituent and kinetic deuterium isotope effects point to a direct hydrogen atom abstraction mechanism.



Oxidation of the *N,N*-dimethylbenzamides using TPPFeCl-Bu^{t}OOH (10^{-3} mol dm^{-3} TPPFeCl; 2 mol equiv. Bu^{t}OOH) in CH_2Cl_2 at 30°C results in the direct formation of the corresponding *N*-formyl-*N*-methylbenzamide (equation). No evidence for the formation of an unstable *N*-hydroxymethyl-N-methylbenzamide intermediate could be obtained. The reactions were monitored by hplc and plots of initial rate, v_i, *versus* initial substrate concentration, S_i, result in plots that exhibit saturation indicative of complex formation between substrate and active oxidant, and from which values of k and k/K_m can be obtained (Table).

Table Values of k and k/K_m for the oxidation of *N,N*-dimethylbenzamides, $ArCONMe_2$, and for their corresponding ionisation potentials

Ar	$10^3k/s^{-1}$	$10^3k/K_m/l\ mol^{-1}\ s^{-1}$	I.P./eV[b]
4-$MeOC_6H_4$	1.59	2.29	9.26
Ph	1.22	2.61	9.55
	0.22[a]	0.47[a]	
4-ClC_6H_4	1.44	2.44	9.66
4-$NO_2C_6H_4$	0.81	2.43	10.01

[a] For $PhCON(CD_3)_2$ [b] Calculated using the AM1 SCF m.o. method

These show that neither k nor k/K_m are significantly dependent on the aryl substituent. Nor is there any correlation with the calculated ionisation potentials of the amides. However, oxidation of *N,N*-di(deuteriomethyl)benzamide displays kinetic deuterium isotope effects in both k and k/K_m of 5.6. These are large and indicative of a hydrogen atom transfer reaction.[4] The anodic oxidation of amides proceeds by electron transfer with the formation of an amide cation radical, the subsequent breakdown of which involves proton loss as evidenced by kinetic deuterium isotope effect of 1.4-1.7.[5] The clear absence of a substituent effect in our work argues that such a cation radical cannot be involved in the TPPFeCl-Bu^{t}OOH catalysed oxidation. We have not yet identified the species, TPPFe(IV)O$^{+\bullet}$ or Bu^{t}O$^\bullet$, that brings about hydrogen atom abstraction.[6] However, both the saturation kinetics and possible formation of an intermediate by uv-visible spectroscopy (λ_{max} 409nm, ε *ca.* 7.5×10^4) suggests that the ferryl-oxo complex is the oxidising species. A mechanism consistent with these observations is as follows:

$$Fe^{IV}(O^\bullet) + ArCONMe_2 \underset{K_m}{\rightleftharpoons} Fe^{IV}(O^\bullet)\cdot ArCONMe_2 \xrightarrow{k} Fe^{IV}(OH)\cdot ArCONMeCH_2^\bullet \longrightarrow \text{products}$$

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