Bimolecular Reactions of Mutagenic *N*-Acetoxy-*N*-alkoxybenzamides and *N*-Methylaniline

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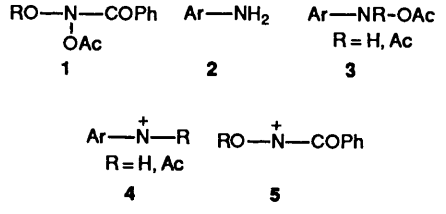
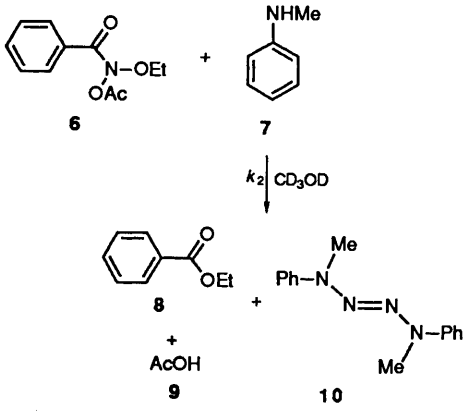
N-Methylaniline reacts bimolecularly with mutagenic *N*-acetoxy-*N*-alkoxybenzamides and the resultant *N*-alkoxy-*N*-(*N*-methylanilino)benzamides undergo a novel, concerted rearrangement to alkylbenzoates and 1-methyl-1-phenyldiazene which dimerizes to 1,4-dimethyl-1,4-diphenyltetrazene.

N-Acetoxy-*N*-alkoxybenzamides (**1**) are a new class of chemical mutagens.[1,2,3] They are analogous to the ultimate carcinogens derived from the metabolism of the aromatic amines (**2**), namely *N*-acetoxy-*N*-arylamines (**3**) or their sulfate esters, both of which have been widely implicated in the mutation of nucleic acids.[4] These metabolites are believed to react with DNA, nucleotides and model aromatic amines by either an S_N1 or an S_N2 process and evidence for both modes of reaction has recently emerged. Certainly, S_N1 reactivity has been found under solvolytic conditions and here an electrophilic, resonance-stabilised arylnitrenium ion (**4**) is generated.[5] However, both Boche and Novak have recently reported bimolecular reactions for model aromatic amine metabolites.[6]



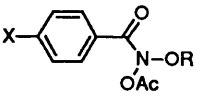
N-Acetoxy-*N*-alkoxybenzamides are direct acting mutagens, in that enzymatic activation is not required for their biological activity and, as such, they would be expected to modify DNA directly.[1] By analogy with the metabolites from the aromatic amines, these mutagens are electrophilic in that they possess a good leaving group at nitrogen, but they are also sources of electrophilic *N*-alkoxynitrenium ions (**5**). Using semi-empirical and *ab initio* molecular orbital methods we recently compared *N*-aryl- (**4**) and *N*-alkoxy-*N*-acylnitrenium ions (**5**) and found them to benefit from similar resonance stabilisation.[7] Thus the biological activity of **1** might well be derived from either of the two possible modes of nucleophilic substitution characterised as S_N1 and S_N2. However, solvolytic studies have shown that these mutagens react rather slowly in aqueous organic medium unless an acid catalyst is present, in which case *N*-alkoxy-*N*-acylnitrenium ions (**5**) are generated along with acetic acid in an $A_{Al}1$ process.[2,3] From studies on a range of *para*-substituted mutagens, there appears to be no obvious correlation between the rates of such acid-catalysed reactions and the levels of mutagenesis as determined in the Ames test.[3] Thus the formation of electrophilic *N*-alkoxynitrenium ion intermediates is unlikely to be a determining factor in their mutagenesis. We report now that *N*-acetoxy-*N*-alkoxybenzamides also undergo bimolecular substitution at nitrogen with an aromatic amine.

N-Methylaniline (**7**) reacts with *N*-acetoxy-*N*-ethoxybenzamide (**6**) in [$^2H_4$]methanol to yield ethyl benzoate (**8**), acetic acid (**9**) and 1,4-dimethyl-1,4-diphenyltetrazene (**10**), which crystallises from the reaction mixture (Scheme 1). The reaction is characterised by a deep red colouration and appears to be



Scheme 1

promoted by polar solvents in that **6** and **7** were unchanged after 36 h in neat acetonitrile. Addition of water, however, led to a rapid, deep red colouration and precipitation of the tetrazene. NMR analysis indicated concurrent formation of **8** and **9**. The disappearance of **11a** and *N*-methylaniline **7** was monitored in



11a; X = H, R = Bu
b; X = MeO, R = Bu
c; X = NO_2, R = Bu
d; X = Br, R = Bu
e; X = Cl, R = Bu
f; X = Ph, R = Bu
g; X = Bu^t, R = Bu
h; X = Me, R = Bu

[$^2H_4$]methanol by 300 MHz ^1H NMR spectroscopy using the acetoxy methyl (δ 2.12) and *N*-methyl (δ 2.82) resonances respectively and the reaction follows bimolecular kinetics (Fig. 1). 66% of the mutagen was consumed after 15.5 min and acetic acid, butyl benzoate and tetrazene were formed at a rate commensurate with the disappearance of **11a**.

Reaction rate constants and Arrhenius activation parameters for the bimolecular reaction of *N*-methylaniline with **11a–g** are given in Table 1. The $\Delta S^{\ddagger}$ values are typically negative in keeping with an associative transition state complex.

The rate constant is greater with electron withdrawing *para*-substituents but, unlike the acid-catalysed solvolysis reactions in which nitrenium ion character is developed β to the aromatic ring (σ^+ correlation with $\rho = -1.5$),[3] the role of the *para*-substituents in these reactions is predictably small and the rate

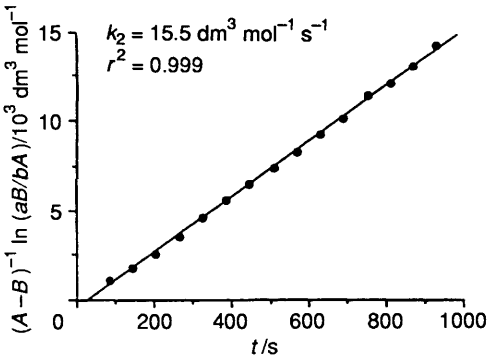


Fig. 1 Second order fit of the disappearance of **11a** and **7** in [$^2H_4$]methanol at 288 K; *A* and *B*—initial concentrations of **7** and **11a**; *a* and *b*—their concentrations at time *t*

Table 1 Arrhenius parameters and bimolecular rate constants for reaction of *N*-acetoxy-*O*-butyl benzohydroxamates (**11a–g**) and *N*-methylaniline (**7**) at 308 K [a]

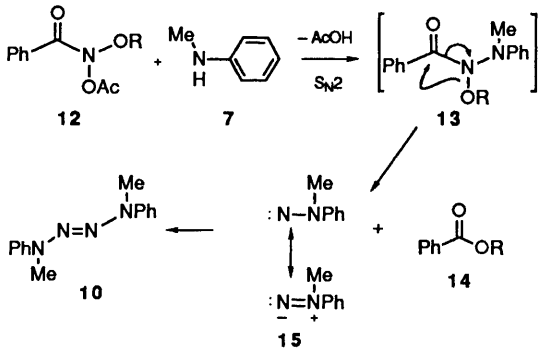
Compound	X	E_a/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	k_2^{308}/dm^3 mol^{-1} s^{-1} [b]	*r*
11a	H	53.98	−42.78	69.1	0.999
b	MeO	55.85	−63.38	48.2	0.993
c	NO_2	47.36	−62.42	86.2	0.983
d	Br	56.59	−33.98	71.7	0.991
e	Cl	37.59	−94.92	78.4	0.996
f	Ph	46.84	−66.17	67.2	0.991
g	Bu^t	55.17	−38.94	68.9	0.994

[a] CD_3OD. [b] From Arrhenius data at 308 K.

data at 308 K correlate with Hammett σ values with a low but positive ρ value of 0.15.

The rates are substantially faster than the acid-catalysed solvolysis to nitrenium ions.* Furthermore the rate constants are some four orders of magnitude greater than those obtained for the reactions of anilines with metabolites of the aromatic amines.[6]

The mechanism of tetrazene formation is depicted in Scheme 2. Since uncatalysed nitrenium ion formation is at best extremely slow,[3] rate-limiting attack of the aniline on an ion



Scheme 2

pair is unlikely and, while nucleophile-assisted ionisation cannot be precluded, the data are most consistent with an S_N2 process. The intermediate *N*-alkoxy-*N*-(*N*-methylanilino)-benzamides (**13**) are unstable and undergo a novel rearrangement to alkyl esters (**14**) and 1,1-diazene (**15**) which is responsible for the deep red colouration. 1-Methyl-1-phenyldiazene is unstable at room temperature and dimerises to **10** which, apart from acetic acid and the ester, is the only other product observable by ^1H NMR spectroscopy. 1,1-Diazenes which are sufficiently stable under the reaction conditions[8] and even kinetically stable, sterically hindered 1,1-diazenes are known to dimerise to tetrazenes.[9] An ultraviolet spectrum of the reaction mixture displayed absorptions at 344 nm due to the tetrazene and one at 462 nm which disappeared in time. Dervan and co-workers reported that in polar media, 1,1-diazenes have a weak n–π* absorption in this region.[9] The diazene (**15**) must however be present in low concentration since no *N*-methyl resonance other than those for **7** and **10** could be observed in ^1H NMR spectra of the reaction mixtures even when the reaction was carried out at 258 K.

The rearrangement of **13** to **14** and **15** appears to be concerted, since a crossover experiment using a mixture of *N*-acetoxy-*N*-butoxytoluamide (**8h**) and *N*-acetoxy-*N*-ethoxybenzamide (**6**) afforded good yields of butyl toluate and ethyl benzoate as the only detectable esters (HPLC). The driving force is most likely the reduction in electron–electron repulsion at the amide nitrogen. Clearly, amides geminally substituted with electronegative atoms are destabilised by lone pair–lone pair repulsion. In addition, unlike simple alkylnitrenes, aminonitrenes are stabilised by overlap between the filled p-orbital of the amino substituent and the vacant orbital on the nitrene. In fact stable 1,1-dialkyldiazenes exhibit a typical N=N stretch absorption just above 1600 cm^{-1} in their IR spectra.[9]

AM1 molecular orbital calculations[10] predict that the rearrangement of the model compound *N*-dimethylamino-*N*-methoxyacetamide (**16**) to 1,1-dimethyldiazene (**17**) and methyl acetate (**18**) is exothermic by 94.6 kJ mol^{-1}. The gas-phase activation energy is 160.6 kJ mol^{-1} (Fig. 2). From a complete mapping of the potential energy surface for the reaction, the process is concerted and the reactants and products are connected by a transition state in which the methoxy group is equidistant (1.85 Å) from both the acetamide nitrogen and carbonyl carbon and orthogonal to the N–C=O plane.

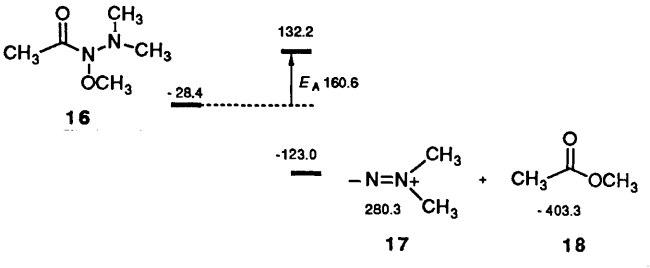


Fig. 2 AM1 energies (kJ mol^{-1}) for rearrangement of *N*-dimethylamino-*N*-methoxyacetamide to 1,1-dimethyldiazene and methyl acetate

The formation of the 1,4-dimethyl-1,4-diphenyltetrazene in these reactions and the bimolecularity of the substitution reaction are real evidence that *N*-acetoxy-*N*-alkoxybenzamides are susceptible to nucleophilic attack at the amide nitrogen. We are currently investigating the reactivity of other primary and secondary amines to establish whether this is a general reaction of these mutagens. *In vivo* and *in vitro* studies have shown that DNA reacts with metabolites from the aromatic amines at the 2-amino substituent of guanine residues. While the exact mechanism for this process is yet to be established, it results in adduct formation and alteration of hydrogen bonding in that region. The preliminary results reported here suggest that mutagenic *N*-acetoxy-*N*-alkoxybenzamides might be expected to modify nucleic acids through a bimolecular substitution

* For example, for identical concentrations of **11b**, equivalent rates of acid-catalysed solvolysis (in CD_3CN/D_2O) and bimolecular reaction with aniline (in [$^2H_4$]methanol) at 308 K would require [H^+] = 143 × [methylaniline].

process resulting in the formation of a 1,1-diazene at this position or at the 4-amino groups of adenine or cytosine.

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