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SOLVOLYSIS AND MUTAGENESIS OF N-ACETOXY-N-ALKOXYBENZAMIDES - EVIDENCE FOR NITRENIUM ION FORMATION

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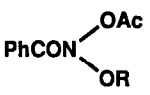
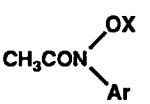
Mutagenic N-acetoxy-N-butoxybenzamides undergo acid catalysed solvolysis in acetonitrile/water. Kinetic data and a Hammett σ^+ correlation (ρ=-1.4) indicate nitrenium ion formation in the rate determining step. Levels of mutagenicity mirror the rates of solvolysis suggesting nitrenium ion involvement.

Recently we described the synthesis and mutagenesis of the new class of geminally substituted amides the N-acetoxy-N-alkoxybenzamides (1). [1] These compounds are N-alkoxy analogues of N-acetoxy-N-arylacetamides (2) which are widely believed to be the potent metabolites derived from carcinogenic aromatic amines such as 2-aminofluorene (4). [2] These metabolites and their sulfate counterparts (3) are thought to react as electrophiles towards nucleotides and considerable effort is currently devoted to establishing whether substitution of acetate or sulfate occurs by an S_N1 or an S_N2 process. [3] Novak *et al* [4] and Boche *et al* [5] have recently presented evidence for an S_N2 reaction by anilines at nitrogen. The former mechanism would however involve the intermediacy of a highly reactive though resonance stabilised nitrenium ion (5).

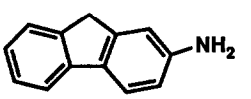


(1)

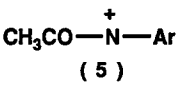
(a) R=Et
(b) R=Bu
(c) R=Bz
(d) R=Oct



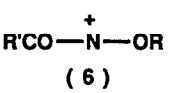
(2) X=Ac
(3) X=SO_3^-



(4)



(5)



(6)

Our molecular orbital studies have indicated that an N-alkoxy-N-acyl nitrenium ion (6) would benefit from a similar stabilisation to that of (5) due to overlap between the vacant orbital on nitrogen and a lone pair orbital on the neighbouring oxygen atom. [6] Significantly all N-acetoxy-N-alkoxybenzamides we have so far subjected to the Ames test have been found to be mutagenic **without** metabolic activation [7] and by analogy with (2) the mutagens may either act directly as an electrophile towards DNA/RNA or solvolysis intracellularly results in formation of a nitrenium ion which intercepts nucleic acids at a nucleophilic center. Accordingly we have engaged in a series of studies to establish whether the solvolysis of (1) involves nitrenium ion intermediacy.

Initial ^{1}H n.m.r. studies in CD_3CN/D_2O (*ca*4:1) indicated that N-butoxy-N-acetoxybenzamide (1b) reacted giving a variety of products including quantitative formation of acetic acid. Monitoring the disappearance of the acetoxyl methyl singlet in the ^{1}H n.m.r. spectrum of (1b) at 308K indicated a poor correlation with unimolecular or pseudo unimolecular kinetics but autocatalysis by acetic acid was evidenced by an excellent fit (r^2=0.999) to integrated rate equation [1] for the solvolysis of esters of weak carboxylic acids in which $[S]_o$ and $[A]_t$ are the initial concentration of (1b) and the concentration of acetic acid respectively, k is the unimolecular or pseudo unimolecular rate constant, K' is the dissociation constant of acetic acid and K is the pre-equilibrium constant for protonation of (1b) (Scheme 1) [8]

$$AcOH \overset{K'}{\rightleftarrows} AcO^- + H^+$$

$$H^+ + S \overset{K}{\rightleftarrows} SH^+$$

$$SH^+ \xrightarrow{k} \text{products} + AcOH$$

Scheme 1

$$kK\sqrt{(K'.[S]_o)}\ t = \ln\frac{[\sqrt{[S]_o}+\sqrt{[A]_t}]}{[\sqrt{[S]_o}-\sqrt{[A]_t}]} \qquad [1]$$

The composite rate constant ($kK.\sqrt{K'}$) at 308K was found to be 8.58×10^{-5} $l^{1/2}mol^{1/2}s^{-1}$ however derivation of the first order rate constant, k, requires a knowledge of the dissociation constant K' for acetic acid under these conditions as well as K.

Upon addition of a solution of sulphuric acid in D_2O the reaction obeyed pseudo-unimolecular kinetics consistent with a rapid reversible protonation followed by a slow decomposition to acetic acid and products (Scheme 2). Since under these conditions water (D_2O) is in a relatively small excess compared to dilute aqueous solutions, the rate may best be represented by equation [2].

$$H_3O^+ + S \underset{}{\overset{K}{\rightleftarrows}} SH^+ + H_2O$$

$$SH^+ \xrightarrow{k} \text{products} + AcOH$$

Scheme 2

$$-\frac{d[S]}{dt} = \frac{d[AcOH]}{dt} = k[SH^+] = k.K.\frac{[S][H_3O^+]}{[H_2O]} = k'.[S] \qquad [2]$$

where the pseudo unimolecular rate constant $k' = \frac{k.K}{[H_2O]}.[H_3O^+] = k_H^X[H_3O^+]$.

Pseudo unimolecular rate constants k' for sulphuric acid catalysed solvolysis of (1b) in CD_3CN/D_2O (adjusted to a constant ratio of 3.81:1 [9]) were found to be linearly dependent upon the sulphuric acid concentration (Figure 1) and the gradient afforded a composite rate constant k_H^X of 4.82×10^{-2} (±0.002) $lmol^{-1}s^{-1}$ at 308K. From the intercept, k_o, the rate constant for uncatalysed solvolysis, was at least three orders smaller. (Table1).

Figure 1. Dependence of k' on $[H_2SO_4]$ for solvolysis of (1b) in CD_3CN/D_2O at 308K

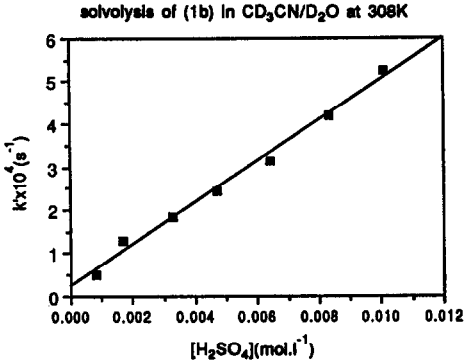
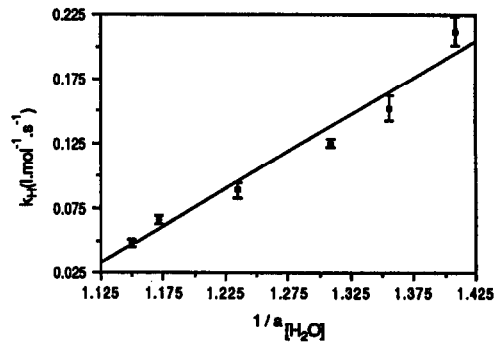


Figure 2. Rates of solvolysis of (1b) in different CD_3CN/D_2O compositions at 308K



Using activities taken for the corresponding mole fractions of water in acetonitrile, [10] experiments at different compositions of CD_3CN/D_2O showed that k_H^X was also inversely proportional to the activity of D_2O in CD_3CN which supports a unimolecular process in the rate determining step (Figure 2). From Scheme 2 and equation [2], a solvent (D_2O) induced S_N2 process would make k_H^X independent of the activity of water.

Solvolysis of *p*-substituted-N-acetoxy-N-butoxybenzamides (7) [11] under identical conditions to those described for (1b) and at a minimum of four different acid concentrations gave the rate constants k_H^X in Table 1.

Rapid reversible protonation of N-acetoxy-N-butoxybenzamide occurs most favourably at the amide and ester carbonyls. [13] Attack at the latter could be followed by:

i. unimolecular dissociation into nitrenium ion and acetic acid (analogous to the $A_{Al}1$ mechanism of hydrolysis of tertiary alkyl esters) (scheme 3, path i);

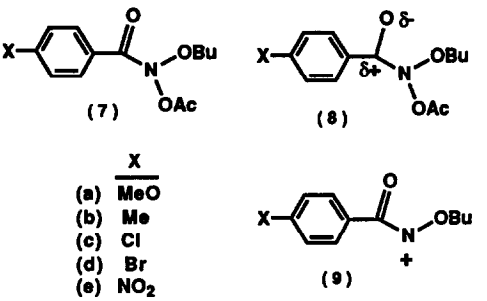
ii. solvent (water) assisted displacement of acetic acid in a bimolecular process (normal S_N2 at nitrogen) (scheme 3, path ii);

iii. solvent (water) attack at the carbonyl leading to acyl bond cleavage (normal $A_{Ac}2$ hydrolysis of esters) (scheme 3, path iii).

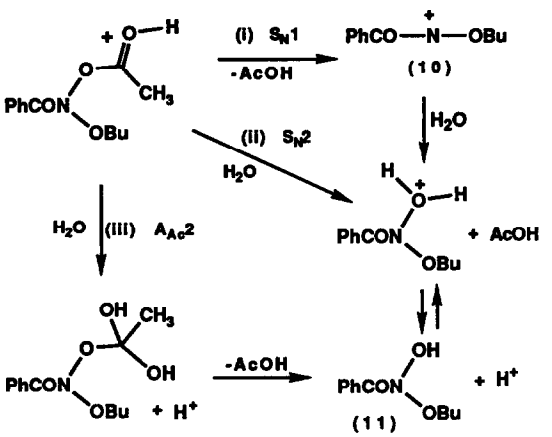
Table1. Rate constants for solvolysis of N-acetoxy-N-butoxybenzamides in CD_3CN/D_2O[1] at 308K.

X		k_H^X x10^2/ lmol^{-1}s^{-1}		k_ox10^5/s^{-1}	
H	(1b)	4.82	(0.21)	2.31	(1.3)
MeO	(7a)	59.13	(6.08)	1.47	(14.3)
Me	(7b)	17.93	(1.25)	13.40	(8.5)
Cl	(7c)	2.04	(0.23)	4.24	-
Br	(7d)	2.38	(0.14)	-0.43	(1.5)
NO_2	(7e)	0.49	(0.05)	2.40	(0.7)

[1] $CD_3CN:D_2O$ 3.81:1



It is unlikely that mechanisms (ii) and (iii). would account for the variation in k_H^X in the series (1b,7a-e). In a solvent induced S_N2 process, the charge build-up at the nitrogen would be small and somewhat removed from the electronic effects of the *para*-substituent (S_N2 reactions at benzylic positions correlate with Hammet σ values with ρ between +1 and -1) [13] In the case of pathway (iii) the *para*-substituent is remote from the ester carbonyl and hydrolyses of *para*-substituted benzoate esters are themselves rather insensitive to substituent effects (correlation with σ, ρ=-0.5) [14] On the other hand, $\log(k_H^X/k_H^H)$ gave an excellent correlation with Hammett σ^+ values (r^2=0.966) with ρ=-1.4 ±0.13 (Figure 3). This together with a poor correlation with Hammett σ values indicates the formation of a positive charge on nitrogen in the rate determining step. The low Hammett ρ value is consistent with nitrenium ion formation beta to the aromatic ring and rate enhancement by activating substituents can be ascribed to a diminution of positive charge at the amide carbon i.e. the contribution of (8) is offset by the electron donating substituents thereby facilitating the development of positive charge at nitrogen. A similar correlation (σ^+, ρ=-0.74) has been reported for the acid catalysed decomposition of ω-diazoacetophenones in which carbenium ion character is is developed on the α-carbon. [15] We conclude from this that the rate determining step in the acid catalysed solvolysis of N-alkoxy-N-butoxybenzamides is unimolecular heterolysis into acetic acid and nitrenium ion (9).



Scheme 3

The mutagenicities of (1b) and (7a-e) (Figure 4) broadly mirror their rates of acid catalysed solvolysis and interestingly, the data also correlate with σ^+ (r^2=0.87) with a ρ=-0.29. The lower sensitivity to substituent effects indicates less charge build-up on nitrogen however and an S_N2 mechanism may also be indicated. In any event this structure-activity relationship shows that increased mutagenicity is associated with donor capacity of the benzamide ring and *vise versa.*

Several solvolysis products have been identified by ^{1}H n.m.r. These include butyraldehyde, benzhydroxamic acids, butanol, butyl benzoates and benzoic acids. These could all arise from intermediate N-alkoxybenzohydroxamic acids (11) formed by capture of (10) by water (Scheme 3). Analysis of ^{1}H n.m.r. spectra of reaction mixtures indicated formation of benzhydroxamic acids and butyraldehyde in similar yields which may be ascribed to reversible protonation at the amide nitrogen or carbonyl in (11)

Figure 3. Linear Free Energy fit for solvolysis of (1b) and 7(a-e) in CD_3CN/D_2O at 308K.

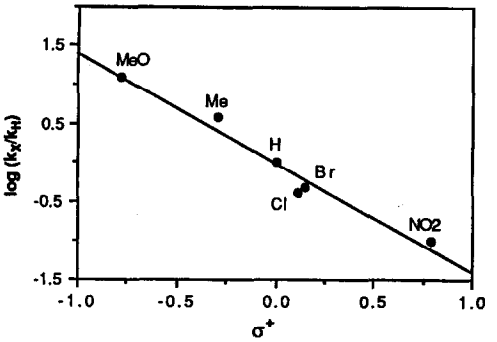
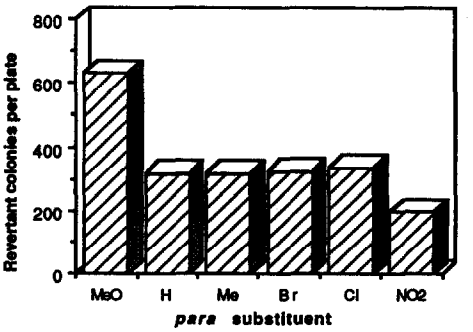


Figure 4. Mutagenicities of (1b),7(a-e) in salmonellaTA100 at 1μmol/plate



followed by elimination of the hydroxamic acids. The mechanisms of formation of these as well as the other products are currently being investigated.

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