The Solvolysis of *N*-Acetoxy-2-acetylaminofluorene and *N*-Acetoxy-4-acetylaminobiphenyl: Delicate Balance between Nitrenium Ion Formation and Hydrolysis

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The solvolysis of *N*-acetoxy-2-acetylaminofluorene in aqueous acetone at neutral pH proceeds exclusively with nitrenium ion formation while under the same conditions, the 4-aminobiphenyl analogue undergoes exclusive acyl–oxygen scission.

Many polycyclic aromatic amines and amides are known mutagens or carcinogens.[1] Of these, the most extensively studied is 2-acetylaminofluorene. It is generally regarded that these compounds owe their biological activity to the ability of their metabolites to undergo dissociation to nitrenium ions.[2] Although the structures of isolated DNA adducts are compatible with this proposal, mechanistic support has been lacking. Scribner *et al.*[3] reported the rates, but not products, of solvolysis of several *N*-acetoxy-*N*-arylacetamides. Subsequent studies have shown that most of these compounds undergo preferred, or exclusive, acyl–oxygen cleavage.[4] We now report that *N*-acetoxy-*N*-acetylaminofluorene (AAAF) (**1**), unlike the closely related biphenyl analogue, undergoes solvolysis with nitrenium ion formation at physiological pH in aqueous solution.

All reactions were carried out in 40% aqueous acetone at 40.00 °C, buffered to pH between 4.0 and 10.0 using acetate, phosphate, and borate buffers. The ionic strength was maintained at 0.25 M with $LiClO_4$. The reaction was monitored by sampling at appropriate intervals and analysing by h.p.l.c. Reactions were followed for at least three half-lives, and were first order with respect to (**1**). Rigorous analysis[5] revealed minor participation (<10%) by the borate buffer alone. The rate data can be fitted to equation (1).† The rate constants thus obtained are given in Table 1 along with other pertinent data.

$$k_{obs.} = k_o + k_{OH}[OH] \quad (1)$$

Six monomeric products were identified: compounds (**7**)—(**12**). The amount of (**7**) produced was pH-dependent and this was the sole product from the hydroxide-catalysed process. Below pH 7, where the rate of reaction was practically pH-independent, the ratio of products remained constant and the yield of (**7**) was <2%. Some higher molecular weight material was also produced. One such product which could be

† Data for reactions utilising borate buffers were extrapolated to infinite dilution by serial buffer dilution.

Table 1. Pseudo-first-order rate constants for the solvolysis of the *N,O*-diacetyl-*N*-arylhydroxylamines (**1**)—(**6**) in acetone–water (60 : 40).

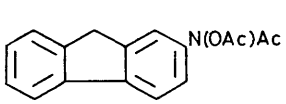
ArN(Ac)OAc	$10^6 \times k_o/s^{-1}$	$k_{OH}/dm^3\,mol^{-1}\,s^{-1}$
(**1**)	88.5 ± 2.7	2.17 ± 0.10
(**2**)	19.0 ± 0.94	1.72 ± 0.09
(**3**)	1.49 ± 0.4	2.02 ± 0.05
(**4**)	1.50 ± 0.05	2.16 ± 0.07
(**5**)	1.43[a]	2.08[a]
(**6**)	1.28[a]	2.67[a]

[a] Estimated from reactions carried out in dioxane–water. Hydroxamic acid was the sole product both in this solvent and in acetone–water.

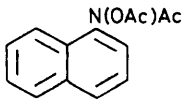
isolated, accounting for *ca.* 25% of the starting material, was tentatively assigned the structure (**13**) on the basis of spectral evidence (mass, i.r., u.v., n.m.r.).

The hydroxide-dependent reaction is an ester hydrolysis. This has been observed in closely related systems.[4] The process defined by k_o clearly involves scission of the nitrogen–oxygen bond and appears to be more complex. Several exploratory studies were made of this process. The rate of reaction increases with solvent polarity ($m = 0.717 \pm 0.04$).[6] It shows no special salt effect (0—0.4 M $NaClO_4$), no common ion effect (0—0.4 M NaOAc), and negligible normal salt effect. The addition of NaOAc led to an increase in the yield of (**8**) and (**9**) from 10 ± 2 to 34 ± 2%. Replacing the buffer with sodium [$^2H_3$]acetate led to the same result, 10% of the product being composed of (**8**) and (**9**) and 24% being [$^2H_3$]-(**8**) and [$^2H_3$]-(**9**). The addition of reducing agents (NaI, $FeCl_2$, $Na_2S_2O_3$, hydroquinone, or sodium ascorbate) led to increases in the yield of (**12**),‡ primarily at the expense of dimeric material with no effect on the yields of (**8**) and (**9**). Only ascorbate increased the rate of reaction.§ When (**1**) was labelled with oxygen-18 specifically in the acetate carbonyl group,[7] starting material recovered after one half life showed no scrambling of the label. Analysis of rearranged (**8**) from this reaction revealed partial scrambling, which can be described as 60% of the product derived from a process involving complete randomisation and 40% from a favoured 1,3 shift.¶

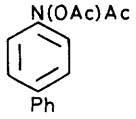
The formation of (**8**)—(**12**) appears to require a heterolysis of the N–O bond to produce acetate–nitrenium ion pairs. Since recovered starting material shows no scrambling of the label, the simplest interpretation is that these ion pairs do not reform starting material. The invariance of the yields of (**8**) and (**9**) on adding reducing agents suggests that they form from an intimate ion pair.[9] We can glean some insight into the nature of this rearrangement since the ^{18}O labelling experiment indicates that the oxygen atom from which the bond is broken is also the preferred atom to which the new bond is formed. Evidently this is a rather structured short-lived



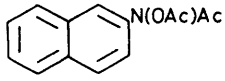
(**1**)



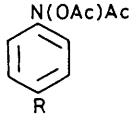
(**2**)



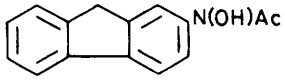
(**3**)



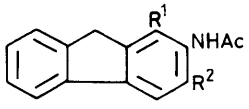
(**4**)



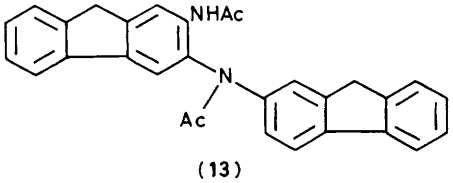
(**5**), R = H
(**6**), R = Me



(**7**)



(**8**) R^1 = OAc, R^2 = H
(**9**) R^1 = H, R^2 = OAc
(**10**) R^1 = OH, R^2 = H
(**11**) R^1 = H, R^2 = OH
(**12**) R^1 = R^2 = H



(**13**)

ion-pair intermediate. A similar conclusion has been reached for other systems.[10] The increased yields of (**8**) and (**9**) with added acetate or deuterioacetate indicate that there are also other routes to these products.

The conversion of nitrenium ions into amines or amides has been reported previously[11,12] and attributed to singlet–triplet inter-system crossing[11] and to reduction.[12] In the present case this is clearly a reduction (hydroquinone, ascorbate); the addition of tribromo- or trichloro-acetate had no effect on the yields of the amide at concentrations up to 0.5 M.

At this point we can begin to generalise on the chemistry of this class of putative 'ultimate carcinogens,' (**1**)—(**6**). From Table 1 it is seen that all undergo a base-catalysed hydrolysis at pH >8 with comparable rates. All also undergo a pH-independent reaction at and below neutral pH, but the nature of this reaction is exceptionally sensitive to the nature of the aryl ring. For (**3**)—(**6**) this reaction is exclusively an uncatalysed ester hydrolysis while for (**1**) and (**2**) it is N–O bond cleavage.

From the relative constancy of the rates of the hydroxide-catalysed reactions, it is reasonable to assume that the corresponding uncatalysed hydrolyses would also occur at comparable rates.[13] The observed N–O bond scission for (**1**)

‡ The yields of (**12**) were dependent on the identity and concentration of the reducing agent. Typically the yield increased from 6% in the absence of reducing agent to a plateau value of 90% at *ca.* 0.1 M iodide. Under these conditions, the sole products were (**8**), (**9**), and (**12**).

§ The rate increased approximately linearly with concentration to a 25% increase for 0.1 M ascorbate.

¶ Isolation and analysis, performed by mass spectrometry, were carried out under conditions shown not to result in partial scrambling of the label. The fragmentation of a number of hydroxamic acids and their derivatives has recently been analysed in some detail.[8]

and (**2**) must therefore be due to some facilitation of this process in these cases. This contention is supported by experimental data for these compounds,[14] for their carbocationic counterparts,[15] and by molecular orbital calculations.[3]

It now becomes obvious why attempts to arylamidate nucleosides with (**1**) have met with modest success,[16] while those with (**3**)—(**6**) have not.[17,4d] It is interesting that (**2**) has been reported to lead only to acetylation of deoxyguanosine.[4a] It appears highly likely that appropriate changes in reaction conditions may have quite spectacular effects on the course of these reactions.

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