Flash Photolysis Observation and Lifetimes of 2-Fluorenyl- and 4-Biphenylylacetylnitrenium Ions in Aqueous Solution

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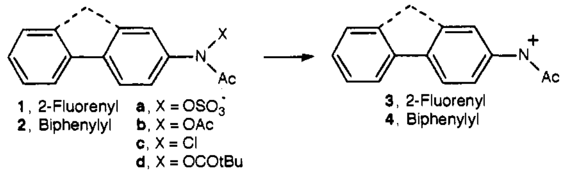
Received January 31, 1994

The carcinogens 2-(acetylamino)fluorene and 4-(acetylamino)-biphenyl are believed to interact with cellular targets by metabolic conversion to the hydroxamic acid esters **1a**,**b**, and **2a**,**b**, followed by N–O heterolysis to nitrenium ion intermediates **3** and **4**.[1]



(1)

Considerable indirect evidence exists for the intermediacy of such electrophiles in studies with these and other model systems.[2] However, reports of their direct observation are limited to some diarylnitrenium ions, obtained electrochemically in acetonitrile,[3] and *tert*-butyl(2-acetylaryl)nitrenium ions, observed in the same solvent with laser flash photolysis (LFP).[4] In this paper we report the LFP observation of cations **3** and **4**, the first characterization of nitrenium ions derived from widely studied carcinogens. These cations have been observed in aqueous solution, allowing their lifetimes to be directly measured in the solvent where they would be formed in biological systems. This information is important in determining whether such cations can survive long enough to react with biological nucleophiles. Previous studies have estimated lifetimes of nitrenium ions by application of the "azide-clock" method,[5] in which selectivity ratios $k_{az}:k_w$ are converted into absolute rate constants k_w with the assumption that azide reacts at the diffusion limit.[6] The present study also evaluates this assumption.

We employed as precursors the pivalate **1d** for **3** and both the sulfate **2a** and the *N*-chloride **2c** for **4**. In each case, 248-nm excitation results in a strong absorbance with λ_{max} in the vicinity of 450–460 nm, plus a weaker absorbance at 300–350 nm. The situation with **2a** is shown in Figure 1. The absorbance at higher wavelength decays with excellent exponential kinetics; the absorbance at lower wavelength shows little decay at times up to 100 μs.[7]

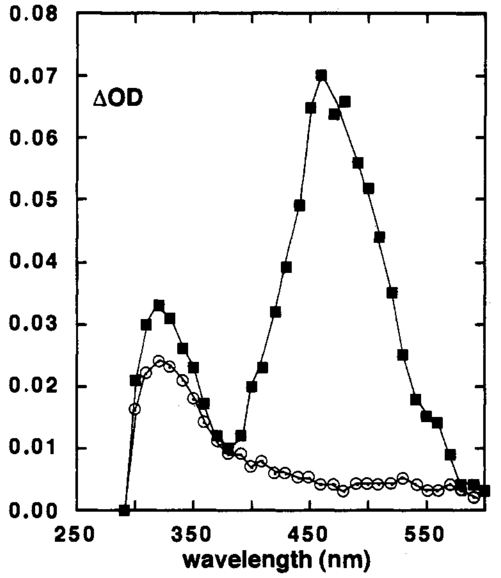


Figure 1. Transient absorption spectra obtained upon 248-nm excitation of **2a** in aqueous solution containing 0.5 M $NaClO_4$ (■) immediately after laser pulse and (○) after 5 μs.

Table 1. Products of 248-nm Irradiation and Solvolysis of *N*-Chloro-4-phenylacetanilide (2c) in pH 4.5, 0.02 M Acetate Buffer, 0.5 M $NaClO_4$

product	photolysis[a]	solvolysis[b]
4-hydroxy-4-phenyl-2,5-cyclohexadienone (**5**)	10	15
4-hydroxy-3-phenylacetanilide (**6**)	50	77
3-hydroxy-4-phenylacetanilide (**7**)	2	2
2-hydroxy-4-phenylacetanilide (**8**)	3	3
2-chloro-4-phenylacetanilide (**9**)	16	4
4-phenylacetanilide (**10**)	20	0

[a] After 5-min irradiation at 254 nm; 40% **2c** unreacted, yields are based upon reacted **2c**. [b] After 27 h in the dark at 20 °C.

We propose that the 450-nm absorbance is due to nitrenium ion **3** or **4**, with the following evidence. (i) A product study with **2c** shows that nitrenium ion **4** does form on irradiation. This is illustrated in Table 1, by way of a comparison with solvolysis results, where products are explained by trapping of an intermediate nitrenium ion (chloride **9** arising from the ion pair in the absence of added chloride).[5b,8] Irradiation, under conditions where there is no thermal reaction, results in similar products, most notably **5** and **6** as major products in the same 1:5 ratio. The product profile is not identical, irradiation resulting in a greater quantity of **9**, plus the reduction product **10**. However, the conclusion is that >60% of the products of irradiation arise from the same intermediate as formed in solvolysis. (ii) Precursors **2a** and **2c** result in the same band at higher wavelength, identical in both shape and kinetics of decay. (iii) The 450-nm absorbances show the decay characteristics of a cationic intermediate, no effect of oxygen but efficient quenching by azide.[9] A modest retarding effect of added $NaClO_4$[10] and a pronounced kinetic stabilization

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(7) There is clearly some overlap of the two bands. The 450-nm absorbance does not decay to 0, and there is also a small amount of decay at 300–350 nm, this occurring with the same rate constant as observed at 450 nm.

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Table 2. Rate Constants and Rate Constant Ratios for Decay of Nitrenium Ions 3 and 4 and Carbenium Ion 14 (20 °C)

ion	conditns	k_s, s^{-1}	k_{az}, $M^{-1}\,s^{-1}$	$k_{az}:k_s$(LFP),[a] M^{-1}	$k_{az}:k_s$(Pr),[a] M^{-1}
3	water, $\mu = 0$	1.3×10^5			
	water, $\mu = 0.5$[c]	7.7×10^4	4.2×10^9	5.4×10^4	6.2×10^4 [d]
4[e]	water, $\mu = 0$	1.14×10^7	9.1×10^9	8.0×10^2	
	water, $\mu = 0.5$[c]	5.9×10^6	5.1×10^9	8.6×10^2	10.2×10^2 [f]
	CF_3CH_2OH	1.12×10^4			
14	water, $\mu = 0$	1.5×10^7			

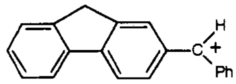
[a] Ratio calculated from directly measured rate constants. [b] Calculated from products of ground-state solvolysis. [c] Maintained with $NaClO_4$. [d] This work, see footnote 11. [e] Precursor **2c**. Using **2a**, k_s is 1.05 s^{-1} at $\mu = 0$, and 6.3×10^6 s^{-1} at $\mu = 0.5$. [f] Reference 5b.

in 2,2,2-trifluoroethanol[9a] (Table 2) are also typical of cations, these effects being similar to ones observed with carbenium ions. (iv) With both systems, the ratios $k_{az}:k_w$ obtained directly with LFP are similar to ones obtained through analysis of products of solvolysis reactions (Table 2).[12] This observation is the most important. It demonstrates that the transient is identical with the ground-state intermediate, and also makes a cation radical very unlikely for the identity of the LFP intermediate.

The identity of the second transient is still not established, although its presence is clearly consistent with the additional products found on irradiation. Either a radical obtained by homolysis[9a,10b,13] or a triplet nitrenium ion obtained by heterolysis in the triplet excited state[14] would account for **10**, although perhaps not the additional **9**.[15] This transient shows no decay over 100 μs with O_2 (saturated) nor with $(Me_3Si)_3SiH$ (0.05 M in 90% acetonitrile), species which may quench such radicals and triplets.

The LFP observation of nitrenium ions **3** and **4** provides the opportunity for directly examining the kinetic behavior of these electrophiles in aqueous solution. The results do verify that azide does react at rates typical of diffusion control, as assumed in the "azide-clock" approach.[5] This had previously been established with LFP for carbenium ions,[9] but not nitrenium ions. Perhaps more important is the basic observation that **3** and **4** are long-lived in water. On the basis of azide trapping, the parent phenylnitrenium ion[16] and its 2,6-dimethyl derivative[5a] have quite short lifetimes in water, 200 ps and 1.4 ns, respectively, leading to the suggestion[5a] that these are unlikely to be biologically relevant electrophiles. This statement, however, cannot be made with **3** and **4**, which are much longer lived. The addition of the *p*-phenyl group results in a kinetic stabilization much greater than expected, for example, on the basis of σ^+.[17] This effect is especially pronounced when the entire system is forced to be planar. Thus the fluorenylnitrenium ion **3** is 50000-fold longer lived then the parent phenylnitrenium ion $PhNH^+$.

The remarkable kinetic stability of **3** is further illustrated through a comparison with the carbenium analog **14**. This ion


14

has been generated by LFP of the acetate, as previously described for other diarylmethyl cations.[9a,13] Here there is no ambiguity in the assignment of the transient, since the spectrum (λ_{max} at 545 nm) agrees with that of authentic cation in 96% H_2SO_4. This carbenium ion is 100-fold more reactive in water than the nitrogen analog, despite the presence of a destabilizing acetyl substituent in the latter and a stabilizing phenyl in the former.[18] Kinetic stability of arylnitrenium ions relative to benzylic cations has been suggested previously on the basis of selectivities,[19] but this is the first time that it has been demonstrated by direct measurement. Nitrenium ions differ from carbenium ions in that the former react with heteroatom nucleophiles such as water at a ring carbon, with an ensuing loss of aromaticity in the intermediate species so formed. A possible explanation for the increased kinetic stability is that this results in a significant barrier. In this respect it is interesting that the reaction of nitrenium ions with carbon nucleophiles, most notably C8 of guanine, occurs at the external nitrogen. This process could well have a lower intrinsic barrier, so that, despite the low reactivity of such π-nucleophiles, the reaction may compete with that of solvent.

Acknowledgment. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the American Cancer Society (CN-23K).

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(11) The solvolysis ratio was obtained with **1a** at pH 7.4. The only solvent-incorporated product is 2-(*N*-acetylamino)-4-hydroxyfluorene (**11**); a few percent of rearranged sulfates are also formed. Azide forms two products, 3-azido- and 1-azido-2-(*N*-acetylamino)fluorene (**12** and **13**), in a 2.5:1 ratio. A plot of $1/f_w\{([\mathbf{11}] + [\mathbf{12}] + [\mathbf{13}]):[\mathbf{11}]\}$ versus [azide] is excellently linear with a slope of $(6.2 \pm 0.2) \times 10^4$ M^{-1} and an intercept of 1.

(12) With both the systems, the LFP ratio is about 15% lower than the one obtained through product analysis. Since the error in the two methods is 3–4% (1 standard deviation), this difference would appear to be just outside experimental error. It is possible that the difference is real, since the LFP method measures the initial competition, producing adducts which in the case of nitrenium ions must undergo further reactions to get to the observed products.

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(15) It is possible that the ion pair formed at very short times photochemically is different from that in the thermal reaction, the former showing greater collapse at the ortho carbon. The *o*-chloro product could also arise from a radical pair collapsing at the ortho carbon.

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(18) (a) The acetyl group likely has only a small effect, as shown, for example, by azide trapping results with **4** and its analog without the *N*-acetyl group (a 3-fold difference).[5b] On the other hand, the phenyl substituent in the carbenium ion has a 100–1000-fold kinetic stabilization, as shown, for example, by comparisons of $4\text{-}MeOC_6H_4CHPh^+$ and $4\text{-}MeOC_6H_4CH_2^+$.[9a,18b,c] (b) Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 9507. (c) McClelland, R. A.; Chan, C.; Cozens, F.; Modro, A.; Steenken, S. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1337.

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