PERKIN 2 COMMUNICATION

Tautomers and conjugate base of the nitrenium ion derived from *N*-acetylbenzidine

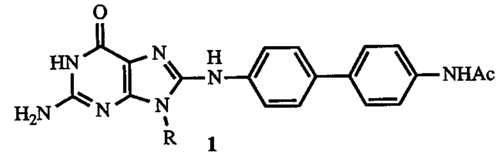
Andrew P. Dicks, Abid R. Ahmad, Raechelle D'Sa and Robert A. McClelland *

Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada

Received (in Cambridge) 27th October 1998, Accepted 16th November 1998

The mono-*N*-acetylated 'nitrenium ion' derived from the carcinogen benzidine exists at physiological pH as a long-lived mixture of the 4′-amino-4-biphenylyl-*N*-acetylnitrenium ion 9 and a neutral conjugate base, the mono-acetylated bis-imine 12, with $pK_a = 7.5$ for the acid–base pair. Also present in very small amounts is the 4′-(acetylamino)-4-biphenylylnitrenium ion 8. This cation is much more reactive than the other two species, and is the source of the major product of the reaction with water. Thus, the *N*-acetylbenzidine 'nitrenium ion' is a very stable system that hides within it a small amount of a highly reactive form.

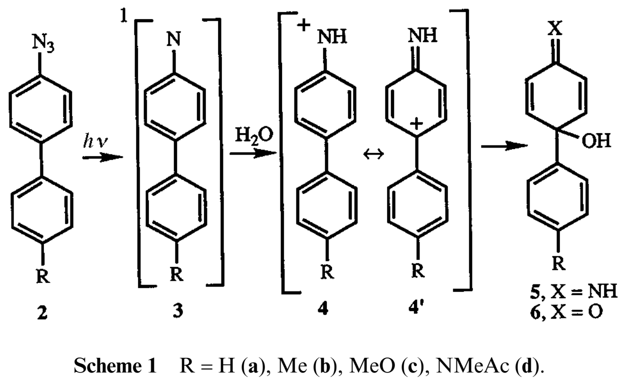
The evaluation of urinary metabolites in workers in the chemical industry exposed to the carcinogen benzidine reveals several DNA adducts, of which the principal one co-chromatographs with **1**.[1]



1

Such arylamination at guanine C8 is typical of arylamine carcinogens,[2] and is attributed to an arylnitrenium ion as the guanine-binding electrophile.[3] An interesting feature of **1** is that it is mono-*N*-acetylated. With the presumption that acetylation has occurred prior to the DNA reaction, the nitrenium ion responsible is the 4′-(acetylamino)-4-biphenylylnitrenium ion. Examination of the structure of this cation reveals that it can exist in both a more stable tautomeric form and a neutral conjugate base form. To evaluate these possibilities we have generated the cation by laser flash photolysis (LFP). Indeed we are able to directly observe its conversions to these more stable forms.

We have previously described LFP studies of the parent 4-biphenylylnitrenium ion **4a**,[4,5] as well as a series of derivatives including **4b** and **4c**.[6] These cations are observed in LFP experiments following irradiation of the appropriate azide **2** in aqueous solution, formed *via* rapid solvent protonation of the initially-formed singlet nitrene **3** (Scheme 1). A feature of these studies has been the observation of pronounced effects on the reactivity of **4** associated with the apparently remote substituents R.[6] This has lead to the conclusion, supported by time-resolved IR,[7] that these cations are better regarded as iminocyclohexadienyl cations, *e.g.* **4′**.



Scheme 1 R = H (**a**), Me (**b**), MeO (**c**), NMeAc (**d**).

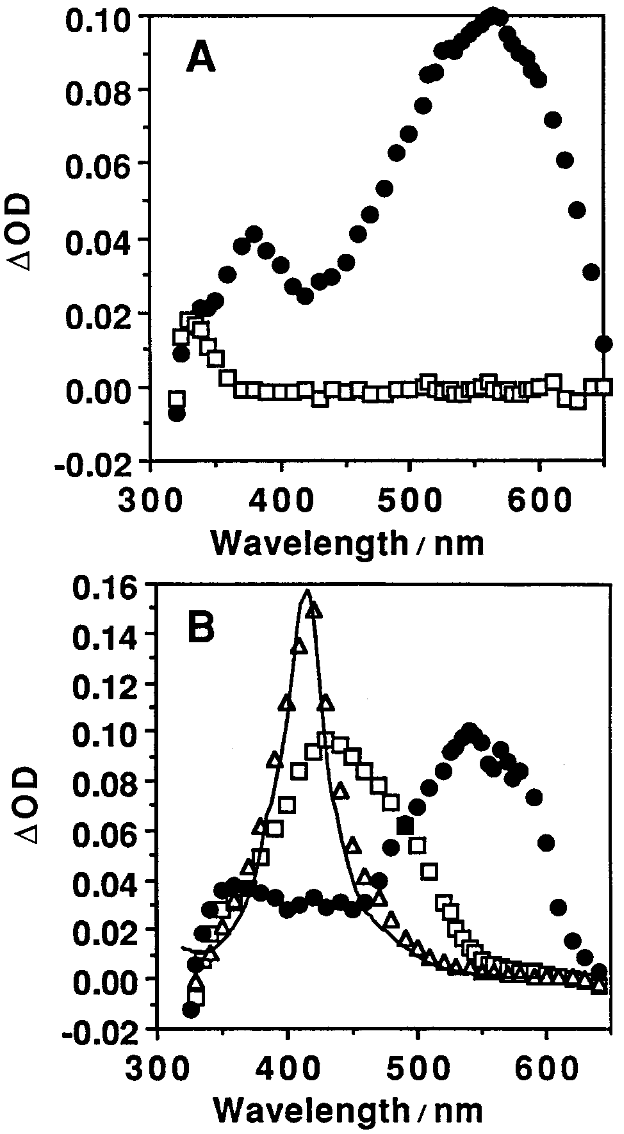
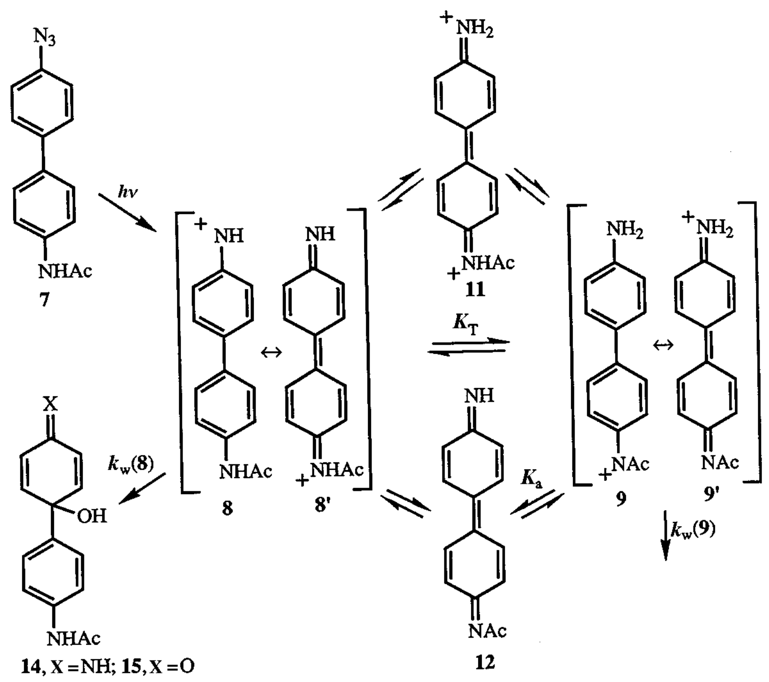


Fig. 1 Transient absorption spectra obtained upon 248 nm laser irradiation. (A) 4′-(*N*-Methylacetylamino)-4-azidobiphenyl **2d** in pH 7 water, with the closed circles the reading immediately after the laser pulse, and the open squares, after completion of the exponential decay. (B) 4′-(Acetylamino)-4-azidobiphenyl **7** in 0.001 M HCl and 0.001 M NaOH. The initial spectra (closed circles) are identical and have been normalized so as to have the same optical densities. The open squares represent the final spectrum in acid, the open triangles the final spectrum in base. The curve is the spectrum in the base solution obtained on a diode array spectrometer ~10 seconds after irradiation, normalized so that the optical densities of this curve and the final spectrum in base are the same at 420 nm.



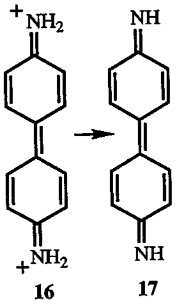
Scheme 2

To model the acetylamino in a system that cannot tautomerize, we prepared the *N*-methyl derivative **2d**. This azide behaved in a manner similar to **2a–c**, producing a nitrenium ion with λ_{max} near 560 nm and a second smaller peak at 380 nm (Fig. 1A). Evidence that this is **4d** comes in the typical, very effective, quenching by azide, with $k_{az} = 8 \times 10^9$ M^{-1} s^{-1}. In addition, the product **6d** is the one expected of this type of nitrenium ion,[8] and there is a long-lived species, presumably **5d**, apparent in the HPLC. Finally, the rate constant k_w for decay of **4d** in water is 3.8×10^4 s^{-1}. This lies between the k_w for **4b** (2.7×10^5 s^{-1})[6] and **4c** (1.6×10^3 s^{-1}),[6] as expected for a substituent whose σ^+ is around −0.6.[9]

While the decay of **4d** results in a spectrum with little residual absorbance above 400 nm,† a very different scenario unfolds with the non-methylated analog. The precursor here is the azide **7**, and as shown in Fig. 1B, this does produce a spectrum immediately after the laser pulse very similar to that of **4d**. With **7** however, there is a pronounced growth at 400–450 nm as the absorbance decays above 500 nm. The spectra at the completion of the growth are pH dependent, with the two extremes in acid and base shown in Fig. 1B, and some mixture of the two at neutral pH. This dependence was investigated by measuring the 'final' OD at 470 nm in a series of solutions of different pH. This was done with the same substrate concentration and laser power to provide a constant concentration of the intermediate. The result was an acid–base titration curve, with constant ODs in acid (pH < 5) and base (pH > 9), and the changeover at intermediate pH. The pK_a obtained from this curve is 7.6 ± 0.1.

The similarity with the spectrum of **4d** shows that the intermediate immediately after the laser pulse is the cation **8**. The behaviour in base is explained by a deprotonation, forming the neutral mono-acetylated bis-imine **12**. Confirmation for such an assignment lies in the close similarity of the 'final' spectrum in base with that of the bis-imine **17**. The latter spectrum was obtained by adding the bis-perchlorate salt of the dication **16**[10] to base and is identical in shape, only shifted 20 nm to lower wavelength. The species produced in acid must be the tautomeric nitrenium ion **9**, and at neutral pH there is some mixture of the two. The pK_a must therefore refer to $\mathbf{9} \rightleftharpoons \mathbf{12} + H^+$. There is less than 1% of **8** in this equilibrium since its absorbance decays to zero at wavelengths greater than 570 nm where **9** and **12** have little absorbance.

These transformations of **8** exhibit catalysis by both acids and bases. The rate constants k^1(obs) extrapolated to zero buffer concentration follow the equation $k^1_H[H^+] + k^1_o + k^1_{OH}$-



16 **17**

[OH$^-$] with $k^1_H = 5.6 \times 10^9$ M^{-1} s^{-1}, $k^1_o = 5.2 \times 10^4$ s^{-1} and $k^1_{OH} = 4.9 \times 10^9$ M^{-1} s^{-1}. The last term must represent hydroxide deprotonating **8**, while the H$^+$ dependence is best explained as a protonation–deprotonation with the dication **11** as an intermediate. We have previously found evidence for dications of biphenylylnitrenium ions, even with the parent system.[4] The k^1_o reaction is probably due to water deprotonating **8**, although there are other possibilities. Catalysis by both the acid and base component of the buffers is also observed; this is currently being investigated in detail.

These products are not indefinitely stable, reacting at considerably longer times leaving little residual absorbance above 350 nm at the completion of the decay. The kinetics have been studied by lamp flash photolysis from pH 4–8. Rate constants extrapolated to zero buffer concentration follow the equation $k^2(\text{obs}) = k^2_w[H^+] : ([H^+] + K_a)$ with $k^2_w = 2.3$ s^{-1} and pK_a = 7.4 ± 0.1. The latter is the same, within experimental error, as the pK_a obtained in the spectroscopic titration. The implication is that in this pH region a cation is the reactive form, and not neutral **12**. In fact at pH 9–11, **12** becomes sufficiently stable that its UV spectrum can be obtained by transferring the irradiated cuvette to a diode array spectrometer. There is a direct reaction of **12** with hydroxide ion at higher pH and it becomes less stable again.

In principle, both cations **8** and **9** can react, with k^2_w being the sum of their contributions—$k^2_w(\mathbf{8}) : K_T + k^2_w(\mathbf{9})$, where K_T is the equilibrium constant [**9**] : [**8**]. An indication of which form is reacting is seen in the products, an HPLC of which shows one major peak, which converts on standing to a second peak. The material responsible for the latter has been isolated from a scaled-up reaction and identified as **15**; the precursor is presumably **14**. The yield of **15** is 70%, and it is a product of the hydration of **8**, not **9**. The conclusion therefore is that at least

70% of the reaction is proceeding by way of **8**, the less stable nitrenium ion.

Although **9** has an electron withdrawing acetyl group on the formal N^+ center when written in this resonance form, this substituent is known to be far less important than ones on the aryl rings.[4,6,9,11,12] As shown with the resonance form **9′**, this cation is more stable than **8** because the NH_2 group in the former can directly conjugate the positive charge. Another way of looking at this is in terms of which of the two nitrogens in **12** is expected to be more basic. This will obviously be the NH nitrogen. In terms of just how much more stable, we estimate the tautomeric equilibrium constant K_T as ~2 × 10^4. This is based on 70% of the reaction proceeding *via* the less stable **8**, and taking the rate constant k_w(**8**) to be equal to k_w(**4d**), *i.e.* $K_T = k_w(\mathbf{4d}) : 0.7k^2_w$.

This study provides the first characterization (and direct observation) of a system where an arylnitrenium ion is converted to both a more stable tautomeric form, as well as to the neutral form that is the conjugate base of the two. At neutral pH, the system decays *via* the cationic forms. The product indicates that the principal form reacting is the less stable tautomer, despite its being disfavored by four orders of magnitude. With respect to the carcinogenicity of benzidine, we show that the mono-*N*-acetylated nitrenium ion derived from this arylamine exists at physiological pH as a mixture of the 4′-amino-4-biphenylyl-*N*-acetylnitrenium ion **9** and its conjugate base, the neutral bis-imine **12**, with $pK_a = 7.5$ for the acid–base pair. By nitrenium ion standards, the system is remarkably long-lived, with lifetimes approaching one second in water alone at neutral pH. Also present is the 4′-(acetylamino)-4-biphenylylnitrenium ion **8**. While the amount of this cation in equilibrium with its tautomer **9** is estimated to be only one in twenty thousand, **8** is a much more reactive species and the majority of the reaction proceeds *via* this form. This nitrenium ion obviously also accounts for the DNA adduct **1** observed in man. Thus, the 'nitrenium ion' derived from *N*-acetylbenzidine is a complex, but very stable, system, that hides within it a small amount of a highly reactive form.

Acknowledgements

The continued financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Notes and references

† There is a small peak at 330 nm, but this is associated with a competing pathway at the stage of the singlet nitrene.[4–6]

1 N. Rothman, V. K. Bhatnagar, R. B. Hayes, T. V. Zenser, S. K. Kashyap, M. A. Butler, D. A. Bell, V. Lakshmi, M. Jaeger, R. Kashyap, A. Hirvonen, P. A. Schulte, M. Dosemeci, F. Hsu, D. J. Parikh, B. B. Davis and G. Talaska, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 5084.

2 A. Dipple, *Carcinogenesis*, 1995, **16**, 437.

3 S. A. Kennedy, M. Novak and B. A. Kolb, *J. Am. Chem. Soc.*, 1997, **119**, 7654.

4 R. A. McClelland, P. A. Davidse and G. Hadzialic, *J. Am. Chem. Soc.*, 1995, **117**, 4173.

5 R. A. McClelland, M. J. Kahley, P. A. Davidse and G. Hadzialic, *J. Am. Chem. Soc.*, 1996, **118**, 4794.

6 D. Ren and R. A. McClelland, *Can. J. Chem.*, 1998, **76**, 78.

7 S. Srivastava, J. P. Toscano, R. J. Moran and D. E. Falvey, *J. Am. Chem. Soc.*, 1997, **119**, 11 552.

8 M. Novak, M. J. Kahley, E. Eigen, J. D. Helmick and H. Peters, *J. Am. Chem. Soc.*, 1993, **115**, 9453.

9 V. M. Csizmadia, K. M. Koshy, K. C. M. Lau, R. A. McClelland, V. J. Nowland and T. T. Tidwell, *J. Am. Chem. Soc.*, 1979, **101**, 974.

10 S. Hunig and P. Richters, *Chem. Ber.*, 1958, **91**, 442.

11 M. Novak, M. J. Kahley, J. Lin, S. A. Kennedy and T. G. James, *J. Org. Chem.*, 1995, **60**, 8294.

12 G. Kohnstam, W. A. Petch and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1984, 423.

Communication 8/08322C