

## REVIEW COMMENTARY

### REACTIVITY OF THE 4-BIPHENYLYL AND 2-FLUORENYLNITRENIUM IONS WITH HETEROCYCLIC AND CARBON NUCLEOPHILES

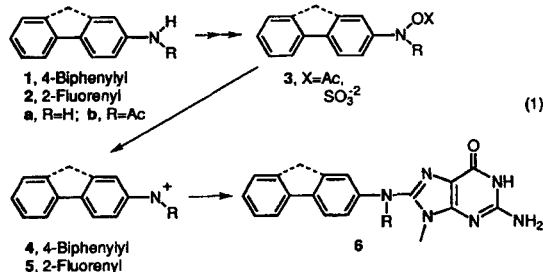
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The carcinogens 4-aminobiphenyl, 2-aminofluorene and their *N*-acetyl derivatives form DNA adducts *in vivo* with the aryl nitrogen attached at C-8 of guanine. These adducts are proposed to arise through the reaction with the DNA base of a nitrenium ion obtained by N-O heterolysis of a hydroxylamine ester obtained metabolically by oxidation and esterification. Routes have now been discovered for the photochemical generation of these nitrenium ions, the *N*-acetyl derivatives via a photoheterolysis reaction and the *N*-H derivatives by protonation of the singlet nitrene photochemically generated from the azide precursor. The nitrenium ions are observed as transient intermediates with laser flash photolysis, and the kinetics of their reaction with various nucleophiles directly monitored. These results, coupled with competition kinetics experiments, show that the nitrenium ions derived from the above amines and amides are relatively long-lived in water, with a remarkably high selectivity in water for reaction with 2'-deoxyguanosine and its 5'-phosphate. The C-8 adduct is the product of this reaction. These behaviours differ considerably from those of analogous carbenium ions. Reactions with vinyl ethers have also been investigated, and also show significant differences between the two types of electrophile.

#### INTRODUCTION

The carcinogens 4-aminobiphenyl (**1a**), 2-aminofluorene (**2a**) and their *N*-acetyl derivatives are generally accepted to undergo a two-fold metabolic activation to *O*-acetate or *O*-sulfate esters **3**, followed by N-O heterolysis to an aryl nitrenium ion (**4** and **5**):<sup>1</sup>



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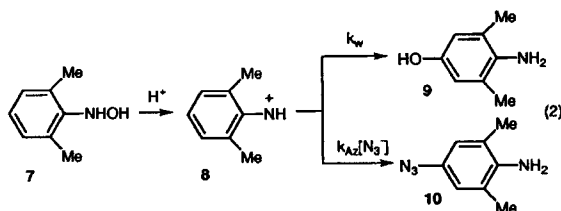
The critical cellular target appears to be DNA, and, indeed, a covalent adduct **6** involving the C-8 carbon of guanine residues can be observed as a major product *in vivo*.<sup>2</sup> Analogous adducts are found in model studies in which the esters **3** undergo solvolysis in the presence of guanine derivatives.<sup>3</sup>

While the structure of this adduct is consistent with a reaction of guanine and a nitrenium ion, unambiguous evidence for such an intermediate has been lacking. As will be described, carbenium ion analogs of **4** and **5** have a very short lifetime in water, exhibit a low selectivity towards guanine in competition with water and, when they do react, do so at a different position in the ring. Thus the following questions arise: (1) do aryl nitrenium ions, in particular those derived from carcinogenic amines, have sufficient lifetimes in aqueous solution to be viable intermediates?; (2) do such nitrenium ions show any reaction with guanine in competition with the solvent, especially at physiological concentrations of the heterocycle?; and (3) if there is a reaction with guanine, does the reaction result in the C-8 adduct?

Within the past 3 years, competition kinetics experiments carried out by the Novak group at Miami University and laser flash photolysis experiments at Toronto have provided positive answers to each of these questions. These experiments provide the subject of this paper.

### AZIDE-CLOCK EXPERIMENTS

The first experiment providing a lifetime of an aryl-nitrenium ion involved the hydroxylamine **7** undergoing an acid-catalyzed Bamberger rearrangement in the presence of azide ion:<sup>4</sup>



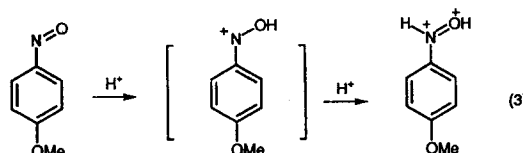
Two products are observed, the normal rearrangement product **9** and the *p*-azido adduct **10**. The latter forms in increasing relative amount with increasing concentration of azide, but does so with no increase in the rate of the reaction. This is classic evidence for an intermediate cation, in this case **8**, forming in a rate-determining step, and then partitioning between the solvent and the added nucleophile. The product data are fitted by the standard equation for competition kinetics providing the ratio  $k_{Az}/k_w = 7.51 \text{ mol}^{-1}$ . With the assumption widely employed for carbenium ions that azide reacts at  $ca\ 5 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$ , the diffusion limit, the lifetime ( $1/k_w$ ) of **8** is around 1 ns. Although this number does indicate that this nitrenium ion can exist as a free cation in water, its lifetime is very short, leading to the suggestion that such an intermediate would be unlikely to survive long enough to react with DNA.

Somewhat surprising, then, were similar experiments reported in 1993 by the Novak group for the 4-biphenylnitrenium ions **4a** and **4b**.<sup>6</sup> These showed  $k_{Az}/k_w$  ratios of the order of  $10^3$ , indicating longer-lived cations, possibly of sufficient lifetime to reach and react with biological targets.

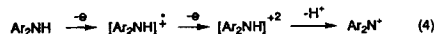
### LASER FLASH PHOTOLYSIS (LFP)

Unlike the situation with carbenium ions, reports of the direct observation of nitrenium ions have been very limited. Attempts to observe such ions under superacid conditions have generally failed.<sup>7,8</sup> The cation  $\text{Ph}_2\text{C}^+\text{CN}$  is argued to have an important nitrenium resonance contributor  $\text{Ph}_2\text{C}=\text{C}=\text{N}^+$ .<sup>7</sup> On the other hand,

nitrosobenzene, whose *O*-protonation would result in a nitrenium ion, is found to be diprotonated in superacids:<sup>8</sup>



There are several reports of diarylnitrenium ions, obtained by electrochemical oxidation/deprotonation of the amine in acetonitrile:<sup>9</sup>



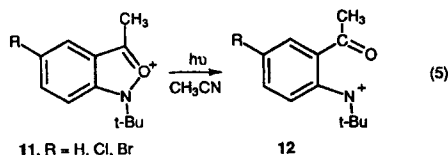
These ions are not stable, however, and have only been characterized by their absorption spectra.

Flash photolysis is an alternative approach for the direct study of intermediate cations, having the particular advantage of providing detailed kinetic information. The technique employs a short pulse of light to generate some intermediate, which is observed and studied, usually by absorption spectroscopy. There are three basic requirements if the approach is to be successful:

- The intermediate must be observable. This normally means that it must have an absorption spectrum with a reasonable extinction coefficient at its  $\lambda_{\text{max}}$ . Instruments that operate in the nanosecond time regime are usually capable of detection from 220 to 700 nm, although the signal-to-noise ratio can be a problem below 300 nm. 'Reasonable' in terms of extinction coefficient depends on the quantum efficiency for production of the intermediate. We have found that cations with extinction coefficients of  $10^4$  produce good signals when the quantum yield for their formation is greater than 0.05.
- The intermediate must survive at least as long as the pulse of light used for its generation. Probably the most widely used excitation source these days is a laser with a pulse width in the nanosecond range (20 ns at Toronto). This means that, based on the azide clock estimates, the ion **8**, and also the parent phenylnitrenium ion ( $1/k_w \approx 500 \text{ ps}$ ),<sup>10</sup> could not be observed in water, but the biphenyl derivatives **4a** and **4b** would be accessible.
- There must be a photochemical reaction generating the desired intermediate. This was a potential problem with nitrenium ions, since little literature precedent existed for their photochemical formation.

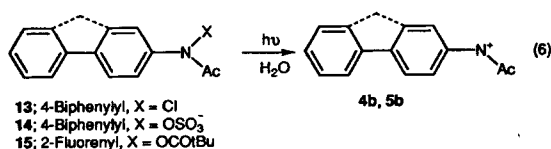
The first report of the successful application of LFP involved the ions **12**, observed in acetonitrile, following

irradiation of **11**:<sup>11</sup>



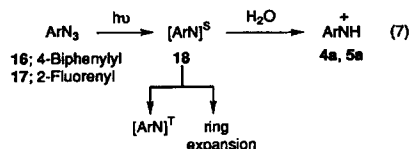
This reaction is a photoheterolysis, with a neutral leaving group, the ketone carbonyl.

At the same time, our group at Toronto was examining the photochemical behavior of the *N*-acetyl derivatives **13–15**, the same precursors that had been employed by the Novak group in their azide-clock studies. These undergo a photoheterolysis in aqueous solution, now with an anionic leaving group, producing transients with  $\lambda_{\max} \approx 450$  nm that could be assigned to the nitrenium ions **4b** and **5b**:<sup>12</sup>



No such transient was observed in acetonitrile, probably a reflection of the need for a very polar medium to allow charge separation. Even in water the photoheterolysis is not the only pathway, since there are other transients and products.

The ground-state solvolytic lability of precursors lacking the *N*-acetyl group precludes their use. However, the corresponding nitrenium ions **4a** and **5a**, also with  $\lambda_{\max} \approx 450$  nm, can be observed with the azide precursors **16** and **17**:<sup>13</sup>



Although the details of the photochemical events have not been conclusively established, what appears to be happening is solvent protonation of an initially formed singlet nitrene. This occurs in competition with the reactions normally observed for such a species, intersystem crossing to a more stable triplet, or ring expansion to a dihydroazepine.<sup>14</sup> In highly aqueous solutions, protonation is the dominant pathway, providing, for example, 84% of the products for irradiation of **16** in 20% acetonitrile. The possibility of protonating aryl nitrenes had been suggested previously by Takeuchi and co-workers.<sup>15</sup> That work, however, employed strong acids. The surprising feature with **16** and **17** is the efficient protonation by water alone. The indication is that the singlet 2-fluorenyl- and 4-biphenylnitrenes are relatively strong bases in water. *p*-Dialkylaminoarylnitrenes have been reported to show similar behaviour.<sup>16</sup>

Several criteria were employed to identify the transient nitrenium ions of equations (6) and (7):

- irradiation of the precursors **13–17** results in products appropriate for such an intermediate;
- the transients are unaffected by oxygen, a radical and triplet trap, but quenched by azide, an effective trap for cations; and
- the ratios of absolute rate constants  $k_{Az}$  and  $k_w$  directly measured by LFP are, within experimental error, the same as ratios determined by the competition kinetics method for the ground-state solvolysis. This is an important observation because it not only assists in establishing the transient as a nitrenium ion, but is also excellent evidence that the nitrenium ion is the same intermediate as formed in a ground-state reaction.

#### ABSOLUTE RATE CONSTANTS: WATER AND AZIDE

Absolute rate constants  $k_{Az}$  and  $k_w$  along with their ratios, are given in Table 1. The nitrenium ions **4** and **5** do indeed have reasonable lifetimes in water, and for a given aryl group there is surprisingly little difference in

Table 1. Absolute rate constants for nitrenium ions reacting with solvent and azide (20 °C; ionic strength 0.5 M, adjusted with NaClO<sub>4</sub>).

Nitrenium ion	$k_w$ (s <sup>-1</sup> )	$k_{Az}$ (l mol <sup>-1</sup> s <sup>-1</sup> )	$k_{Az}/k_s^a$	$k_{Az}/k_s^b$
4-Biphenyl ( <b>4a</b> ) <sup>c</sup>	$1.8 \times 10^6$	$5.0 \times 10^9$	$2.8 \times 10^3$	$2.9 \times 10^{3d}$
<i>N</i> -Acetyl-4-biphenyl ( <b>4b</b> ) <sup>c</sup>	$5.9 \times 10^6$	$5.1 \times 10^9$	$8.6 \times 10^2$	$1.0 \times 10^{2d}$
2-Fluorenyl ( <b>5a</b> ) <sup>c</sup>	$3.4 \times 10^4$	$4.0 \times 10^9$	$1.2 \times 10^5$	
<i>N</i> -Acetyl-2-fluorenyl ( <b>5b</b> ) <sup>c</sup>	$7.7 \times 10^4$	$4.2 \times 10^9$	$5.6 \times 10^4$	$6.2 \times 10^{4e}$

<sup>a</sup> Ratio of absolute rate constants.

<sup>b</sup> Obtained from ground-state solvolysis by method of competition kinetics.

<sup>c</sup> Ref. 13.

<sup>d</sup> Ref. 6.

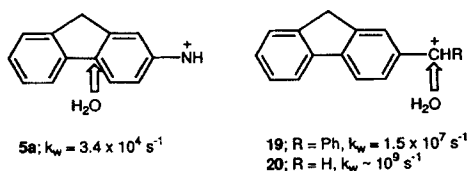
<sup>e</sup> Ref. 12.

$k_w$  for the NH and NAc derivatives. The nature of the aryl group, however, does have a significant effect, the 2-fluorenyl derivatives being about two orders of magnitude longer lived than the 4-biphenyl systems. It is worth noting that the Novak group has shown that  $\log k_w$  for arylnitrenium ions is very poorly correlated by  $\sigma^+$ .<sup>17</sup>

Also of interest are the  $k_{AZ}$  values, which are of the order  $4 \times 10^9 - 5 \times 10^9 \text{ l mol}^{-1}$ . Hence the reactions of azide and nitrenium ions are, at the very least, approaching diffusion control, as assumed in the application of the azide-clock method. Previous LFP studies have also shown similar  $k_{AZ}$  values for carbenium ions in aqueous solution.<sup>18</sup> Thus, in their reactions with azide, carbenium and nitrenium ions exhibit the same behavior, reacting at or near the diffusion limit (although this will not be true for highly stabilized examples).

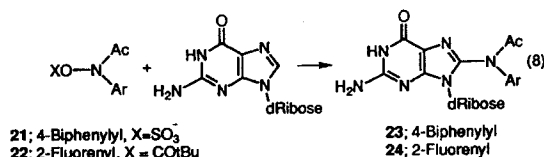
#### AQUEOUS LIFETIMES OF CARBENIUM AND NITRENIUM IONS

The surprising difference lies in the lifetimes in water. One such comparison involves the 2-fluorenyl analogs **5a**, **19** and **20**. In this case the carbenium ion accessible by nanosecond LFP is the diarylmethyl derivative **19**. The simple benzylic-type ion **20** can be estimated to be 100-fold more reactive, based on other carbenium ions where  $\alpha$ -phenyl is replaced by H (e.g.  $4\text{-MeOC}_6\text{H}_4\text{C}^+\text{HPh}$ ,<sup>19</sup>  $k_w = 2.0 \times 10^6 \text{ s}^{-1}$ , and  $4\text{-MeOC}_6\text{H}_4\text{CH}_2^+$ ,<sup>20</sup>  $k_w = 2.0 \times 10^8 \text{ s}^{-1}$ ). The conclusion is that the 2-fluorenyl nitrenium ion has a  $10^5$ -fold greater kinetic stability in water than the 2-fluorenylcarbenium ion. As shown in the above structures, benzylic cations normally react with water at the external carbon, whereas arylnitrenium ions react in the ring. One simple explanation is that the loss of aromaticity associated with the latter results in a larger kinetic barrier.



#### GUANINE DERIVATIVES

Although the lifetimes of the 4-biphenyl and 2-fluorenylnitrenium ions are longer than initially expected, their reactions with water are still relatively fast. The question then arises as to whether guanine can compete. This question was first addressed by the Novak group through solvolysis studies with the *N*-acetyl derivatives **21** and **22** in the presence of 2'-deoxyguanosine (dG):<sup>21</sup>



While the rate constants for the disappearance of **21** or **22** were independent of the concentration of dG, significant amounts of the C-8 adducts **23** or **24** were formed, e.g. 75% of **23** from **21** in 10 mM dG and >95% of **24** from **22** in 5 mM dG. These observations provided the first evidence for the formation of a free nitrenium ion in a rate-limiting step, followed by partitioning between dG and solvent. The partitioning ratios  $k_{dG}/k_w$ , calculated in the standard manner,<sup>21</sup> are given in Table 2.

These experiments clearly show a relatively high selectivity for the nucleoside. Direct evidence that these nitrenium ions are effectively trapped by guanine has recently been obtained with LFP, in experiments in which the decay of the 4-biphenylnitrenium ions **4a** and **4b** was followed in the presence of dG and its 5'-monophosphate derivative.<sup>22</sup> Millimolar concentrations of the guanine derivatives result in rate accelerations, with second-order rate constants for the quenching in the range  $1 \times 10^9 - 2 \times 10^9 \text{ l mol}^{-1}$  (Table 2). Evidence that this quenching represents the reaction of equation (8) is shown by the excellent agreement of the  $k_{dG}/k_w$  ratios measured for **4b** by LFP and competition kinetics.

Hence the arylnitrenium ions **4** and **5** do react with guanine even at low concentrations of the latter, and do so to form the C-8 adduct. This behavior contrasts with

Table 2. Selectivities and absolute rate constants for the reaction of nitrenium ions and 2'-deoxyguanosine (dG) and 2'-deoxyguanosine-5'-phosphate (dGMP) (20 °C, 5% acetonitrile–Water, ionic strength 0.5 M, adjusted with NaClO<sub>4</sub>)

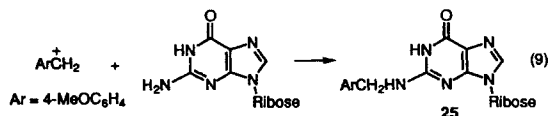
Nitrenium ion	$k_{dG}/k_w$ (1 mol <sup>-1</sup> ) <sup>a</sup>	$k_{dG}/k_w$ (1 mol <sup>-1</sup> ) <sup>b</sup>	$k_{dG}$ (1 mol <sup>-1</sup> s <sup>-1</sup> )	$k_{dGMP}$ (1 mol <sup>-1</sup> s <sup>-1</sup> )
<i>N</i> -Acetyl-4-biphenyl (4b)	290	330	$1.9 \times 10^9$	$1.3 \times 10^9$
4-Biphenyl (4a)			$2.1 \times 10^9$	$1.81 \times 10^9$
<i>N</i> -Acetyl-2-fluorenyl (5b)	8000		$(6.2 \times 10^8)^c$	

<sup>a</sup> Competition kinetics.<sup>21</sup>

<sup>b</sup> Ratio calculated from absolute rate constants LFP.

<sup>c</sup> From competition kinetics ratio, using LFP value for  $k_w$ .

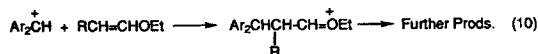
that of analogous carbenium ions. In unpublished LFP studies, we have found that the decay of 4-MeOC<sub>6</sub>H<sub>4</sub>CH<sup>+</sup>Ph, a cation with similar *k<sub>w</sub>* to **4a**, is not accelerated by 5 mM dG in water.<sup>22</sup> The 4-methoxybenzyl cation, obtained solvolytically from the chloride, does alkylate guanosine.<sup>23</sup> The selectivity for the nucleoside is very low. Moreover, there is no C-8 adduct, the principal product being the N-2 adduct **25**:



### VINYL ETHERS

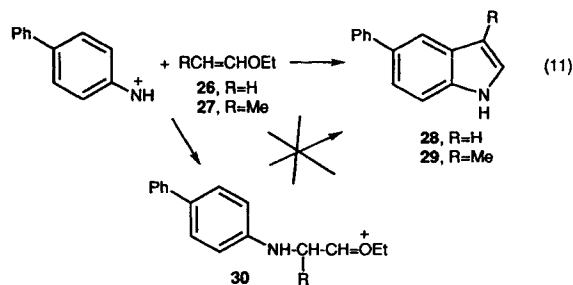
Vinyl ethers represent another class of nucleophiles exhibiting different patterns of reactivity in comparing carbenium and nitrenium ions. With diarylmethylcarbenium ions, studied by LFP in 100% acetonitrile,<sup>24</sup> ethyl vinyl ether shows a reactivity almost identical with that of water, expressing the rate constant for the latter ion in bimolecular terms (Table 3). Were this same pattern to apply in aqueous solution at 55 M water, then even 1 M ethyl vinyl ether would only quench 2% of the cation. However, much lower concentrations of vinyl ethers result in significant accelerations of the decay of the arylnitrenium ions. Second-order rate constants are given in Table 3. As with the guanine derivatives, vinyl ethers clearly showed an enhanced selectivity compared with water in their reactions with nitrenium ions.

The products are also interesting. Carbenium ions react with alkenes in Markovnikov fashion,<sup>25</sup> and with vinyl ethers would give intermediate oxocarbeniums:



With **4a**, the only nitrenium ion thus far investigated, the products with two vinyl ethers, **26** and **27**, have been

identified as indoles, **28** and **29** respectively:



Although the mechanism is still uncertain, the regiochemistry in **29** rules out the formation of the oxocarbenium **30** resulting from N-C bond formation.

### CONCLUSION

The three questions posed in the introduction can now be answered in the affirmative:

- (a) The nitrenium ions **4** and **5** derived from the carcinogenic amines **1** and **2** are relatively long-lived in water.
- (b) These nitrenium ions do react selectively with the guanine of 2'-deoxyguanosine and 2'-deoxyguanosine-5'-phosphate.
- (c) The product of this reaction is the C-8 adduct.

These nitrenium ions differ in all three respects from analogous carbenium ions. The reactions with vinyl ethers represent a fourth system where significant differences are observed.

A number of important questions remain unanswered however,:

- (1) What are the detailed mechanisms for the formation of the C-8 adduct with guanine<sup>26</sup> and the indoles with vinyl ethers?
- (2) What are the origins of the different reactivity

Table 3. Absolute rate constants for the reactions of carbenium ions and nitrenium ions with vinyl ethers.

Cation	<i>k</i> <sub>2</sub> (H <sub>2</sub> O) <sup>a</sup>	<i>k</i> <sub>2</sub> (CH <sub>2</sub> =CHOEt) <sup>a</sup>	<i>k</i> <sub>2</sub> (CH <sub>3</sub> CH=CHOEt) <sup>a</sup>
(4-ClC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> CH <sup>+</sup> <sup>b</sup>	1.7 × 10 <sup>8c</sup>	1.7 × 10 <sup>8</sup>	7.7 × 10 <sup>8</sup> (Z); 1.2 × 10 <sup>9</sup> (E)
(4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> CH <sup>+</sup> <sup>b</sup>	4.3 × 10 <sup>6c</sup>	6.3 × 10 <sup>6</sup>	
<i>N</i> -Acetyl-4-biphenyl ( <b>4b</b> ) <sup>d</sup>	2.1 × 10 <sup>5c</sup>	2.3 × 10 <sup>8</sup>	9.4 × 10 <sup>8f</sup>
4-Biphenyl ( <b>4a</b> ) <sup>d</sup>	6.8 × 10 <sup>4c</sup>	2.2 × 10 <sup>8</sup>	1.4 × 10 <sup>9f</sup>
2-Fluorenyl ( <b>5a</b> ) <sup>d</sup>	2.5 × 10 <sup>2c</sup>	1.4 × 10 <sup>7</sup>	2.8 × 10 <sup>8f</sup>

<sup>a</sup> Units l mol<sup>-1</sup> s<sup>-1</sup>.

<sup>b</sup> Ref. 24; solvent is 100% acetonitrile.

<sup>c</sup> True bimolecular rate constant.

<sup>d</sup> Acetonitrile-water (20:80).

<sup>e</sup> Obtained by dividing *k*(decay) in the solvent by the concentration of water (*ca* 44 M).

<sup>f</sup> Obtained for 3:1 *cis-trans* mixture.

patterns exhibited by carbenium ions and nitrenium ions?

- (3) Do these nitrenium ions also show selectivity for guanine in DNA?
- (4) Do the nitrenium ions derived from other mutagenic amines, e.g. the food mutagen heterocyclic amines, show high selectivity towards guanine?
- (5) Do the reactivities of arylnitrenium ions correlate with mutagenicity/carcinogenicity?

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