Nitrenium Ions from Food-Derived Heterocyclic Arylamine Mutagens

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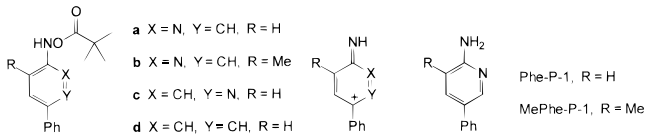
Heterocyclic arylamines, the products of the pyrolysis of proteins and amino acid mixtures, are known mutagens to *Salmonella* in the presence of rat liver homogenates.[1] These materials are carcinogenic in laboratory animals, and are assumed to be carcinogenic in humans.[2] Similar to their carbocyclic analogues, they are promutagens and procarcinogens requiring metabolism into hydroxylamines and subsequently into carboxylic or sulfuric acid esters of the hydroxylamines.[1−3] Because of this, and similarities in DNA adducts derived from the two classes of amines,[4] it is believed that esters of heterocyclic *N*-arylhydroxylamines yield *N*-arylnitrenium ions that are responsible for the carcinogenic effects of these compounds. Although the intermediacy of arylnitrenium ions in the hydrolysis of the carbocyclic esters is well established,[5] as is the involvement of these cations in the formation of DNA adducts,[6] no reports of the generation or chemistry of heterocyclic nitrenium ions derived from these mutagens have appeared.[7]

The esters **1a**−**c** were synthesized to test the hypothesis that heterocyclic nitrenium ions can be generated from their decomposition.[8,9] The esters **1a** and **1b** are derivatives of the mutagen 2-amino-5-phenylpyridine (Phe-P-1), a pyrolysis product of phenylalanine, and the synthetic mutagen 2-amino-3-methyl-5-phenylpyridine (MePhe-P-1).[4a,9a] These esters are heterocyclic



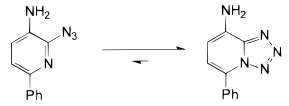
1 **6**

analogues of the carbocyclic ester **1d** derived from the carcinogen 4-aminobiphenyl.[5a] All three heterocyclic esters show pH dependent hydrolysis kinetics (Figure 1) consistent with spontaneous uncatalyzed hydrolysis of the conjugate base of a hydrolytically unreactive acid. The apparent ionization constant, K_a, and the limiting hydrolysis rate constant, k_o, can be obtained from fits of the kinetic data to eq 1. The pH dependence of initial UV

$$k_{obsd} = k_o K_a/(K_a + 10^{-pH}) \quad (1)$$

absorbance (Figures S1−S3 of the Supporting Information) confirms the presence of an ionization equilibrium. Since this was not observed for **1d**,[5a] the pK_a values are assigned to the deprotonation of the endocyclic pyridyl nitrogens of the conjugate acids of **1a**−**c**. The pK_a and kinetic parameters for **1a**−**c** are collected in Table 1. There is good agreement between the pK_a values determined from the UV titrations and the kinetic fits. The endocyclic nitrogens of **1a**−**c** reduce the magnitude of the rate constant k_o compared to that for **1d**, with *o*-pyridyl N (**1a,b**) having a larger effect than *m*-pyridyl N (**1c**).

N_3^- has no significant effect on the hydrolysis rate constants for **1a**−**c** (Table S1 in the Supporting Information), but has a marked effect on product distributions. Figure 2 shows that for **1c** the hydrolysis product **2**[8] is replaced by the azide adduct **3**[8] as the N_3^- concentration increases, but the yield of the rearrangement product **4**[8] is unaffected by N_3^- up to 0.20 M. The adduct **3** is the tetrazole tautomer of the likely initial adduct **5**. In solution



5 **3**

or in the solid state most 2-azidopyridines are less stable than their tetrazole tautomers.[10] These results are consistent with the mechanism of Scheme 1 that is similar to that used to explain the hydrolysis behavior of **1d** and other esters of carbocyclic *N*-arylhydroxylamines and *N*-arylhydroxamic acids.[5]

The rate constant ratio k_{az}/k_s can be determined by fits of the product yield data to eq 2 or 3, where [S] is the yield of solvent-

$$f_s = [S]/([S] + [Az]) = 1/(1 + (k_{az}/k_s)[N_3^-]) \quad (2)$$

$$f_{az} = [Az]/([S] + [Az]) = (k_{az}/k_s)[N_3^-]/(1 + (k_{az}/k_s)[N_3^-]) \quad (3)$$

derived products and [Az] is the yield of N_3^- adducts.[5,11] These ratios are reported in Table 1 for **1a**−**c** and **1d**. The hydrolyses of all three heterocyclic esters yield reactive electrophiles generated after the rate-limiting step of the reaction. These intermediates appear to be the nitrenium ions **6a**−**c** that behave similarly to the nitrenium ions derived from their carbocyclic analogues. Comparison of k_{az}/k_s for **6a**−**c** and **6d** show that the endocyclic N considerably reduces the selectivity of these ions. If k_{az} is

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(1) Sugimura, T. *Environ. Health Perspect*. **1986**, *67*, 5−10. Hatch, F. T.; Knize, M. G.; Felton, J. S. *Environ. Mol. Mutagen*. **1991**, *17*, 4−19.

(2) Ohgaki, H.; Matsukura, N.; Morino, K.; Kanachi, T.; Sugimura, T.; Takayama, S. *Carcinogenesis* **1984**, *5*, 815−819. Sugimura, T. *Mutat. Res*. **1985**, *150*, 33−41. Eisenbrand, G.; Tang, W. *Toxicology* **1993**, *84*, 1−82.

(3) Shinohara, A.; Saito, K.; Yamazoe, Y.; Kamataki, T.; Kato, R. *Cancer Res*. **1986**, *46*, 4312−4367. Snyderwine, E. G.; Wirth, P. J.; Roller, P. P.; Adamson, R. H.; Sato, S.; Thorgiersson, S. S. *Carcinogenesis* **1988**, *9*, 411−418. Meerman, J. H. N.; Ringer, D. P.; Coughtrie, M. W. H.; Bamforth, K. J.; Gilissen, R. A. H. J. *Chem.-Biol. Interact*. **1994**, *92*, 321−328.

(4) (a) Saris, C. P.; van Dijk, W. J.; Westra, J. G.; Hamzink, M. R. J.; van de Werken, G.; Zomer, G.; Stavenuiter, J. F. C. *Chem.-Biol. Interact*. **1995**, *95*, 29−40. (b) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Biochem. Biophys. Res. Commun*. **1980**, *96*, 355−362. (c) Snyderwine, E. G.; Roller, P. P.; Adamson, R. H.; Sato, S.; Thorgiersson, S. S. *Carcinogenesis* **1988**, *9*, 1061−1065.

(5) (a) Novak, M.; Kahley, M. J.; Eiger, E.; Helmick, J. S.; Peters, H. E. *J. Am. Chem. Soc*. **1993**, *115*, 9453−9460. (b) Davidse, P. A.; Kahley, M. J.; McClelland, R. A.; Novak, M. *J. Am. Chem. Soc*. **1994**, *116*, 4513−4514. (c) Novak, M.; Kahley, M. J.; Lin, J.; Kennedy, S. A.; Swanegan, L. A. *J. Am. Chem. Soc*. **1994**, *116*, 11626−11627. (d) McClelland, R. A.; Davidse, P. A.; Hadzialic, G. *J. Am. Chem. Soc*. **1995**, *117*, 4173−4174.

(6) Novak, M.; Kennedy, S. A. *J. Am. Chem. Soc.* **1995**, *117*, 574−575. Kennedy, S. A.; Novak, M.; Kolb, B. A. *J. Am. Chem. Soc*. **1997**, *119*, 7654−7664.

(7) The heterocyclic 2-imidazolylnitrenium ion has been characterized as a very long-lived species: Bolton, J. L.; McClelland, R. A. *J. Am. Chem. Soc*. **1989**, *111*, 8172−8181.

(8) See the Supporting Information.

(9) (a) Lutgerink, J. T.; Stavenuiter, J. F. C.; Zomer, G.; Hamzink, M.; van Dijk, P.; Westra, J. G.; Kriek, E. *Carcinogenesis* **1989**, *10*, 1957−1960. (b) Stavenuiter, J. F. C.; Verrips-Kroon, M.; Bos, E. J.; Westra, J. G. *Carcinogenesis* **1985**, *6*, 13−19. (c) Stavenuiter, J.; Hamzink, M.; van der Hulst, R.; Zomer, G.; Westra, G.; Kriek, E. *Heterocycles* **1987**, *26*, 2711−2716. (d) Setliff, F. L. *J. Chem. Eng. Data* **1970**, *15*, 590−591. (e) Novak, M.; Lagerman, R. K. *J. Org. Chem*. **1988**, *53*, 4762−4769.

(10) Boyer, J. H.; Miller, E. J. *J. Am. Chem. Soc.* **1959**, *81*, 4671−4673. Wentrup, C.; Winter, H.-W. *J. Am. Chem. Soc*. **1980**, *102*, 6161−6163.

(11) Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 4689−4691; **1982**, *104*, 4691−4692; **1984**, *106*, 1383−1396. Kemp, D. S.; Casey, M. L. *J. Am. Chem. Soc*. **1973**, *95*, 6670−6680. Rappoport, Z. *Tetrahedron Lett*. **1979**, 2559−2562.

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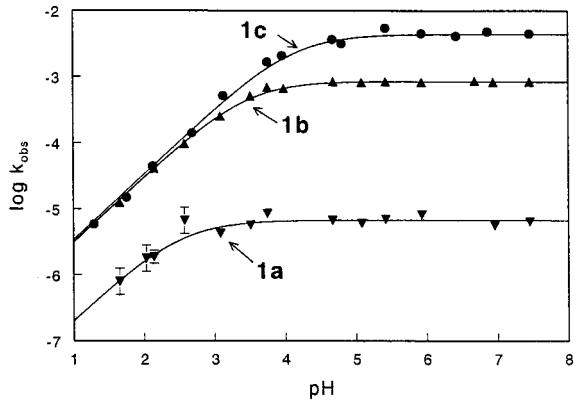


Figure 1. log k_{obs} vs pH for **1a**−**c**. Theoretical lines were obtained from fits to eq 1. Kinetic parameters are reported in Table 1.

Table 1. Kinetic Parameters for **1a**−**d**[a]

	pK_a			
ester	UV data[b]	kinetic data[c]	k_o (s^{-1})[c]	k_{az}/k_s (M^{-1})
1a	2.83 ± 0.05	2.5 ± 0.3	(6.7 ± 0.4) × 10^{-6}	10 ± 2
1b	3.34 ± 0.05	3.43 ± 0.04	(8.5 ± 0.1) × 10^{-4}	300 ± 50
1c	3.95 ± 0.07	4.11 ± 0.09	(4.4 ± 0.2) × 10^{-3}	79 ± 11
1d			(1.3 ± 0.2) × 10^{-1} [d]	(2.9 ± 0.2) × 10^3 [e]

[a] Determined in 5% vol/vol CH_3CN-H_2O at μ = 0.5 and T = 20 °C, unless otherwise indicated. Buffers used to maintain pH were 0.02 M Na_2HPO_4/NaH_2PO_4, NaOAc/AcOH, $NaHCO_2/HCO_2H$, and $HClO_4$ solutions at pH ≤ 2.5. [b] The initial UV absorbance vs pH was fit to a standard titration curve (see the Supporting Information). [c] Obtained from a fit of k_{obs} vs pH to eq 1. [d] Measured at 0 °C; see ref 5a. [e] See ref 5a.

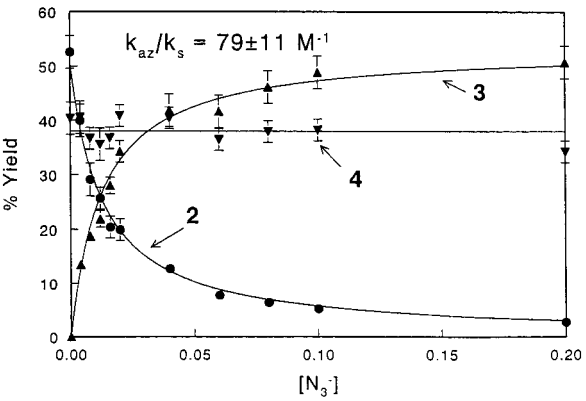
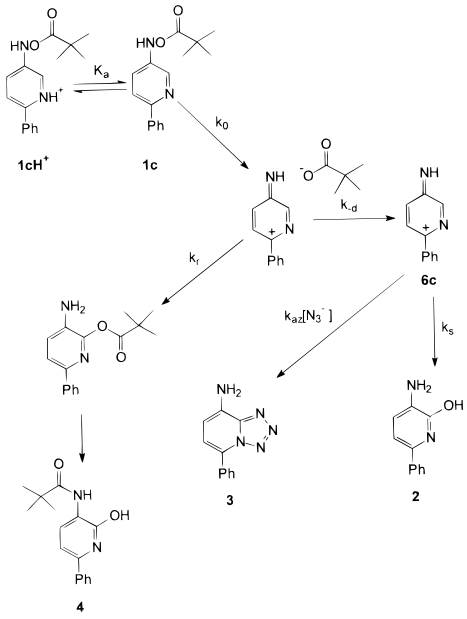


Figure 2. Product yields as a function of $[N_3^-]$ at pH 7.0 for **1c**. Product yield data for **2** and **3** were fit to eqs 2 and 3, respectively, to obtain the theoretical lines for those compounds and the value of k_{az}/k_s. The theoretical line for **4** is the average value of its yield for $[N_3^-]$ from 0.0 to 0.2 M.

diffusion limited, as it is for **6d**,[5d] these reduced selectivities are due to increased k_s for the heterocyclic ions. This is consistent with the expected effect on nitrenium ion stability of replacement of aromatic ring carbons by the more electronegative nitrogen atom. The lower selectivity of **6a** compared to **6c** is expected because the *o*-pyridyl N will more strongly interact with the positive charge of **6a** than will the *m*-pyridyl N of **6c**.

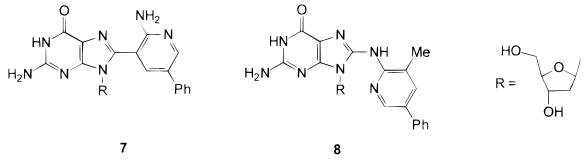
The effect of the *o*-methyl in **6b** (a 30-fold increase in k_{az}/k_s compared to **6a**) is considerably larger than the effect of two methyl groups in the (2,6-dimethylphenyl)nitrenium ion compared to phenylnitrenium ion (ca. 7-fold increase in k_{az}/k_s).[12] This may be due to greater localization of charge at the ortho carbon of **6a** and **6b**. The reaction of **6b** with both solvent and N_3^- is

Scheme 1



complicated by the lack of sites on the proximal ring of the nitrenium ion that can lead to aromatic products after initial nucleophilic attack. The products of N_3^- and solvent reaction with **6b** are under investigation. All three initial N_3^- adducts decompose into new products during isolation.

The acetic acid esters analogous to **1a** and **1b** yield the adducts **7** and **8** upon reaction with 2′-deoxyguanosine (d-G).[4a] The



adduct **8** is formed in much greater yield than **7** under identical conditions (ca. 15-fold in DNA studies and ca. 5-fold in saturated d-G solutions). The structure of **7** is unusual since most aromatic amine carcinogens yield adducts similar to **8**, but the relative yields of **7** and **8** are consistent with our observation that **6b** is 30-fold more selective toward N_3^- than **6a.** The selectivity of carbocyclic arylnitrenium ions toward d-G, $k_{d\text{-}G}/k_s$, is smaller than k_{az}/k_s by a factor of ca. 3−8.[6] For example, $k_{d\text{-}G}/k_s$ for **6d** is 1.1 × 10^3 M^{-1}.[6] If that is true for heterocyclic arylnitrenium ions, **6a**−**c** will have small but measurable $k_{d\text{-}G}/k_s$ values. Since Phe-P-1 and MePhe-P-1 are weakly mutagenic heterocyclic amines,[1] it is not surprising that **6a** and **6b** are relatively unselective ions. We are preparing esters of a wide variety of heterocyclic arylhydroxylamines with mutagenicities toward *Salmonella* that vary over a factor of 10^4. Measurement of k_{az}/k_s and $k_{d\text{-}G}/k_s$ for nitrenium ions derived from these esters will show if there is a correlation of mutagenicity with nitrenium ion selectivity as there is for carbocyclic nitrenium ions.[13]

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Supporting Information Available: Synthesis of **1a**−**c**, isolation and characterization of **2**−**4**, Table S1, and Figures S1−S3 (7 pages). See any current masthead page for ordering information and Web access instructions.

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(12) Fishbein, J. C.; McClelland, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 2824−2825.

(13) Novak, M.; Lin, J.; Kayser, K. J. To be submitted to *J. Am. Chem. Soc*.