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Chemistry of carcinogenic and mutagenic metabolites of heterocyclic aromatic amines†,‡

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ABSTRACT: Ester derivatives of the hydroxylamine metabolic oxidation products of heterocyclic amines (HCAs) are the ultimate mutagenic and carcinogenic compounds derived from this ubiquitous class of chemical carcinogens that are produced during the cooking of protein-containing foods. Considerable work has been done on the mechanism of formation of the HCAs during cooking processes, their detection at ppb levels in food products, the metabolism of the HCAs and the detection and characterization of DNA adducts of the HCA metabolites, but until recently very little work had been done to characterize the chemistry of the carcinogenic/mutagenic metabolites themselves. This paper reviews our recent work on the chemistry of model carcinogens from this class. The kinetics of their decomposition in aqueous solution, identification of reaction products and the characterization of the reactivity and selectivity of heterocyclic nitrenium ions generated during their reactions are presented. The implications of these results with respect to mutagenicity and carcinogenicity are discussed. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: heterocyclic aromatic amines; decomposition kinetics; carcinogens; mutagens; nitrenium ions; azide clock; 2′-deoxyguanosine adducts

INTRODUCTION

It has been known since the late 1970s that broiling and frying of meats and fish generates compounds, subsequently identified as heterocyclic amines (HCAs), that are mutagenic to *Salmonella typhimurium* after activation by mammalian liver homogenates.[1,2] These compounds are also found in commercial food flavorings and sauces, beverages and tobacco smoke.[2,3] They have been shown to be carcinogens in laboratory animals and are assumed to be human carcinogens.[2,4] A list of selected HCAs is presented in Scheme 1 along with the common metabolic pathways required for activation into their ultimate mutagenic and carcinogenic forms, the sulfuric or acetic acid esters of the corresponding hydroxylamines.[2,5,6]

The nitrenium ion hypothesis was developed for the ester metabolites of carbocyclic aromatic amines in the late 1960s based largely on the structure of the ultimate carcinogens and DNA adducts.[7] This hypothesis held that these compounds were subject to heterolytic N—O bond cleavage resulting in a reactive nitrenium ion that was ultimately responsible for genetic damage through reaction with DNA bases. The HCAs are metabolized in the same manner as are mutagenic and carcinogenic carbocyclic aromatic amines and generate very similar DNA adducts. For that reason, it has been assumed, without supporting evidence, that the HCA metabolites also generate nitrenium ion intermediates that are responsible for the deleterious effects of these compounds.[2] The hypothesis for the carbocyclic esters received considerable experimental support from work performed in the laboratories of Novak, McClelland, and Falvey during the 1990s, and it is now clear that the carbocyclic esters do react via an S_N1 ($D_N + A_N$) mechanism with DNA bases and other nucleophiles in aqueous solution.[8–11] No experimental support for a similar process in the heterocyclic esters had been published prior to 1998, and given the added complication of acid–base chemistry related to the heterocyclic rings, and the unknown effect of the heterocyclic substituents on nitrenium ion stability, it was not clear whether these compounds did react in a similar fashion to their carbocyclic analogues.

In an effort to understand the chemistry of these ubiquitous carcinogens, we have synthesized and studied a series of ester derivatives of heterocyclic hydroxylamines and hydroxamic acids, **1a**–**k** (Scheme 2).[12–16] The hydroxylamine esters are the physiologically relevant compounds, but in some cases have proven to be too reactive to isolate. We have resorted to the more stable hydroxamic acid esters in these cases.[14–16] In carbocyclic cases it has been shown that the *N*-acetyl group slows the

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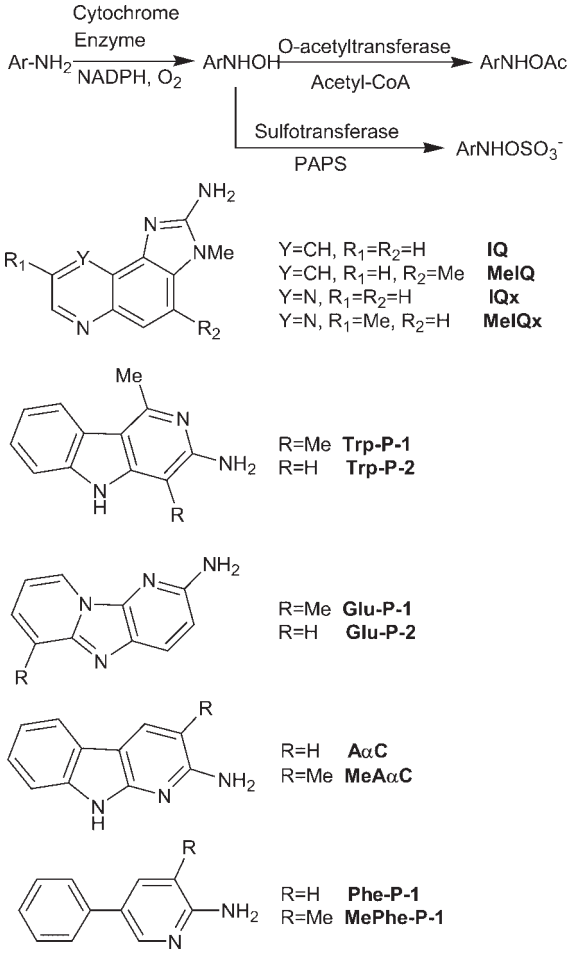
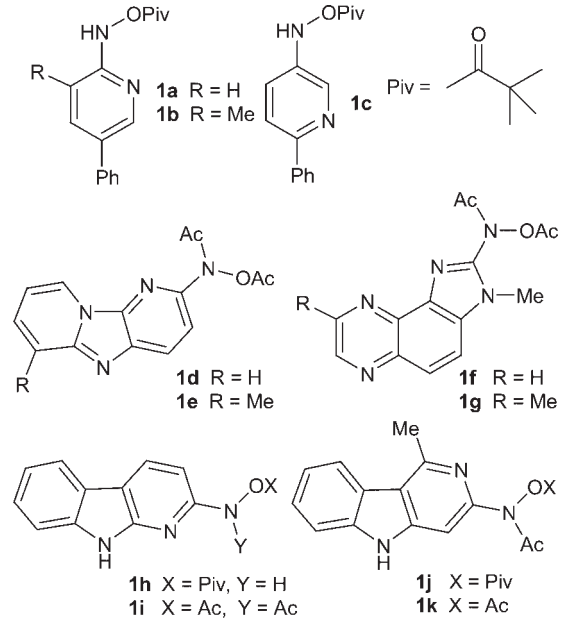


Scheme 1



Scheme 2

N—O bond cleavage by a factor of up to 10^5, but does not otherwise alter the basic chemistry of the ester, and has a minimal effect on the reactions and reactivity of the resulting nitrenium ion.[8,17]

The aim of this paper is to provide a review of the mechanistic studies undertaken with these esters, to interpret those results and to consider the biological implications of the chemistry that has been discovered. The synthesis of the HCAs and their ester derivatives is beyond the scope of this review and has been described elsewhere.[12–16,18]

KINETICS

All of the esters undergo decomposition in acidic to neutral aqueous solution with rate constants (k_{obs}) that are pH dependent, but independent of buffer concentration at the low concentrations ($\leq$0.1 M) used in our studies.[12–16] Log k_{obs} vs pH data are shown in Figs 1 and 2. All of the esters exhibit pH-independent decomposition rates near neutral pH and a kinetic pK_a that varies from ca 0.0 to 4.0 (Table 1). The kinetic pK_a values correspond closely to those obtained from spectrophotometric titration of the esters (Table 1). The protonated hydroxylamine esters (**1aH**$^+$–**1cH**$^+$, **1hH**$^+$) are unreactive under acidic conditions (Fig. 1),[12,13] but the protonated hydroxamic acid esters exhibit a variety of behaviors under acidic conditions (Fig. 2).[14–16] A rate law that includes all kinetic terms observed for **1a–k** is given by

$$k_{obs} = [k_H(10^{-pH})^2 + k_A(10^{-pH}) + k_B K_a]/(K_a + 10^{-pH}) \quad (1)$$

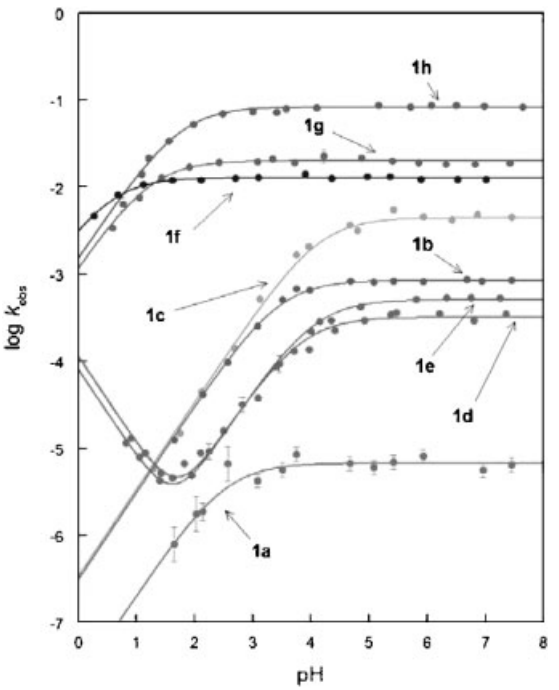


Figure 1. Log k_{obs} for ester disappearance vs pH for **1a–h** at 20 °C

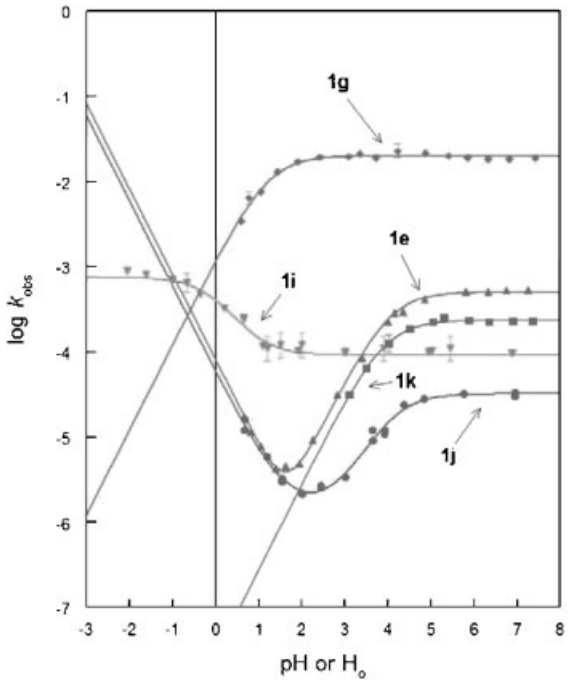
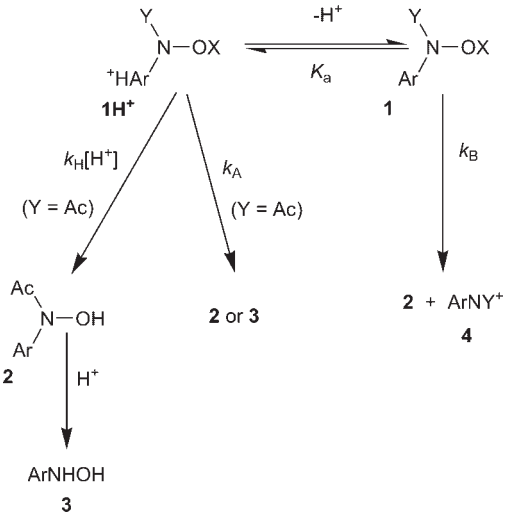


Figure 2. Log k_{obs} vs pH for disappearance of selected hydroxamic acid esters. Data for **1i** taken at 40 °C, all others at 20 °C



Scheme 3

Individual rate constants obtained for all esters are collected in Table 1. The first term of Eqn (1) (k_H) corresponds to the acid-catalyzed decomposition of the conjugate acid of the ester.[14] For each ester for which this term has been observed (**1d**, **e**, **j**), the initial reaction product is the corresponding hydroxamic acid, **2** (Scheme 3).[14,16] This species is subsequently hydrolyzed into the hydroxylamine, **3**. Unlike their carbocyclic analogues, these heterocyclic hydroxylamines are not subject to rapid Bamberger rearrangement, so they are easy to detect as the final hydrolysis products.[14] The second term of Eqn (1) (k_A) corresponds to spontaneous decomposition of the conjugate acid of the ester. It has been observed only for **1i** and **j**.[15,16] In both cases the final product is the corresponding hydroxylamine, **3**. For **1j** this product is generated through hydrolysis of the

Table 1. Rate constants for decomposition of **1a**–**k** and comparison with carbocyclic esters

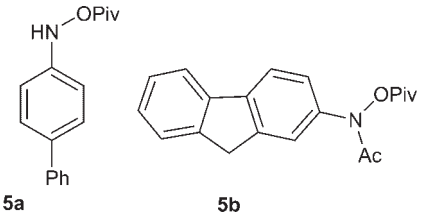
	pK_a				
Ester[a]	Titration	Kinetics	$10^5 k_H$ ($M^{-1} s^{-1}$)	$10^6 k_A$ (s^{-1})	$10^4 k_B$ (s^{-1})
1a[b]	2.83 ± 0.05	2.5 ± 0.3			0.067 ± 0.004
1b[b]	3.34 ± 0.05	3.43 ± 0.04			8.5 ± 0.1
1c[b]	3.95 ± 0.07	4.11 ± 0.09			44 ± 2
1d[c]	4.17 ± 0.08	3.82 ± 0.06	12 ± 1		3.2 ± 0.2
1e[c]	4.21 ± 0.05	4.04 ± 0.03	8.1 ± 0.5		5.1 ± 0.2
1f[c]		0.49 ± 0.03			129 ± 2
1g[c]	1.02 ± 0.19	1.21 ± 0.07			199 ± 4
1h[d]		1.73 ± 0.06			830 ± 20
1i (40 °C)[e]	−0.04 ± 0.09	−0.08 ± 0.07		780 ± 40	0.98 ± 0.04
1i (20 °C)[e]					0.15 ± 0.02
1j[f]	4.11 ± 0.03	4.12 ± 0.07	5.8 ± 0.6	1.5 ± 0.2	0.32 ± 0.02
1k[f]	4.15 ± 0.06	3.92 ± 0.04			2.4 ± 0.1
5a (0 °C)[g]					1400 ± 100
5b[h]					2.0 ± 0.3

[a] Conditions: 5 vol.% CH_3CN–H_2O, $\mu = 0.5$ ($NaClO_4$), $T = 20$ °C.
[b] Source: Ref. 12.
[c] Source: Ref. 14.
[d] Source: Ref. 13.
[e] Source: Ref. 15.
[f] Source: Ref. 16.
[g] Source: Ref. 17.
[h] Source: Ref. 19.

intermediate **2j**,[16] but for **1i** the kinetic data show that the hydroxylamine is generated directly from **1iH**$^+$ with no intermediate hydroxamic acid involved (Scheme 3).[15] The k_H and k_A terms of the rate law are important only for the less reactive hydroxamic acid esters. The very reactive **1f** and **1g** do not exhibit these terms, presumably because of their rapid decomposition via the pathway governed by the third term of Eqn (1).[14] For technical reasons it was not possible to extend the kinetic study for **1k** into sufficiently acidic media to observe the k_H and k_A terms.[16]

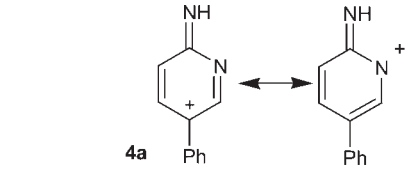
The kinetic terms that dominate under acidic conditions are not physiologically relevant, and the hydroxamic acid esters are not the physiologically relevant carcinogens/mutagens. The third term of the general rate law (k_B) does dominate under physiologically relevant pH conditions, and it is observed for both hydroxylamine and hydroxamic acid esters.[12–16] The *N*-acetyl group of the hydroxamic acid esters appears to decrease the pK_a by 1–2 units and to decrease the magnitude of k_B by a factor of 10^3–10^4.[15] This indicates that all of the naturally formed hydroxylamine acetic acid esters would be maximally reactive via the k_B path under physiological conditions. The range of reactivities spanned by the hydroxylamine esters is predicted to be about 10^7 at 20 °C with lifetimes ranging from ca 0.01 to 1.5×10^5 s. It is clear that under physiological conditions the more reactive esters are too unstable to act at a site remote from their point of generation.

The k_B term corresponds to spontaneous decomposition of the neutral form of the ester (Scheme 3).[12] This is consistent with either N—O bond cleavage to generate a nitrenium ion species, **4**, or with an uncatalyzed acyl transfer to the aqueous solvent to generate the corresponding hydroxamic acid or hydroxylamine, **2** or **3**.[12,16] The latter reaction could occur with intramolecular nucleophilic catalysis through a five-membered ring transition state if a 2-pyridyl N is available.[16] Product analysis shows that both reactions do occur, although N—O bond cleavage is the exclusive reaction of the hydroxylamine esters (**1a–c**, **h**) and the more reactive hydroxamic acid esters (**1d–g**).[12–14]

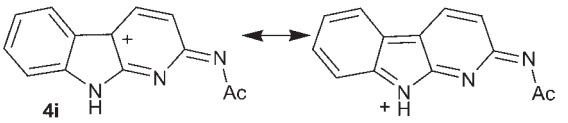


5a **5b**

Some direct kinetic comparisons to carbocyclic esters that undergo exclusive N—O bond cleavage are possible. The 4-aminobiphenylyl derivative **5a** is the carbocyclic analogue of **1a** and the *N*-acetyl-2-aminofluorene derivative **5b** is a carbocyclic analogue of **1i**. The leaving group ability of the pivaloyloxy group of **5b** should be nearly identical with that of the acetoxy group of **1i**. No pK_a is detected for the decomposition of **5a** or **5b** in the moderately acidic to neutral pH range because of the absence of a heterocyclic N in the structure of either **5a** or **5b**.[17,19] The 2-pyridyl N of **1a** has a significant rate retarding effect in excess of 2×10^4-fold from comparison of k_B for **1a** at 20 °C and **5a** at 0 °C. The 9-indolyl N of **1i** has a very different effect. Comparison of k_B for **1i** and **5b**, both at 20 °C, shows that **5b** is only 13-fold more reactive than **1i**. N—O bond cleavage is the dominant neutral pH reaction for **1i** ($>90\%$).[15] Since the rate-retarding 2-pyridyl N is present in **1i**, the 9-indolyl N must have a rate accelerating effect in excess of 1.5×10^3-fold. These opposite effects on reaction rates are understandable if comparisons of the expected stabilizing/destabilizing effects of the two heterocyclic atoms on their respective nitrenium ions, **4a** and **4i**, are considered. Similar considerations for other heterocyclic nitrenium ions suggest that the imidazolyl rings in **4d–g** should stabilize the ion and accelerate N—O bond cleavage. This appears to be the case.[14]



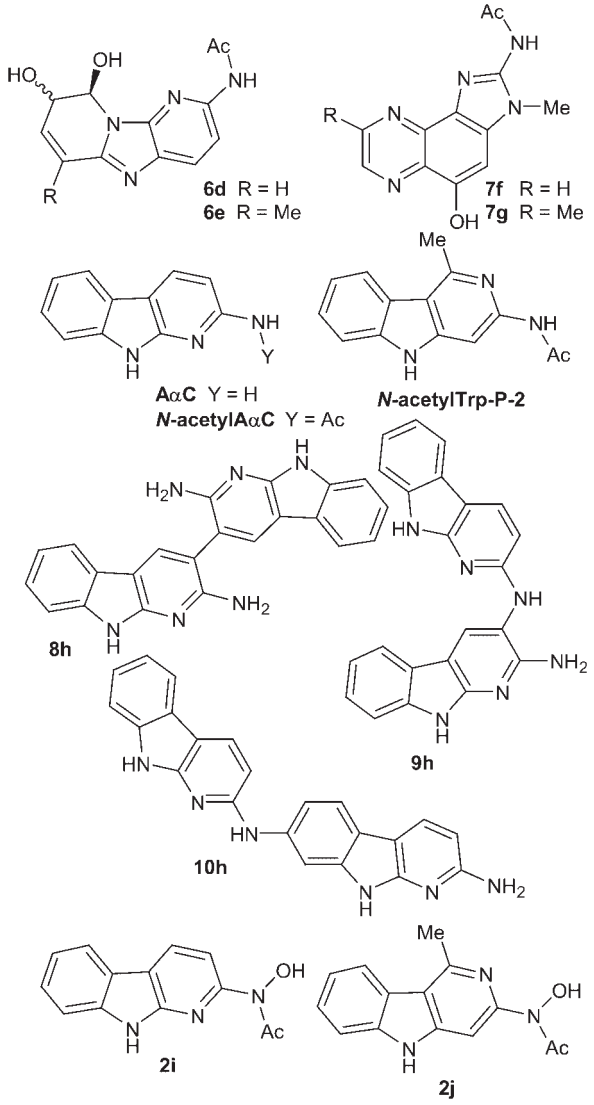
4a



4i

REACTION PRODUCTS

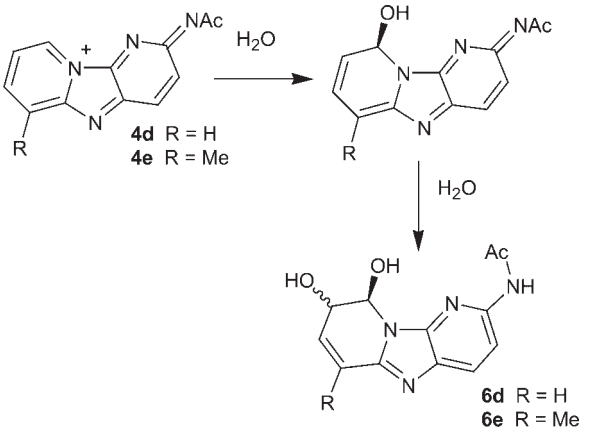
The major reaction products from the decomposition of the model esters at neutral pH in aqueous solution in the absence of non-solvent nucleophiles are exemplified in Scheme 4. Four different classes of products are observed: apparent nucleophilic aromatic substitution and addition products (**6d**, **e**, **7f**, **g**), reduction products (**AαC**, ***N*-acetylAαC**, ***N*-acetylTrp-P-2**), dimers (**8h–10h**) and hydroxamic acids (**2i**, **j**).[12–16] The hydroxamic acid products are found only for the unreactive esters **1i**, **j** and **k**.[15,16] Available data indicate that the hydroxamic acids are generated by an acyl transfer reaction: decomposition of **1i** in $H_2{}^{18}O$ generates **2i** with a maximum of 6% incorporation of excess ^{18}O into the hydroxamic acid,[15] changing the leaving group in the ***N*-acetylTrp-P-2** derivatives **1k** and **1j** from acetoxy to pivaloyloxy



Scheme 4

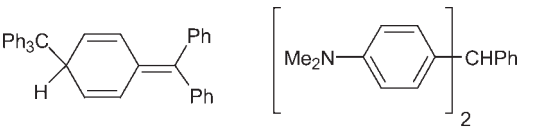
reduces k_{obs} by a factor of 7.5 and reduces the yield of **2j** from ca 95 to 40%[16] and, finally, decomposition of **1i–k** in phosphate buffers containing N_3^- leads to an N_3^--dependent increase in the rate constant for decomposition of the esters and a corresponding increase in the yields of the hydroxamic acids **2i** and **j**.[15,16] No apparent rate increase was noted in phosphate buffers at buffer concentrations up to 0.06 M, but the rate increases noted in the presence of the much more strongly nucleophilic N_3^- are quite modest. For example, k_{obs} increases by less than 2.5-fold for **1i** in pH 6.9 phosphate buffer as $[N_3^-]$ increases from 0.0 to 0.01 M.[15]

The other products noted in Scheme 4 appear to be generated by N—O bond cleavage reactions. The phenol and diol products are consistent with attack of H_2O on a nitrenium ion species, but do not require the existence of such a species.[12–14] The diol addition products such as **6d**, **e** do require the consecutive attack of two molecules of H_2O as in Eqn (2).[14]



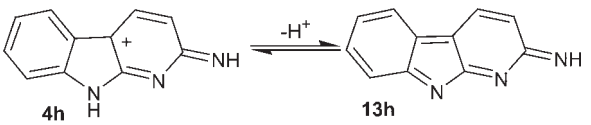
(2)

Significant yields of the reduction products are only observed for the α-carboline and γ-carboline derivatives **1h–k**.[13,15,16] Ordinarily, such products are assumed to be derived from H· scavenging by triplet nitrenium ions, but the triplet scavenger Ph_3CH[20] does not increase the yield of **AαC** or generate the characteristic radical coupling product **11** when **1h** decomposes in aqueous solution saturated with Ph_3CH.[13] The H^- donor **12**[21] (4.0×10^{-5} M) does increase the yield of **AαC** at neutral pH from 14 to 37% during the decomposition of 1×10^{-5} M **1h**.[13] This suggests that formation of **AαC** is a singlet-state reaction.



11 **12**

The carboline-derived nitrenium ions are unique in that they can readily deprotonate to generate a neutral quinone imine methide conjugate base **13** [Eqn (3)] under mildly acidic pH conditions.[13,15] The neutral intermediate **13h** appears to be the more readily reduced species since the yield of **AαC** decreases at lower pH.[13] The apparently high propensity for reduction of the neutral intermediate may be responsible for the significant yields of the reduction products from the carboline derivatives. The source of reducing equivalents in these reactions is not clear, but the phenol products generated by apparent nucleophilic aromatic substitution could be the responsible species.[13]



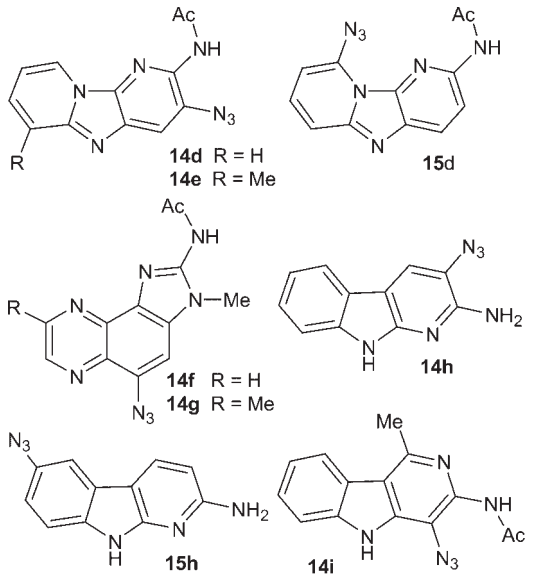
(3)

Dimeric products are only observed in cases in which the reduction products are also generated in significant yield.[13,15] They appear to be derived from nucleophilic trapping of the nitrenium ion **4** or its conjugate base **13** by the amine or amide reduction product.[13] Similar trapping

reactions have previously been documented for selective carbocyclic nitrenium ions.[22] The dimerization is also reminiscent of that of the α-(*N*,*N*-dimethylthiocarbamoyl)-4-methoxybenzyl carbocation that reacts efficiently with its own elimination product to form a dimer in 50:50 TFE–H_2O.[23] The yields of the dimeric products are pH dependent for **1h**, but the kinetics of decomposition of the ester remain cleanly first order and k_{obs} does not vary with dimer product yields.[13] This suggests that the dimeric products are produced in a post-rate-limiting step of a multi-step reaction mechanism.

REACTIONS WITH NON-SOLVENT NUCLEOPHILES

The reactions of **1a–j** with non-solvent nucleophiles including N_3^-, 2′-deoxyguanosine (d-G), and in some cases buffer components such as AcO^- and HPO_4^{2-}, provide the most definitive evidence for the generation of nitrenium ions during the decomposition of the esters.[12–14] All of these esters react with N_3^- to generate products of apparent nucleophilic aromatic substitution. Representative examples are provided in Scheme 5. These N_3^- adducts are generated with no detectable increase in the rate of decomposition of the esters **1a–h**, even under conditions in which the yields of **14** and **15** exceed 80–90%.[12–14] This, of course, constitutes classical evidence for an S_N1, or D_N+A_N, reaction mechanism. The situation is more complicated for **1i** and **j** because these esters are also subject to N_3^--catalyzed acyl transfer reactions described above.[15,16] In both of these cases N_3^- increases the rate of decomposition of the ester, but only the yield of the hydroxamic acid product **2i** or **j** correlates with this rate acceleration. The yield of the N_3^- adducts is not correlated with the N_3^--induced rate acceleration of these compounds, so it was concluded that these adducts were also generated by a nitrenium ion pathway.[15,16] The ester **1k** decomposes almost entirely via an acyl transfer process that is subject to N_3^- catalysis, but even in this case small amounts of the N_3^- adduct can be detected, indicating a minor N—O bond cleavage pathway for this ester.[16]



Scheme 5

Table 2 provides a compilation of $\log(k_{az}/k_s)$ or $\log(k_{az}/k_c)$ values determined for the nitrenium ions **4a–i** and the conjugate bases **13h–j** by competition methods that rely on the determination of product yields as a function of $[N_3^-]$. This 'azide clock' method has been well documented for carbenium and nitrenium ions.[17,24,25] In these ratios k_{az} is the second-order rate constant for reaction of the ion with N_3^-, k_s is the pseudo-first-order rate constant for reaction of the ion with solvent and k_c is given by Eqn (4).[13] This term contains first-order terms for reduction (k_{red}) and reaction with solvent (k_s), and a term that depends on the second-order rate constant (k_{dim}) for formation of the dimeric products and the concentration of the amine or amide reduction product.[13]

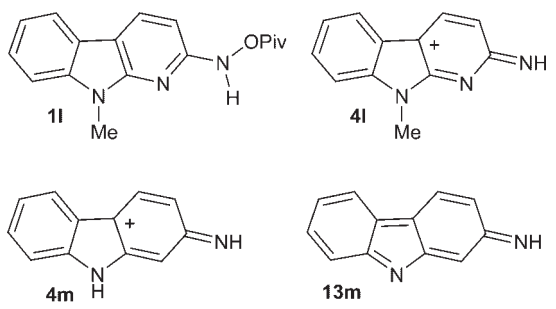
$$k_c = k_s + k_{red} + k_{dim}[\text{reduction product}] \qquad (4)$$

Table 2. Azide/solvent and d-G/solvent selectivities for **4**, **13** and selected carbocyclic ions, **16**

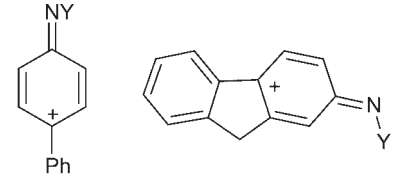
Ion or its conjugate base[a]	Rate constant in denominator	Log (k_{az}/k_s) or log (k_{az}/k_c)	Log ($k_{d\text{-}G}/k_s$) or log ($k_{d\text{-}G}/k_c$)
4a[b]	k_s	1.00	
4b[b]	k_s	2.48	1.92
4c[b]	k_s	1.90	
4d[c]	k_s	6.36	4.49
4e[c]	k_s	6.71	4.70
4f[c]	k_s	4.72	2.96
4g[c]	k_s	5.08	2.92
4h[d]	k_c	4.54	3.91
13h[d]	k_c	3.08	2.38
4i[e]	k_c	3.62	
13i[e]	k_c	2.65	
13j[f]	k_c	4.59	
4l[d]	k_c	4.65	3.94
4m[g]	?	5.83	
13m[g]	?	4.33	
16a[h]	k_s	2.97	2.51
16b[i]	k_s	3.45	3.04
16c[j]	k_s	4.76	3.88
16d[k]	k_s	5.07	4.46

[a] Conditions: 5 vol.% CH_3CN–H_2O, $\mu = 0.5$, $T = 20\,^\circ C$, unless otherwise indicated.
[b] Source: Ref. 12.
[c] Source: Ref. 14.
[d] Source: Ref. 13.
[e] Source: Ref. 15, $T = 40\,^\circ C$.
[f] Source: Ref. 16.
[g] Source: Ref. 26, 20 vol.% CH_3CN–H_2O, $\mu = 0.1$.
[h] Source: Refs 9, 17, 27, 28.
[i] Source: Refs 9, 17, 28, 29.
[j] Source: Refs 9, 19, 27, 28.
[k] Source: Refs 28, 29.

Experimentally, these ratios are determined under conditions in which the k_{dim} term is a minor contributor to k_c so this rate constant can be treated as the sum of the first two terms of Eqn (4).[13] The k_{az}/k_c ratio is reported only for those esters that exhibit significant yields of reduction product (**1h–j**). This ratio is a lower limit for k_{az}/k_s for these species. For these same species k_{az}/k_c is pH dependent, decreasing as the pH increases from 3 to 8.[13,15] Two limiting selectivity ratios can be calculated. The limiting ratio at low pH is interpreted to be k_{az}/k_c for the cations **4h** or **i**, whereas that limiting ratio under neutral pH conditions is attributed to the neutral conjugate bases, **13h** or **i**.[13] Based on yields of the reduction product derived from **1h**, k_{az}/k_c underestimates k_{az}/k_s by a factor of ca 2–3 at low pH and ca 5–10 at neutral pH.[13] For **1j** the selectivity ratio has been measured only at neutral pH and is assumed to be that of **13j**.[16] The interpretation of the pH dependence is supported by two lines of evidence. The 9-methyl analogue of **1h**, **1l**, decomposes with almost identical rate constants as **1h** to generate an intermediate trapped by N_3^- in a pH-independent fashion in the pH range 3–8 with a selectivity that is comparable to that observed for **1h** under acidic conditions.[13] Since **4l** cannot form a conjugate base in the same way as **4h**, the lack of pH dependence in the N_3^- trapping of the 9-methyl analogue is consistent with the interpretation given above. McClelland and Licence have directly observed the 2-carbazolylnitrenium ion **4m** generated by laser flash photolysis of the corresponding azide.[26] They reported kinetic and spectrophotometric evidence for its deprotonation to its conjugate base **13m**. Table 2 contains the rate constant ratios for **4m** and **13m** determined from direct measurements of the individual rate constants. The pK_a of 5.8 observed for **4m** is consistent with the estimate of 3.0 for **4h** obtained from the pH-dependence of N_3^- trapping.[13,26] They also showed that the 9-methyl analogue of **4m** did not deprotonate under acidic to mildly basic (pH 10) conditions, although both cations could be protonated to their dicationic conjugate acids, both of which have pK_as of about 1.8.[26] The magnitude of k_{az} for **4m** of $2.7 \times 10^9\ M^{-1}\ s^{-1}$ is within a factor of two of the diffusion-controlled limit for k_{az} of ca $5 \times 10^9\ M^{-1}\ s^{-1}$ determined from observations of a wide variety of carbocyclic nitrenium ions.[27–30]



Most of the heterocyclic ions have azide/solvent selectivities that are less than or of similar magnitude to those of the familiar 4-biphenylyl- and 2-fluorenylnitrenium ions **16a–d** (Table 2). Only the *N*-acetyl-Glu-P-2 and *N*-acetyl-Glu-P-1 ions **4d** and **4e** are significantly more selective for N_3^-.[14] Since the directly measured k_{az} is $(4–5) \times 10^9\ M^{-1}\ s^{-1}$ for **16a–d**, it is likely that k_{az} for the heterocyclic ions, except **4d** and **4e**, are at the diffusion-controlled limit.[27,29] Carbocyclic ions with azide/solvent selectivities similar to **4d** and **4e** have k_{az} within a factor of two of the diffusion controlled limit, so it is likely that $k_{az} \geq 2 \times 10^9\ M^{-1}\ s^{-1}$ even for these ions.[30]



The azide/solvent selectivities for the cationic species vary over a range of almost six orders of magnitude. Figure 3 shows that there is no apparent correlation of the logarithm of these selectivities with log k_B. If k_{az} for these ions is at or near the diffusion limit, this plot indicates that there is no correlation of the aqueous solution lifetimes of the ions, $1/k_s$, with the rate constant for their formation from precursors with nearly identical acetate and pivalate leaving groups (k_B). A similar phenomenon was observed previously for carbocyclic nitrenium ions.[19] The underlying reason for this appears to be that the energies of the transition states for generation of the nitrenium ions by N—O bond cleavage and for their reaction with solvent by attack of H_2O on the ring respond very differently to substituent effects due to their very different structures.[19,31]

The ions **4b**, **d–h** and **l** and the conjugate base **13h** have been shown to react efficiently with d-G predominately to form C-8 adducts such as **17f**, **g**.[12–14] The minor N-2

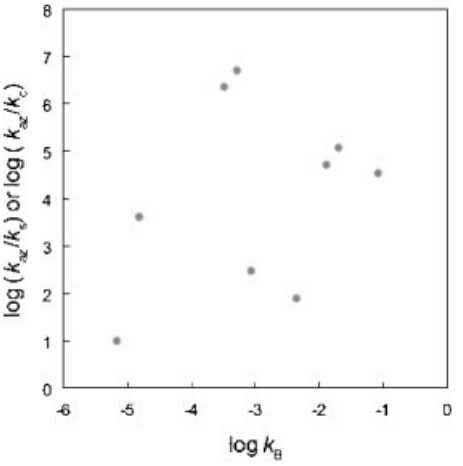
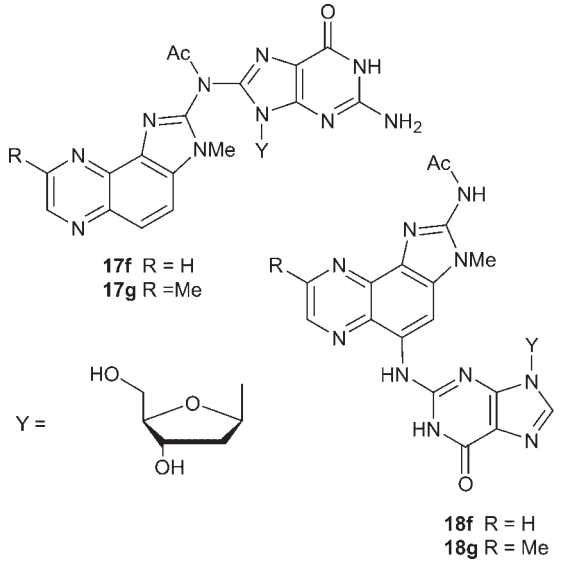


Figure 3. Log(azide/solvent selectvity) for **4** vs log k_B for **1**.

adducts **18f**, **g** (ca 20% of the d-G adduct yield) are also formed in two cases.[14] The same adducts have been observed from *in vivo* or *in vitro* experiments involving the corresponding HCAs or their metabolites.[2,32,33] In these experiments C-8 adducts also predominate. Only **IQ** and **MeIQx** derivatives have been unequivocally shown to generate minor N-2 adducts.[32] The available $\log(k_{d\text{-}G}/k_s)$ or $\log(k_{d\text{-}G}/k_c)$ selectivities are listed in Table 2. Based on measurements of carbocyclic ions, the diffusion-controlled limit for $k_{d\text{-}G}$ is ca $(1\text{–}2) \times 10^9\ M^{-1}\ s^{-1}$.[9,28] The ions **4b**, **h** and **l** appear to have $k_{d\text{-}G}$ in that range based on comparisons of azide/solvent and d-G/solvent selectivities in Table 2. The ions **4d–g** have significantly smaller $k_{d\text{-}G}$, apparently in the range $(4\text{–}9) \times 10^7\ M^{-1}\ s^{-1}$ based on the same comparisons.[14] Some carbocyclic ions also have $k_{d\text{-}G}$ in that range.[8,28,34] The reasons for this are not clear because the ions that have $k_{d\text{-}G}$ significantly below the diffusion-controlled limit do not all have very high azide/solvent selectivities that would suggest the onset of activation-controlled reactions for weaker nucleophiles such as d-G. It has been suggested that those ions with a dominant resonance structure in which all heavy atoms have an octet of electrons and the formal positive charge is localized on a heteroatom other than the nitrenium N may have reduced ability to form C-8 adducts because of reduced electrophilic reactivity on the nitrenium N.[34] All of the carbocyclic and heterocyclic nitrenium ions with reduced $k_{d\text{-}G}$ do share this structural feature, but so do at least two ions (**4h**, **l**) that appear to have diffusion-limited $k_{d\text{-}G}$.[13]



BIOLOGICAL IMPLICATIONS

The kinetic data show that the ester metabolites of the HCAs are reactive at physiological pH. At this pH the acetic acid esters are predominately deprotonated and they decompose via heterolytic N—O bond cleavage to generate nitrenium ion species. The reactivity of the ester is very dependent on structure with some of the acetic acid esters of the hydroxylamines having aqueous solution lifetimes measured in the millisecond range and others with lifetimes measured in days. Sulfuric acid esters were not examined in these studies, but based on comparisons available for carbocyclic cases, the sulfate leaving group will increase N—O bond cleavage rates by $(2\text{–}4) \times 10^2$-fold.[19,35] This would reduce the lifetimes of even the least reactive sulfuric acid esters into the ca 5 min range under physiological conditions. Nonetheless, it is clear that there are considerable differences among these carcinogenic esters in transportability from the site of their generation because of these differences in their hydrolytic stability. Because the acetyltransferases (NAT) and sulfotransferases (SULT) involved in the last step of activation are widely distributed in various organs and tissues in rodents, it is unlikely that any correlation of ester hydrolytic reactivity and organ specificity can be found in these animals.[6] The distribution of the human isozyme NAT2 that is apparently the major isozyme responsible for acetylation of heterocyclic hydroxylamines in humans is more limited than found for rodent NAT1 or NAT2, but can still be detected in liver, intestinal epithelium and, to a lesser extent, in pancreas, lung and esophagus.[6] Any such correlations are also likely to be confounded by the recent findings that some heterocyclic hydroxylamines are preferentially activated by human NAT2 (**IQ**) while others are preferentially activated by human SULT1A1 (**PhIP**) or SULT1A2 (**Phe-P-1**) that are expressed in a wide variety of tissues.[36]

Our data show that all the heterocyclic nitrenium ions examined react with d-G in aqueous solution to generate C-8 or N-2 adducts identical in structure with those found in DNA.[2,32,33] The relative proportion of these two adducts found in DNA treated with the carcinogens is similar to that found for monomeric d-G.[14,32] For the ester metabolites of the carbolines such as **AαC** and **Trp-P-2**, the nitrenium ion initially generated by N—O bond cleavage will be deprotonated to form its neutral conjugate base **13** under physiological conditions.[13,15] These also react with d-G to form the same adducts as the corresponding nitrenium ion, but less efficiently.[13] There can be little doubt that the nitrenium ions and/or their conjugate bases are responsible for the DNA lesions caused by the carcinogenic metabolites of HCAs. At this time it is not known how or whether the structure of DNA affects nitrenium ion selectivity for reaction with individual d-G residues within the DNA polymer. This will be examined in the future.

Recently, we have shown that log[(histidine revertants)/(nanomoles of amine)], log*m*, correlates ($r_{adj}^2 = 0.5491$, slope $= 0.82 \pm 0.18$ for TA 98, $r_{adj}^2 = 0.6338$, slope $= 0.59 \pm 0.12$ for TA 100) with $\log(k_{az}/k_s)$ of the electrophilic species present at neutral pH (the nitrenium ion or its conjugate base) for a series of mutagenic

amines, including five HCAs, in *Salmonella typhimurium* TA 98 (18 amines) and TA 100 (15 amines).[37] Log*m* for monocyclic amines was noticeably smaller (ca 2 log units) than that for bicyclic or tricyclic amines that have nitrenium ions of similar selectivity. A multiple variable linear regression model that included $\log(k_{az}/k_s)$, a ring index variable that distinguished monocyclic amines from other amines, and *C*log*P* (the calculated logarithm of the octanol–water partition coefficient of the amine)[38] as independent variables predicted the observed log*m* with good accuracy ($r_{adj}^2 = 0.8913$, RMSE = 0.83 for TA 98, $r_{adj}^2 = 0.9011$, RMSE = 0.55 for TA 100). These results suggest that nitrenium ion selectivity may be one of several critical variables that determine the mutagenic potential of carbocyclic and heterocyclic aromatic amines. −LogTD50 in mice [the negative logarithm of the dose in mmol (kg body mass)$^{-1}$ day^{-1} required to halve the probability of the animal remaining tumorless to the end of its standard life span] for a series of 12 amines, including four HCAs, correlates with $\log(k_{az}/k_s)$ with $r_{adj}^2 = 0.5357$ and slope $= 0.31 \pm 0.08$.[37] A two-parameter regression model including $\log(k_{az}/k_s)$ and *C*log*P* as independent variables adequately predicted the carcinogenicity data ($r_{adj}^2 = 0.7606$, RMSE = 0.53), although so did several two parameter models that included *C*log*P* and other variables such as the number of rings or the number of π-electrons.[37]

The role of nitrenium ion selectivity in determining the carcinogenicity potential of the corresponding amines is difficult to assess from these correlations. This is not unexpected since mammalian carcinogenesis is a much more complicated process than bacterial mutagenesis and correlations of carcinogenicity data generally provide poorer fits than do correlations of mutagenicity data with the same independent variables.[39] Nevertheless, the demonstration that the heterocyclic nitrenium ions or their conjugate bases react with d-G to generate C-8 or N-2 adducts identical with those obtained from *in vitro* and *in vivo* experiments with DNA makes it clear that nitrenium species are involved in carcinogenesis, although factors other than nitrenium selectivity may control the carcinogenicity potential of a particular amine.[2,12–14,32,33,38]

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