Purpose: To gain a fundamental understanding of the fragmentation mechanisms of PAH-purine adducts produced by collisional activation.

Methods:

- High and low-energy CAD provide the patterns of fragmentation. Free purine fragmentations characterized by isotopic labeling.
- Theoretical calculations: PM3 for all minima, transition states, and reaction paths; DFT for starting species and products.

Results:

- All fragmentations are highly endothermic. At internal energies below fragmentation, protons become mobile about the adducts.
- Sites of proton attachment define entry points into cascades of rearrangements leading to fragmentation.
The first critical event in the carcinogenesis of polycyclic aromatic hydrocarbons (PAH) is the metabolic activation of these compounds [1-4]. The activated products subsequently react with the bases of DNA, which in turn may cause misreading upon replication [5,6]. Such adducts have been isolated from in vitro and in vivo studies of DNA damage [7]. Our interests involve adducts formed by reaction of DNA bases with PAH's activated by one-electron oxidation [1,2].

The patterns of the fragmentation of these PAH-base adducts have been characterized by tandem mass spectrometry [7-10]. But, studies of the fundamentals that underpin these and related fragmentation reactions are few, and only the free bases have been studied thoroughly [11,12].

The purpose of this study is gain an understanding, via theoretical modeling, of the fragmentation pathways and corresponding ionic structures manifest in the fragment-ion spectra of the PAH-base adducts.
References


Acknowledgments

This research project was supported by the NIH-supported Mass Spectrometry Research Resource at Washington University (Grant No. P41RR00954).

This poster will be available on the web a couple weeks after ASMS.  
Website: http://www.chemistry.wustl.edu/~msf
Methods

One-electron oxidation of the polycyclic hydrocarbons (PAH's), anthracene, benzo[a]pyrene and dibenzo[a,l]pyrene, produces radical cations with the charge localized at a unique site for each PAH, C-9, C-6 and C10, respectively, which become sites for efficient nucleophilic attack by the bases of DNA (Figure 1). The most abundant adducts formed include the C8 and N7 isomers of the dibenzo[a,l]pyrene-gaunine and benzo[a]pyrene-gaunine adducts (DBP- and BP-Gau) and the N1, N3, N7 and N6 isomers of DBP-Ade and the N7 and N6 isomers of BP-Ade. Fragmentations of the [M + H]^+ precursors of these adducts have been studied by CAD (collisionally-activated decomposition) in our laboratory both at Washington University and the University of Nebraska [8-10]. In addition, CAD coupled with isotopic labeling has been used to characterize the fragmentation of protonated guanine and adenine [11,12].
Characteristic Fragmentations: The CAD spectra of \([M + H]^+\) of the various positional isomers are characterized by some features derived from the PAH moiety of the adducts:

- Fragmentations that involve the linkage between the base and the PAH or are in proximity on the imidazole ring are characterized by a pair of ions separated by the mass of \(H_2\). For the DBP-10- adducts, the fragments having the loss of \(H_2\) are more abundant, which reflects the ease of loss of \(H_2\) and the concomitant formation of a C-C bond between carbons 1 and 14, thus relieving significant steric strain that otherwise would force the ring system to be non-planar.

- The \([M + H]^+\) ions of the PAH-purine adducts, although they are even-electron, closed-shell ions, give rise to abundant fragment ions that are odd-electron, open-shell species, unlike the \([M + H]^+\) ions of the protonated purine bases. Two factors are necessary for such partitioning of charge: the final step in the fragmentation is likely a simple bond cleavage; and the PAH moiety possesses a lower IP (ionization potential) than either Gua or Ade, which necessarily extends to fragments incorporating the PAH core and the corresponding neutral.

The CAD spectra of the \([M + H]^+\) precursors of the various positional
isomers, although very similar, do indicate that there are some fragmentations characteristic of certain precursors. These differences serve to distinguish between certain of the isomers.

- Collision activation (CA) of the [M + H]$^+$ of the DBP-10-C8-Gua and BP-6-C8-Gua adducts produces fragments of large abundance (base) at $m/z$ 327 (ArCN$^+$•) and small abundance (~10%) at $m/z$ 302 (ArH$^+$•). But CA of the [M + H]$^+$ of the corresponding DBP-10-N7-Gua and BP-6-N7-Gua adducts instead produces an $m/z$ 302 of large abundance (base) and an $m/z$ 327 (ArNC$^+$• in this case) of much lesser abundance (Table 1).

- Among the DBP-10-Nx-Ade isomers, CA (collisional activation) of the [M + H]$^+$ of the DBP-10-N6-Ade adduct leads to the production of a small but significant abundance of the ArNH2$^+$• product, unlike the other isomers. In addition, the [M + H]$^+$ of the DBP-10-N7-Ade isomer, upon CA, produces an enhanced abundance of the [M + H - NH$_3$]$^+$ ion (~2x) relative to the [M + H]$^+$ of the other positional isomers (Table 2).

We have applied theoretical modeling to these characteristic fragmentations in search of reasonable mechanisms for their generation. In addition, we have consulted the fragmentations of protonated Gua and Ade as characterized by $^{13}$C and $^{15}$N labeling [11,12] (Table 3).
Theoretical Calculations: For structural and mechanistic elucidation, we have performed theoretical calculations on the adducts and their subsequent rearrangements, decomposition products and transition states, to characterize the associated potential energy surfaces.

To facilitate the modeling, most calculations have been performed using anthracene as the PAH rather than BP or DBP. This surrogate PAH maintains critical characteristics of BP or DBP, IP of PAH < IP of Ade, Gau and similar attachment site geometries and reactivities (Figure 1 and Table 5), while presenting a less formidable target for the calculations.

- Theoretical modeling by the PM3 [13] semi-empirical algorithm was used for general structure and transition-state determination and associated energetics. This algorithm was chosen because the size of most of the ions is too large to be realistically accommodated by ab-initio calculations.

The semi-empirical PM3 algorithm was part of the Spartan v.5.01 package (Wavefunction, Inc.), which was running on a Silicon Graphics Indigo II workstation.

- To characterize initial adducts and products more fully, density functional theory (DFT) has been selected instead of formal ab initio methods for the
computational effort. DFT requires less computational overhead, incorporates dynamic correlation, and has less spin contamination and associated problems [14]. DFT usually performs adequately giving proper geometries, energies, and frequencies, but may fail for certain small highly-symmetrical systems [15,16].

Geometries were optimized by using B3LYP/6-31G(d,p) and vibrational frequency analyses were done at this level to confirm the minima. From the vibrational frequency analyses, we calculated thermal-energy corrections from the scaled zero-point energies and fundamental vibrational frequencies [17] to attain standard conditions (T = 298.15 K, p = 1.0 atm).

The DFT calculations were performed by using the Gaussian 98 suite of programs (Gaussian, Inc.) which were running on a Silicon Graphics Power-challenge workstation [18].

The DFT calculations applied to the initial adducts, final products, and key intermediates is a work in progress.

The structure space for the [M + H]⁺ each PAH-purine isomer was scouted extensively for potential candidates for fragmentation pathways on the potential energy surface. All potential candidates were verified as true local minima and screened on basis of connections to other ions along a
fragmentation pathway characterized by reasonable mechanisms and true transition states.

In addition, the potential energy surfaces for protonated Ade and Gua have been similarly investigated for comparison with regard to losses in common with the \([M + H]^+\) ions of the PAH adducts (data not shown).

The structure of all ions were characterized as true local minima by frequency analysis (no imaginary frequencies). Likewise, all transition states were characterized by having a single imaginary frequency. Where rotational isomers exist, the presented structure represents the most stable form.

**Note:** The calculations of theoretical modeling yield information about the potential-energy surface, but fragmentation is dependent upon kinetic processes.
Results and Discussion

**Theoretical modeling results:** The potential energy surfaces for the [M + H]⁺ ions of the C8 and N7 isomers of Anth-Gua (anthracyl-Gua) the N7 and N6 isomers of Anth-Ade have been determined by the PM3 algorithm. In addition, similar calculations were performed on the free Gua and Ade bases as a guide to NH₃ loss from the adducts. Significant findings include:

- All fragmentations are highly endothermic, requiring 60-100 kcal/mol of total internal energy to promote from the most stable form of the precursor to the most favorable products.
- The most favorable site for protonation of the C8-Gua isomer is N7 as it is for the free base (Figure 1). But for the N7-Gua isomer, the most favorable site is N9, unlike N1 for the free base.
- At internal energies >50 kcal/mol but below that required for fragmentation, protons in the precursor ions become mobile, migrating about the ring systems.
(Schemes 1 and 2). Some configurations accessed by the mobile protons define entry points to series of rearrangements that lead to products.

- The decompositions, which generate ArCN⁺⁺ or ArNC⁺⁺ from the [M + H]+ of the C8 and N7 Anth-Gua isomers, take place by two step mechanisms instead of concerted cycloreversion reactions, in accord with previous reports for radical systems [20]. Each step involves the simple cleavage of a bond (Scheme 3).

- The proposed mechanisms (Scheme 3) for the production of ArCN⁺⁺ or ArNC⁺⁺ and ArH⁺⁺ from the [M + H]+ of the C8 and N7 Anth-Gua isomers can rationalize the relative abundance distributions of these products. The rate-limiting step in the formation of ArCN⁺⁺ from Anth-C8-Gua requires ~10 kcal/mol less energy than the rate-limiting step for the formation of ArH⁺⁺. But for the Anth-N7-Gua isomer, the rate-limiting steps to ArNC⁺⁺ and ArH⁺⁺ favor the latter by >20 kcal/mol even though that decomposition pathway is convoluted. The proposed mechanisms thus account for experimental results.

- The mechanism for loss of NH₃ from the [M + H]+ of the isomeric Anth-Ade adducts is proposed by analogy to that made to account for the results of isotopic studies involving the free base Ade [12]. Also by analogy, it is proposed that N1 and N⁶ become equivalent in giving rise to NH₃ (Scheme 4).
• The enhanced formation of [M + H - NH₃]⁺⁺ from the N7 isomer of the Anth-Ade adducts can be rationalized by a proposed mechanism that involves cyclization with the C1 site on the anthracene followed by loss of NH₃ (Scheme 4). This pathway is in addition to the common pathway for loss of NH₃ proposed for the Anth-Ade adducts.

• The formation of ArNH₂⁺⁺ only from the [M + H]⁺ of the N⁶ Anth-Ade isomer is rationalized by realizing that the ArNH₂⁺⁺ product is just an aryl substituted NH₃ which has been stripped of an electron because it possesses a lower IP than the other product of the decomposition (Scheme 4).

• The final steps in all proposed mechanisms involve a simple cleavage of a single bond, as postulated by charge partitioning between the products.
Conclusions

We have applied theoretical PM3 and DFT calculations to characterize the structures of precursor and fragment ions from PAH-purine adducts formed by reaction of PAH's activated by one-electron oxidation with the purine bases of DNA. We have proposed mechanisms for the generation of distinctive fragment ions using anthracene as a surrogate PAH.

- The fragmentations are highly endothermic by 60-100 kcal/mol, an energy greater than that required to promote proton mobility about the ring systems. Various proton attachment sites define entry points leading to fragmentation.

- The generation of ArCN$^{+*}$ or ArNC$^{+*}$ from the C8- and N7-Gua adducts proceeds by two step processes rather than concerted cycloreversion reactions. The relative abundance of these products with regard to ArH$^{+*}$ can be accounted for by the proposed mechanisms for their formation.

- Loss of NH$_3$ from the Nx-Ade adduct isomers is proposed to follow a mechanism analogous to that for the free purine bases. The ArNH$_2^{+*}$, formed only from the N$^6$ isomer, is an aryl-substituted NH$_3$ which carries the charge.
Protonation Sites of Guanine and Adenine

Guanine

\[
\text{C}_5\text{H}_5\text{N}_5\text{O} \ (151 \text{ d})
\]

\[
\begin{align*}
\Delta \Delta H \ (\text{kcal/mol}) &= -\Delta PA \\
\text{N7} &= 0.00 \\
\text{O6} &= 6.50 \\
\text{N3} &= 17.69
\end{align*}
\]

Adenine

\[
\text{C}_5\text{H}_5\text{N}_5 \ (135 \text{ d})
\]

\[
\begin{align*}
\Delta \Delta H \ (\text{kcal/mol}) &= -\Delta PA \\
\text{N1} &= 0.00 \\
\text{N3} &= 2.22 \\
\text{N7} &= 7.58
\end{align*}
\]

Values were calculated by density functional theory (DFT) and are accurate with experimental data to within 3 kcal/mol

Table 1

Fragmentation by CA of PAH-Gua Adducts

[M + H]⁺ precursors (m/z 452)

<table>
<thead>
<tr>
<th>Fragment Ion</th>
<th>m/z</th>
<th>Rel. Abundance</th>
<th>Rel. Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>[(M + H) - NH₃]⁺</td>
<td>435</td>
<td>medium</td>
<td>medium</td>
</tr>
<tr>
<td>Ar—CN⁺⁺</td>
<td>327</td>
<td>large</td>
<td>&gt;</td>
</tr>
<tr>
<td>C₂₄H₁₂N⁺⁺</td>
<td>314</td>
<td>small</td>
<td>~</td>
</tr>
<tr>
<td>Ar—H⁺⁺</td>
<td>302</td>
<td>small</td>
<td>&lt;&lt;</td>
</tr>
<tr>
<td>[(Ar—H) - H₂]⁺⁺</td>
<td>300</td>
<td>medium</td>
<td>~</td>
</tr>
<tr>
<td>Ar⁺⁺</td>
<td>301</td>
<td>medium</td>
<td>medium</td>
</tr>
</tbody>
</table>

From reference 9.
Analogous results for the benzo[a]pyrenyl-6- (BP-6-) adducts are reported in reference 8.

Dibenz[a,l]pyrenyl-10- (DBP-10-)

Ar =

![](image)
Table 2

Fragmentation by CA of PAH-Ade Adducts

\[ [M + H]^+ \text{ precursors (m/z 436)} \]

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>([(M + H) - NH_3]^+)</td>
<td>419</td>
<td>medium</td>
<td>small</td>
<td>large</td>
<td>medium</td>
</tr>
<tr>
<td>(\text{Ar—NC}^+)</td>
<td>327</td>
<td>small</td>
<td>small</td>
<td>small</td>
<td></td>
</tr>
<tr>
<td>(\text{Ar—NH}_2^+)</td>
<td>317</td>
<td></td>
<td></td>
<td></td>
<td>small</td>
</tr>
<tr>
<td>(\text{C}<em>{24}\text{H}</em>{12}\text{N}^+)</td>
<td>314</td>
<td>small</td>
<td>small</td>
<td>small</td>
<td>small</td>
</tr>
<tr>
<td>(\text{Ar—H}^+)</td>
<td>302</td>
<td>medium</td>
<td>medium</td>
<td>medium</td>
<td>large</td>
</tr>
<tr>
<td>([(\text{Ar—H}) - H_2]^+)</td>
<td>300</td>
<td>large</td>
<td>large</td>
<td>large</td>
<td>large</td>
</tr>
<tr>
<td>(\text{Ar}^+)</td>
<td>301</td>
<td>medium</td>
<td>medium</td>
<td>medium</td>
<td>medium</td>
</tr>
</tbody>
</table>

From reference 10.
The benzo[a]pyrenyl-6- (BP-6-) N7Ade adduct is reported in reference 8.
Table 3

Fragmentation by CA of Guanine and Adenine

\([M + H]^+\) precursors

<table>
<thead>
<tr>
<th>Guanine(^1)</th>
<th>Adenine(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{C}_5\text{H}_5\text{N}_5\text{O}\ (151\ d))</td>
<td>(\text{C}_5\text{H}_5\text{N}_5\ (135\ d))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decomposition</th>
<th>Source</th>
<th>Decomposition</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>- (\text{NH}_3)</td>
<td>N1 and N(^2) equally</td>
<td>- (\text{NH}_3)</td>
<td>N1 and N(^2) equally</td>
</tr>
<tr>
<td>- (\text{NH} = \text{C} = \text{NH}) or (\text{NH}_2\text{CN})</td>
<td>N1-C2-N(^2)</td>
<td>- (\text{NH}_2\text{CN})</td>
<td>N1-C6-N(^6)</td>
</tr>
<tr>
<td>(\text{NH} = \text{C} = \text{NH}_2^+)</td>
<td>N1-C2-N(^2)</td>
<td>- (\text{HCN})</td>
<td>N1-C2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\text{NH}_4^+)</td>
<td>N1 !</td>
</tr>
</tbody>
</table>

Decompositions were determined by \(^{13}\text{C}\) and \(^{15}\text{N}\) labeling and comparison with mono-methylated analogs in conjunction with MS/MS.

The indicated sources for the decomposition are almost exclusive in all cases.

Data from references 11 and 12
### Table 4: Known Thermochemistry

<table>
<thead>
<tr>
<th>Compound</th>
<th>IP (eV)</th>
<th>$\Delta H_f$[ion] (kcal/mol)</th>
<th>$\Delta H_f$[neutral] (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$N-NH$_2$</td>
<td>7.85</td>
<td>181</td>
<td>1</td>
</tr>
<tr>
<td>N$_2$H$_5$</td>
<td>7.80</td>
<td>229</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>9.25</td>
<td>233</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>8.14</td>
<td>224</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>7.41</td>
<td>240</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>7.89</td>
<td>230</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>7.45</td>
<td>227</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>7.12</td>
<td>233</td>
<td>69</td>
</tr>
<tr>
<td>CN</td>
<td>7.80</td>
<td>267</td>
<td>87</td>
</tr>
</tbody>
</table>

From reference 19
Scheme 1: Proton migration

[M + H]$^+$ of C8-Guanine adduct

Heats of Formation (kcal/mol) vs Protonation Site

Heats of formation are given for indicated sites of proton attachment (minima) or bridging sites of transition states.

All heats of formation are presented in kcal/mol. The stationary states in indicated in **bold**, and the transition states are given by *italic TS*.

All calculations were performed by PM3 algorithm.
Heats of formation are given for indicated sites of proton attachment (minima) or bridging sites of transition states.

All heats of formation are presented in kcal/mol. The stationary states in indicated in **bold**, and the transition states are given by *italic TS*.

All calculations were performed by PM3 algorithm.
Scheme 3

Proposed Mechanisms of Fragmentation
C8-Guanine adduct

Production of ArCN$^{++}$

Production of ArH$^{+}$

Heats of Formation given in kcal/mol
TS = transition state
Proposed Mechanisms of Fragmentation

N7-Guanine adduct

Production of ArNC$^{+\bullet}$

Production of ArH$^{+\bullet}$

Heats of Formation
given in kcal/mol

TS = transition state
Proposed Mechanisms of Fragmentation

Loss of NH$_3$ from N7 isomer only, geometrically specific

Loss of NH$_3$, analogous for other isomers

N1, N$_6$ scrambling, analogous for other isomers

Loss of NH$_3$ from N7 isomer only, geometrically specific

ArNH$_2$$^+$ generation from the N$_6$ isomer