The action of xanthine oxidase on some 2-substituted adenines

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The purine antagonist 2-fluoroadenine has been found to be oxidized by xanthine oxidase much more rapidly than is adenine (0-aminopurine). Other substituted adenines were compared to investigate the effects of various 2-substituents on the susceptibility to attack at the 8-position, which, in this series, is the single position available for oxidation by xanthine oxidase.

The concentrations of solutions of spectrally pure 2-fluoroadenine (1), 2-chloroadenine (2), 2-iodoadenine (3), 2-methyladenine (4), and adenine (5) were determined by their absorbance values. The activity of the xanthine oxidase preparation was determined by the rate of increase of the optical density at 290 nm of 0.049 mM hypoxanthine in 0.033 M sodium phosphate buffer of pH 7.6. Aqueous solutions of the 2-substituted adenines were added to an excess of the enzyme in buffer. Each rate was determined at at least two enzyme concentrations, and some characteristics of 2-substituents are tabulated for the substituents in the 8-position which might be of potential significance to the rates of oxidation at the 8-position.

### Table I

<table>
<thead>
<tr>
<th>2-Substituent</th>
<th>Oxidation rate</th>
<th>van der Waals radii</th>
<th>Electronegativity</th>
<th>Hammett (10)</th>
<th>Taft (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0.171</td>
<td>1.35 A</td>
<td>4.0</td>
<td>0.06</td>
<td>-0.44</td>
</tr>
<tr>
<td>Cl</td>
<td>0.142</td>
<td>1.80</td>
<td>3.0</td>
<td>0.23</td>
<td>-0.24</td>
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<tr>
<td>OH</td>
<td>0.066</td>
<td>1.40</td>
<td></td>
<td>-0.36</td>
<td>-0.61</td>
</tr>
<tr>
<td>I</td>
<td>0.050*</td>
<td>2.15</td>
<td>2.5</td>
<td>0.28</td>
<td>-0.10</td>
</tr>
<tr>
<td>NH₂</td>
<td>0.0023*</td>
<td></td>
<td></td>
<td>-0.66</td>
<td>-0.76</td>
</tr>
<tr>
<td>OCH₃</td>
<td>0.00084</td>
<td></td>
<td></td>
<td>-0.27</td>
<td>-0.50</td>
</tr>
<tr>
<td>CH₃</td>
<td>0.00084</td>
<td></td>
<td></td>
<td>-0.17</td>
<td>-0.13</td>
</tr>
<tr>
<td>H (adenine)</td>
<td>0.066</td>
<td></td>
<td></td>
<td>0.009</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Calculated from Wyngaarden (6).

From their calculations of nucleophilic localization energies, Peraulet, Valdemoro, and Pullman (8) suggest that the action of xanthine oxidase involves an initial attack by a nucleophilic agent, which implies that an electronegative site in another part of the molecule might favor such an attack.

The values in Table I include rates for adenine, 2,6-diaminopurine, and isoguanine (2-hydroxyadenine) calculated from the data of Wyngaarden (6). Adenine is included, and the values demonstrate the close agreement between the rates derived in two laboratories. Adenine cannot be compared directly since it is oxidized at both the 2- and 8-positions, and more rapidly at the 8-position (7). From such data it may begin to be possible to make deductions regarding the transmission of effects of substituents through this heterocyclic ring system. To this end there are also tabulated for the substituents in the 2-position some characteristics which might be of potential significance to the rate of oxidation at the 8-position.

From their calculations of nucleophilic localization energies, Peraulet, Valdemoro, and Pullman (8) suggest that the action of xanthine oxidase involves an initial attack by a nucleophilic agent, which implies that an electronegative site in another part of the molecule might favor such an attack.
Steric effects are another consideration but are obviously not a primary factor since the van der Waals radii (9) of the 2-substituents correlate poorly with the observed rates of oxidation. The reaction rate does decrease as the size of the halogen substituents increases, but a decrease in the electronegativity of the halogens (9) also correlates with the decreasing reaction rate.

The Hammett sigma constant (σ), often employed in attempting to correlate reaction rate differences among substituted aromatic compounds, reflects inductive and resonance contributions of the substituents. Such studies of the influence of substituents in aromatic compounds are of doubtful applicability to heterocyclic compounds, but certain comparisons are of interest. The sigma constants for substituents in the para- or meta-positions of the benzene ring (10) do not correlate with the present data. Taft (11) has, however, distinguished between the contributions of resonance and inductive effects in the Hammett σ_{para} and his values for the inductive effects (σ_I) and resonance effects (σ_R) are tabulated. Except for the OH and NH_2 substituents, which exist in tautomeric forms in the purines and are not at all comparable to phenolic and aromatic amine groups, the Taft σ_I values do show an interesting rough correlation whereas the σ_R values do not.

The concept (8) that the action of xanthine oxidase involves a nucleophilic attack is compatible with the interpretation that the influence of the group at the 2-position on the reactivity at the 8-position is the result of the introduction of an electronegative site into the molecule.

**SUMMARY**

The oxidation of several 2-substituted adenines by xanthine oxidase is compared and possible correlations with properties of the 2-substituent are considered.

**REFERENCES**

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