A Proposed Model of the Interaction of 4-Nitroquinoline 1-Oxide with DNA

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SUMMARY

Extended Hückel molecular orbital computations were performed on both carcinogenic and noncarcinogenic 4-nitroquinoline 1-oxides (4-NQO's) and related compounds. Distinct correlations between carcinogenicity and several molecular orbital characteristics were found. All correlations between molecular orbital properties and carcinogenicity were modified by one or more of the following structural factors: (a) the absence of a 4-nitro group, (b) the absence of a 1-oxide group, or (c) the presence of bulky substituents in position 2 or 3 of the quinoline ring. Any of the three factors resulted in either noncarcinogenicity or reduced carcinogenicity. Conformational studies indicated that the 4-nitro group was approximately 60° out of the plane of the quinoline ring and that the addition of an electron to 4-NQO was energetically probable.

4-Hydroxyaminoquinoline 1-oxide, a metabolic reduction product of 4-NQO, has been considered a possible proximal carcinogen. The theoretical ease of reduction of the nitroquinolines to the corresponding hydroxyaminoquinoline does not always parallel carcinogenicity.

A model of complex formation between deoxyguanosine in DNA and carcinogenic 4-NQO's and 4-hydroxyaminoquinoline 1-oxides was constructed based upon molecular orbital results and steric factors, which allowed the prediction of the qualitative carcinogenicity of 26 derivatives of 4-NQO. Carcinogenicity may result from complex formation of the 4-nitro derivatives and/or from the interaction of the 4-hydroxyaminoquinoline 1-oxides with DNA.

INTRODUCTION

4-NQO is typical of a series of compounds that have been assayed for carcinogenic activity (5, 6, 13, 23). These compounds vary in carcinogenicity from those more potent than 4-NQO to noncarcinogens (5, 20, 22, 39). Molecular orbital calculations on certain 4-NQO's have been reported (6, 9, 19). The reactivity of 4-NQO's with nucleophilic reagents (e.g., sulphydryl groups) (4) has been used to explain their carcinogenicity (6). Molecular orbital calculations have been used to study the charge-transfer properties of 4-NQO (9) and the possible interaction of the 4-NQO's with DNA (19).

Several investigators have indicated that a metabolic reduction product of 4-NQO, 4-HAQO, may be the proximal carcinogen (13, 37); however, less work has been done on the relative carcinogenicity of the substituted 4-HAQO's than on that of the 4-NQO's. In view of the larger body of data concerning the relative carcinogenicity of the 4-NQO's, molecular orbital studies were made with respect to the direct interaction of the 4-NQO's with DNA and their conversion to the corresponding 4-HAQO's.

The degree of in vitro interaction of the 4-NQO's with DNA is related to carcinogenicity (20), and of the 4-NQO's that interact the quinoline ring was oriented parallel to the base planes (20). This interaction required native DNA (20). This requirement for maximal interaction of 4-NQO with DNA is substantiated by Tm studies (28, 29) and spectral data (20, 25, 28). Spectrophotometric evidence indicated that an n→π* charge-transfer complex is involved in this interaction (26); Tm (29) and spectrophotometric studies (28) have demonstrated that the DNA-4-NQO interaction can be inhibited by urea (28) and was influenced by ionic strength (20, 28), which indicated that charged sites (28) may be involved.

UV flow dichroism studies (20) of the interaction of 4-NQO with purinic and pyrimidinic acids demonstrated that the primary site of interaction involved the purine residues (14, 20). Studies with deoxyribonucleosides have demonstrated a similar interaction of 4-NQO with both deoxyguanosine and deoxyadenosine in solution (24-26). 4-NPO, a noncarcinogen, did not interact (25). The interaction of 4-NQO with synthetic polydeoxygenucleotides has implicated deoxyguanosine as the primary site of interaction (28, 29).

MATERIALS AND METHODS

EHMO calculations (7) modified by the ω technique (36) were performed on 27 4-NQO's of known carcinogenicity and on dGMP with the use of a Univac 1108 or CDC 6600 computer. The input data consisted of Cartesian coordinates,
Paul, Montgomery, and Louis
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clementi coefficients (2), and ionization potentials (31) for each atom of the molecule. The coordinates were obtained from X-ray diffraction data on related compounds (38) due to the lack of coordinates on quinoline or quinoline 1-oxides. The charges were converged to 0.05 unit for the quinolines and 0.1 unit for deoxyguanosine. The coordinates for dGMP in DNA were taken from X-ray diffraction data of the β form of DNA (15).

Some pertinent information obtained from an EHMO calculation are energy levels, total energy, eigenvectors, Mulliken populations, reduced overlap populations, charge matrices, orbital charges, and atomic charges. The energy levels indicate the energies of the occupied and unoccupied energy levels of the molecule. The lowest unoccupied energy level is an index of ease of reduction in aromatic systems (32). The total energy is the summation of the discrete electronic energies of the molecule and is related to stability. The eigenvectors represent the coefficients of the wave functions for a given energy level and are an indication of the electron population of an orbital (3). The Mulliken population (3) is a measure of the π electron density between 2 orbitals in a π system. The total orbital overlap between 2 atoms is given by the reduced overlap population. The charge matrix indicates the electronic charge in an orbital by energy level, while the orbital charges are the electronic population of an orbital for all occupied energy levels. The atomic charges represent the gross charge of an atom. The physical interpretation of these values as well as the EHMO method has been discussed in detail (3, 32, 36).

For computational reasons, the aromatic ring of the NQO's was located in the x-y plane, and the 4-nitro group was located in the y-z plane, resulting in a perpendicular orientation between the 4-nitro group and the quinoline ring. Chart 1 illustrates the chemical nomenclature used in this paper.

Van der Waals models of the 4-NQO's were constructed to determine the steric effect of neighboring hydrogen atoms on the 4-nitro group. Additional molecular orbital calculations on 4-NQO were performed by rotating the 4-nitro group around the C4–N2 bond to determine the most stable configuration.

Van der Waals and Prentice-Hall framework molecular models of the 4-NQO's and DNA were constructed. The models were used to determine whether intercalation or the interaction of complementary external sites on both molecules is stereochemically possible. Van der Waals models represent the effective size of the atoms in a molecule and give an accurate representation of the geometry of the molecules. Framework models represent the location of atoms in a molecule, thus simplifying the visualization of the atomic centers. Steric barriers to intercalation of the 4-NQO's between base pairs in DNA are best examined with Van der Waals models. Both types of models were used to examine external interaction of the molecules. A possible structure for the interaction of deoxyguanosine and 4-NQO is shown in Chart 2. The distance between the oxygen of the 1-oxide group of deoxyguanosine was assumed to be 1.7 Å; this is a probable distance for a charge-transfer interaction (1). The stereochemistry of the complex was examined with the use of a computer-driven plotter.

RESULTS

Conformational studies minimized the total energy of 4-NQO with the 4-nitro group 60° out of the plane of the quinoline ring (−1375.23 e.v.). Perpendicular orientation was slightly less stable (−1375.15 e.v.); an orientation of 30° was more unfavorable (−1374.61 e.v.). This concurs with the steric influence of the 3 and 5 hydrogen atoms of the quinoline ring on the 4-nitro group.

The Mulliken population of the 4-nitro group, the reduced overlap population of the C4–N2 bond, the reduced overlap population of the 1-oxide group (N1–O1), and the energy of CANCER RESEARCH VOL. 31

Chart 1. Ring nomenclature of the 4-NQO's used in this paper.

Chart 2. The postulated complex between 4-NQO and deoxyguanosine. Two sites of interaction occur; a charge-transfer complex between the nitrogen of the nitro group in position 4 with the ring oxygen in deoxyguanosine and a hydrogen bond between the oxygen of the 1-oxide group and a hydrogen of the amino group of deoxyguanosine.
The carcinogenicity values are taken from the data of various workers using a variety of methods, strains of animals, and dosages. Therefore, quantitative ranking of the derivatives is impossible in most cases. Many of the compounds discussed in this paper are now under test in our laboratory with the use of a standard technique to obtain quantitative data as to their relative carcinogenicity.

An analysis of the 4-nitro group Mulliken population (Table 1) disregarding stereochemical properties indicated that, in general, the more potent the carcinogen is, the lower is the degree of \( \pi \) bonding. The \( \pi \) bonding value of the weak carcinogen 6-carboxy-4-nitroquinoline 1-oxide was assumed to be the maximal value compatible with carcinogenic activity. No carcinogens were found in the range of the \( \pi \) bonding of noncarcinogens.

Increased values of the reduced overlap population between the \( C_4 \) and \( N_2 \) atoms were associated with increased carcinogenicity (Table 1), as opposed to decreased \( \pi \) bonding values. The reciprocal relationship is a manifestation of the interaction between these associated regions.

In many instances, lower values of the reduced overlap population between the nitrogen and oxygen of the 1-oxide group were associated with increased carcinogenicity (Table 1), as opposed to decreased \( n \) bonding interaction between these associated regions. Disregarding these \( N_2-O \) group charge-transfer interaction by the bulky 3-methyl group; g, 6-nitro group cannot participate in charge-transfer interaction by the bulky 3-methyl group; h, 1-nitro group cannot participate in charge-transfer interaction; h, 1-nitro group cannot participate in charge-transfer interaction; h, 1-nitro group cannot participate in charge-transfer interaction.

<table>
<thead>
<tr>
<th>Carcinogenicity( ^a )</th>
<th>Reference</th>
<th>Compound</th>
<th>Mulliken Population</th>
<th>Reduced overlap population for ( C_4-N_2 )</th>
<th>Reduced overlap population of the 1-oxide group</th>
<th>Energy level (eV) of lowest unoccupied orbital</th>
<th>Energy level (eV) of highest occupied orbital</th>
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<td>+ (13)</td>
<td>5-Methyl-4-nitroquinoline 1-oxide</td>
<td>0.1715</td>
<td>0.6815</td>
<td>0.7035</td>
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<td>-11.19</td>
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<tr>
<td>- (6)</td>
<td>6-Nitroquinoline</td>
<td>0.1739</td>
<td>(( C_4-N_2 ))</td>
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<td>-11.66</td>
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<td>- (16)</td>
<td>2,4-Dinitroquinoline 1-oxide</td>
<td>0.1842</td>
<td>0.6782</td>
<td>0.7013</td>
<td>-11.45</td>
<td>-12.34</td>
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<td>-11.19</td>
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<td>0.6790</td>
<td>0.7043</td>
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<td>-12.31</td>
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<tr>
<td>+ (13)</td>
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<td>0.1830</td>
<td>0.6790</td>
<td>0.7031</td>
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<td>-12.31</td>
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<tr>
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<td>6-Nitroquinoline 1-oxide</td>
<td>0.1830</td>
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<td>0.6918</td>
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<td>-11.90</td>
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<td>0.1839</td>
<td>0.6784</td>
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<tr>
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<td>0.6988</td>
<td>0.6873</td>
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<td>- (12)</td>
<td>3-Fluro-4-nitroquinoline 1-oxide</td>
<td>0.1912</td>
<td>0.6631</td>
<td>0.7062</td>
<td>-11.59</td>
<td>-12.38</td>
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<td>- (13)</td>
<td>4,5-Dinitroquinoline 1-oxide</td>
<td>0.1913</td>
<td>0.6696</td>
<td>0.7058</td>
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<td>-12.43</td>
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<td>0.7093</td>
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<td>- (20)</td>
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<td>e</td>
<td>0.8983</td>
<td>0.6967</td>
<td>-12.03</td>
<td>-12.25</td>
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<td>e</td>
<td>0.8983</td>
<td>0.6967</td>
<td>-9.94</td>
<td>-12.31</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) The carcinogenicity values are taken from the data of various workers using a variety of methods, strains of animals, and dosages. Therefore, quantitative ranking of the derivatives is impossible in most cases. Many of the compounds discussed in this paper are now under test in our laboratory with the use of a standard technique to obtain quantitative data as to their relative carcinogenicity.

\( ^b \) Compounds that are exceptions to predicted carcinogenicity due to steric factors. In all instances, carcinogenicity is below predicted values, e.g., N-O hydrogen bond by bulky i-butyl group; d, carcinogenicity reduced to level of 4-NQO by partial interference of N-O hydrogen bond by 2-ethyl group; e, no N-O group; f, steric interference of 4-NQO group charge-transfer interaction by the bulky 3-methyl group; g, 6-nitro group cannot participate in charge-transfer interaction; h, 1-nitro group cannot participate in charge-transfer interaction.

\( ^c \) No 1-oxide group.

\( ^d \) Weak carcinogen.

\( ^e \) No nitro group present.

Table 1

Molecular Orbital Calculations on the 4-NQO's
small changes in the overlap population of the nitrogen-oxygen bond, and it is unlikely that chemical attack would occur at this site. The relationship between the reduced overlap population ($C_4-N_2$) and carcinogenicity appeared to result from the interaction of the $\pi$ systems (Mulliken population) of the 4-nitro group and the quinoline ring. The lowest unoccupied and highest occupied energy levels (an index of the ease of chemical reduction and oxidation, respectively) were considered less important than the Mulliken populations because their correlation with carcinogenicity was modified by structural features at certain positions and not at other sterically equivalent positions. If chemical reduction of the 4-nitro group is the primary mechanism in their carcinogenic effect, reduction should be equally hindered by substitution at position 3 or 5. The molecular orbital calculations did not demonstrate that substitution in position 3 should interfere with reduction of the 4-nitro group while substitution in position 5 should not. Thus, the Mulliken population of the 4-nitro group was considered to be the most important molecular orbital index.

The highest occupied energy level of dGMP was $-10.87$ e.v. The lowest unoccupied energy levels of the heterocyclic derivatives (Table 1) could allow a charge-transfer complex involving deoxyguanosine and any of the NQO's to occur. EHMO calculations on 4-NQO with the nitro group oriented $60^\circ$ out of the plane of the quinoline ring indicated that the addition of an electron resulted in a radical with relative stability. The respective energies were $-1375.23$ e.v. (4-NQO) and $-1375.71$ e.v. (4-NQO radical).

Based upon the experimental evidence and the molecular orbital calculations, a possible model (Chart 2) of complex formation was formulated. It is hypothesized that a 2-point attachment exists; 1 site involves a hydrogen bond between the oxygen of the 1-oxide group of 4-NQO and a hydrogen of the amino group of deoxyguanosine, and the 2nd site involves the transfer of a nonbonding electron ($n$) from the ring oxygen of the deoxyribose group to the singly occupied orbital of the nitrogen in the 4-nitro group, which is available due to the nonplanarity of the 4-nitro group. This attachment would result in the observed parallel orientation of 4-NQO with the DNA base pairs. The model requires the location of a 4-nitro group para to a 1-oxide group and the absence of bulky groups at positions 2 and/or 3 of the quinoline ring for interaction.

The ability of the $\pi$ system of the 4-nitro group to accept an electron should be a function of the electron density of the $\pi$ system (1); thus, $\pi$ systems with a low electron density (i.e., low Mulliken values) should more readily accept an electron than $\pi$ systems with higher electron densities. The strength of the complex in the absence of steric hindrance should be a function of the ability of the nitro-group $\pi$ system to accept an additional electron. The derivatives in Table 1 that could be prevented or partially inhibited from interacting with DNA by this mechanism are listed. All of these derivatives exhibit reduced carcinogenicity from predicted levels. Other than these exceptions, there is a qualitative correlation between $\pi$ bonding and carcinogenicity.

In view of similar structural properties, the 4-HAQO's are capable of forming a similar complex with deoxyguanosine with the 4-hydroxyamino group participating in a charge-transfer interaction.

**DISCUSSION**

**Structural, Physiochemical, and Quantum Chemical Properties of the 4-NQO's.** X-ray diffraction on certain quinolines indicates that the nitro group is rotated out of the plane of the ring in the absence of internal hydrogen bonding (33). In this study, the 4-nitro group was assumed to be perpendicular to the ring to facilitate the interpretation of molecular orbital calculations. Greater differences in the molecular orbital values for the 4-NQO's could be induced by the use of more probable $60^\circ$ conformation of the 4-nitro group instead of the $90^\circ$ conformation, due to increased interaction between the $\pi$ systems of the nitro group and the quinoline ring. However, use of the $90^\circ$ approximation did not alter the relative order of the relevant molecular orbital values and is a valid approximation.

The Mulliken population predicts the following strength of complex formation and presumably the following order of decreasing carcinogenicity: 6-chloro-4-nitroquinoline 1-oxide $\approx$ 4-NQO $>$ 6-carboxy-4-nitroquinoline 1-oxide $> 4,6$-dinitroquinoline 1-oxide $> 4$-NPO. This sequence has been verified experimentally (5, 6, 13, 20, 23, 34, 39).

4-NQO is a good electron acceptor (9), and guanosine is the best electron donor of the nucleotides (32). EPR studies have shown that 4-NQO accepts an electron from DNA (27).

The model postulates the transfer of an $n$ electron from deoxyribose, resulting in a $n \rightarrow \pi^*$ (26, 35) charge-transfer complex, which is supported by the structural restraints in the heterocyclic ring. The observed $n \rightarrow \pi^*$ charge transition is commonly found in a localized charge-transfer complex (1, 35).

**Stereochemical Features of the Interaction of the 4-NQO's with Deoxyguanosine.** The model requires that the 4-NQO's possess certain favorable quantum chemical properties that may be modified by structural features. Both a 1-oxide group and a 4-nitro group on the quinoline ring are essential for complex formation, which agrees with in vitro studies (20). It is generally considered that none of the 4-NQO's lacking a 1-oxide or 4-nitro group are carcinogenic (5, 21–23). Recently, 4-nitroquinoline has been reported to be carcinogenic (18). In view of the dosage and the method of synthesis, sufficient 4-NQO may have been present to induce tumor formation. In the 4-NQO's substituted at positions 2 and/or 3, carcinogenicity is reduced or abolished (13, 16), emphasizing the importance of both an unhindered 4-nitro and 1-oxide group for carcinogenicity. Derivatives with bulky substituents (12, 13, 16) in position 2 or 3 of the quinoline ring are generally noncarcinogenic (e.g., 2-t-butyl-4-nitroquinoline 1-oxide, 3-methyl-4-nitroquinoline 1-oxide). This may be a result of interference with hydrogen bond formation or charge-transfer interaction, respectively. Both of these derivatives have favorable molecular orbital values for carcinogenicity but are noncarcinogens (13). The unhindered
para relationship in the carcinogenic derivatives may indicate that the ultimate biological activity of these compounds is dependent upon either interaction at 2 sites or the activation of 1 group by the other. 2-Methyl-4-nitroquinoline 1-oxide appeared to be about as effective a carcinogen as 4-NQO (22). On the basis of \( \pi \) bonding values, it should be a much more potent carcinogen than 4-NQO. Possibly, steric factors resulting in a partial inhibition of hydrogen bond formation reduced the stability of the complex, and hence the carcinogenicity was reduced.

The 3-halo derivatives of 4-NQO are complex, since, in addition to steric features, dehalogenation occurs to varying degrees \([\text{Br} > \text{Cl} > \text{F}]\) (13). The 3-bromo derivative undergoes dehalogenation most readily to yield 4-HAQO. This may explain its carcinogenicity (13).

The 3-chloro derivative, a noncarcinogen except at high dosage levels (12), was predicted to be a marginal carcinogen on the basis of \( \pi \) bonding at usual dosage levels. The relatively small Van der Waals radius of the chlorine atom may allow a low degree of complex formation, or, more likely, limited amounts of 4-NQO were produced by partial dechlorination. The 3-fluoro derivative was predicted to be a noncarcinogen on the basis of \( \pi \) bonding and is somewhat carcinogenic at high dosages (12). Perhaps limited defluorination results in the production of 4-NQO in biologically significant amounts at the greatly increased dosages necessary to induce tumor formation.

After consideration of the above stereochemical factors, there appear to be no major exceptions to the predicted carcinogenicity or noncarcinogenicity of the derivatives examined. In most instances, comparative studies by the same workers using the same in vivo system have not been performed; consequently, the predicted and actual relative carcinogenicity between derivatives with similar \( \pi \) bonding values cannot be unequivocally demonstrated in most cases.

**Chemical Reduction of the 4-NQO's to 4-HAQO's.**

Extensive studies have demonstrated that 4-NQO is converted to 4-HAQO in vivo (37), and presumably a similar enzymatic reduction occurs for other 4-NQO's. The 4-HAQO's have been postulated to be the proximal carcinogens in this class of compounds. Like the 4-NQO's, a 1-oxide group and a 4-hydroxyamino group are necessary for carcinogenicity (13). Neither 8-methyl-4-hydroxyaminoquinoline nor 3-hydroxyaminoquinoline 1-oxide are carcinogens (13).

If the HAQO's interact with DNA by chemical attack through the hydroxyamino group, substituents adjacent to the hydroxyamino group (i.e., position 3 or 5) should inhibit carcinogenicity. While 3-substituted derivatives are poor carcinogens (12, 13), 5-substituted derivatives are not (13). Similar steric effects are applicable to chemical attack by the 1-oxide group. The 8-substituted derivatives are usually carcinogenic (13), and so are several of the 2-substituted derivatives (13). Molecular orbital calculations on a few HAQO's have shown no evident correlation between possible reactive sites on the 4-HAQO's and carcinogenicity.

The concept that the carcinogenic 4-HAQO's interact by a mechanism other than covalent bond formation can be supported by several lines of experimental evidence. Spectrophotometric studies have demonstrated that 4-HAQO interacts with DNA in a manner similar to 4-NQO (14), probably involving an \( \pi^* \pi^* \) interaction (24). 4-HAQO, like 4-NQO, binds specifically with the purines both at the nucleoside and polymer levels (14), resulting in a parallel orientation to the base planes (20). The similarities between the interaction and the structural conformation of the 4-HAQO's and 4-NQO's with DNA suggest a similarity in their interaction with DNA.

The conversion of the 4,6-dinitro- and 4,7-dinitroquinoline 1-oxides to their corresponding 4-hydroxyamino derivatives results in enhanced carcinogenicity (13). This may be a manifestation of increased stability of the charge-transfer complex of the hydroxyamino derivatives compared with corresponding 4-nitro derivatives.

EPR measurements (10) and self-consistent field molecular orbital calculations (11) have shown that, in solution, 4-HAQO results in free radical formation (10). In the presence of DNA, 4-HAQO exhibited a decreased EPR spectrum (27). This is compatible with the proposed model of complex formation of 4-HAQO with deoxyguanosine, since the 4-HAQO radical would accept an electron during complex formation, resulting in a decreased EPR signal.

**Conclusion.** It appears both theoretically and experimentally probable that the carcinogenic 4-NQO's and 4-HAQO's interact with DNA. In addition, the 4-NQO's themselves may have a direct role in the process of carcinogenesis by the 4-NQO's (8). Spectrophotometric evidence of the relationship between the quantity of charge-transfer interaction between several NQO's with deoxyribonucleosides and carcinogenicity (24) has supported previous theories (9, 19) that postulate a direct role in carcinogenesis of certain substituted 4-NQO's.

It is possible that enhanced binding to DNA occurs after the conversion of the carcinogenic 4-NQO's to the 4-HAQO's. In view of the substantial agreement between known carcinogenicity and molecular orbital calculations interpreted with the use of the steric model, the 4-NQO's themselves must be considered as potentially having a direct role in carcinogenesis. The relative importance of the 4-NQO's as opposed to the 4-HAQO's cannot currently be assessed.

If it is assumed that the proposed model of the binding of 4-NQO's and 4-HAQO's to deoxyguanosine in DNA is biologically significant, then these compounds may exert their influence by causing erroneous base selection during DNA replication.

A possible mechanism for disruption of base selection during DNA synthesis could involve a complex of the carcinogenic 4-NQO's (or 4-HAQO's) with deoxyguanosine in native DNA, which results in the stabilization of a tautomeric form of deoxyguanosine that erroneously pairs with deoxythymidine in the next DNA replication. During the next period of DNA replication, the G-T pairs would give rise to a G-C pair and an erroneous A-T pair (17). In this manner, a number of permanent point mutations could be induced in the genome of the progeny. A series of these alterations in the genome of the cell may be of fundamental importance in the role of carcinogenesis (21).
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A Proposed Model of the Interaction of 4-Nitroquinoline 1-Oxide with DNA

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