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An *Ab Initio* Study of the Relationship between Nitroarene Mutagenicity and Electron Affinity

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SUMMARY

Electron affinities, approximated by lowest unoccupied molecular orbital (LUMO) energies, were determined for an extensive group of nitrated polycyclic aromatic hydrocarbons by *ab initio* methods at the STO-3G level. Significant correlations were demonstrated between nitroarene LUMO energy and the corresponding mutagenic activity in *Salmonella typhimurium* strains TA98, TA100, TA1537, and TA1538. An analogous correlation using Hückel calculations was substantially poorer. A correlation between nitro group rotation and LUMO energy was related to π-conjugation about the C—N bond. Analysis of aryl substituent effects on nitrenium ion stability implicated additional nitro substitution in certain systems to be destabilizing. The results suggest a means for predicting nitroarene mutagenic activity and for assessing the role of metabolic intermediates.

Nitrated PAHs are mutagenic in mammalian cell systems (1, 2) and, in some cases, are highly mutagenic in bacterial systems (2). Their presence in the environment poses a potentially widespread health hazard. They were initially found in photocopying toners (3) but have since been identified as byproducts of incomplete combustion processes, notably diesel emissions (2, 4, 5).

In elucidating the mechanisms by which nitroarenes are bacterial mutagens, investigations have shown the enzymatic reduction of the nitro moiety to be an important, if not necessary, metabolic step. This was demonstrated by Rosenkranz and co-workers (2, 6) who showed that there was a dramatic decrease in the mutagenic activity of nitroarenes when tested in nitroreductase-deficient *Salmonella typhimurium* strains. When these strains were treated with the corresponding arylhydroxylamine anaolgues, bypassing the need for the reduction step, the resulting mutagenic activity was equivalent to that initially observed for the non-deficient bacterial strains. In another important study, Howard *et al.* (7) isolated the *in vitro* DNA adducts of 1-nitropyrene generated by the presence of a mammalian nitroreductase. The major component was identified as *N*-(deoxyguanosin-8-yl)-1-aminopyrene. This was also the major metabolic DNA adduct of 1-nitropyrene found in *S. typhimurium* TA1538. Similarly, it was demonstrated (8) that *S. typhimurium* metabolized 1-nitropyrene to 1-aminopyrene and *N*-acetyl-1-aminopyrene and that, during the notably slow reduction step, binding to the bacterial DNA occurred. It is postulated that the nitroreduction step is followed by the formation of electrophilic intermediates, particularly the aryl nitrenium ion, which serve as the ultimate mutagens (9–11). The nitroarene metabolic pathway thus, by reduction, resembles the sequence of arylamine metabolism which involves cytochrome P-450-mediated oxidation of an arylamine to an arylhydroxylamine (12, 13). It should also be noted that metabolic processes leading to ring oxidation can also be a factor in determining nitroarene activity. Fu *et al.* (14) showed that ring hydroxylation of 6-nitrobenzo(*a*)pyrene was involved in promoting mutagenic activity. Furthermore, conversion of reduced nitroarene metabolites to aryl hydroxamic esters, apparently through transacetylase-mediated *O*-acetylation, has been shown to be as important as nitroreduction in the activation of 1,8-dinitropyrene (15, 16).

Given the importance of the nitroreduction process to the overall activity of these compounds and, especially, the rate dependence of DNA binding on the production of the reduced species, as in the case of 1-nitropyrene (8), it is reasonable to conjecture that a logarithmic relationship may exist between the mutagenic assay or rate constant ([revertants]/[mutagen], per unit time at constant T) and the activation energy associated with the reduction step. This assumes the ease of nitroreduction to be the distinguishing feature in mutagenic activation of nitroarenes and neglects contributions from other metabolic processes (i.e., transport) or further biotransformation (15, 16). From rate theory, rate constant (k) and activation energy (Ea) are related by $k \propto e^{-Ea/RT}$. Previous investigations (13, 17, 18) have utilized both experimental and theoretical estimates of the nitroaromatic reduction potential as a measure of this energy barrier. In a study by Klopman *et al.* (17) an

ABBREVIATIONS: PAH, polycyclic aromatic hydrocarbon; LUMO, lowest unoccupied molecular orbital; MNDO, modified neglect of differential overlap; SCF, self-consistent field; a.u., atomic unit; HOMO, highest occupied molecular orbital.

attempt was made to determine whether a correlation existed between the reduction potentials of a selected group of nitroarenes and their corresponding mutagenicity in *S. typhimurium* strains TA98 and TA1538. A linear correlation was sought between the logarithm of the mutagenicity rate constants and the corresponding reduction potentials or electron affinities for 10 nitroarenes, half being nitropyrenes. The reduction potentials in this case were polarographic half-wave reduction potentials and electron affinities approximated by the LUMO energies obtained by Hückel calculations. Correlation between Hückel LUMO energies and experimental half-wave reduction potentials for PAH systems has been demonstrated previously (19). Although application of the Hückel method is limited by its empiricism and insensitivity to molecular structure, the results were highly suggestive that a correlation existed for the mutagens chosen. Recently, Loew *et al.* (18) have also demonstrated correlations between the mutagenic activity of nitrobenzene and nitrotoluene systems in TA100 and LUMO energies obtained by the semi-empirical MNDO method. In this report, we extend the investigation of these correlations to a wider variety of nitroarenes and *S. typhimurium* strains employing *ab initio* molecular orbital calculations to determine the LUMO energies.

Methods

The *ab initio* calculations to be presented were performed with the VAX version of GAUSSIAN-82 (20) on a VAX 11/780 computer. The LUMO energies for the nitroarenes (listed in Table 1) were obtained using the STO-3G basis set (21) to approximate the wave functions. Single point Hartree-Fock SCF calculations were made at the crystallographic X-ray geometries where published structures were available. Otherwise, reasonable estimates were made for the molecular structures. Due to the size of the aromatic systems, *ab initio* geometry optimizations were not computationally feasible[1] for the compounds for which specific X-ray structures were lacking. In these instances, geometries were chosen which corresponded to the nitroarene aromatic ring systems having the same structures as that of the parent arenes and with the nitro groups oriented in one of two ways. One orientation corresponded to the crystallographic geometry of the nitro group in 1,5-dinitronaphthalene (22) in which the NO_2 plane is tilted 48° from the plane defined by the naphthalene ring system, due to the steric interaction between the NO_2 oxygen and an adjacent hydrogen on the opposite ring, with bond lengths, N—O 1.208 Å, C—N 1.486 Å, and an O—N—O angle of 123°. A second orientation was that for the nitro group in 6-amino-2-nitronaphthalene (23), which is coplanar with the ring system with bond lengths, N—O 1.228 Å, C—N 1.458 Å, and an O—N—O angle of 122°. These two orientations reflect the physical environments of the nitro groups in the compounds studied and served as templates for the nitro group geometry.

The *S. typhimurium* mutagenicity assay (24) data were taken from several sources, the results of which have been compiled in a review by Rosenkranz and Mermelstein (2) that gives a summary of the investigations concerned with nitroarene mutagenicity. Given the sometimes widely variable interlaboratory mutagenicity assay results for nitroarenes, the data of Rosenkranz and co-workers (1–3, 6, 15) were used where possible to maintain consistency. The set of nitroarenes chosen for study exhibits a wide range of mutagenic activity, spanning 6 orders of magnitude and a variety of nitro group environments. If the process of nitroreduction is indeed of universal importance to the mutagenic activation of nitroarenes, the set of compounds selected provides a challenging test for correlation.

[1] A single point SCF calculation for the dinitropyrenes, for example, averaged 580 min of central processing unit time.

Results

The nitroarene STO-3G LUMO energies are presented in Table 1 along with the corresponding mutagenic responses for four *S. typhimurium* strains (TA98, TA100, TA1537, and TA1538). Figs. 1–4 reveal that a significant linear correlation exists between the logarithm of the mutagenicity and the LUMO energy of the compound. The appropriateness of using a linear relationship to correlate the data is supported by F statistics of 35.9, 20.5, 38.1, and 39.6, with correlation coefficients of −0.82, −0.75, −0.88, and −0.86, for the strains TA98, TA100, TA1537, and TA1538, respectively. The magnitudes of the F statistics for the four independent sets of analogous correlations dismiss any chance relationship between the LUMO energy of a nitroarene and the degree of mutagenicity exhibited.

Although there is certainly substantial scatter in the correlations presented, for TA100, in particular, it should be noted that the *Salmonella* assay serves mainly as a qualitative measure of mutagenic activity and is thus quantitatively limited. In addition, nitroarenes have been shown to exhibit greater assay variability under identical experimental conditions relative to other compounds. Significant interlaboratory mutagenic variability and substantial intralaboratory variability have been demonstrated as well (2). Interlaboratory variability, as expressed in revertants/nmol, may result from different methods of calculation.

The error of the STO-3G LUMO energies associated with using non-optimized geometries for the single point SCF calculations was estimated to be within 0.01 a.u. This was determined by comparing the non-optimized STO-3G LUMO energies for 1-nitronaphthalene, 2-nitronaphthalene, nitrobenzene, and 1,8-dinitropyrene with the LUMO energies determined by optimizing the nitro group C—N, and O—N bond distances, and the C—C—N—O dihedral angle. Given this sensitivity to geometry, the correlations above were reevaluated by allowing the LUMO energies to randomly relax within a range of 0.01 a.u. and then averaging the statistical fits for 500 samples. The variation in the F statistic and the linear correlation coefficients presented above, however, was not significant. The largest variation occurred in the TA1537 correlation where the average F statistic and correlation coefficient were 36.9 and −0.86, respectively.

Hückel calculations were also performed to determine how mutagenicity and the LUMO energies obtained by this method correlated. Hückel parameters reported previously by Klopman *et al.* (17) were used in calculating the LUMO energies presented in Table 1. However, the Hückel LUMO energies calculated for 1,3,6-trinitropyrene (−0.142), 2,7-dinitro-9-fluorenone (−0.088), and 2-nitrofluorene (−0.348) disagreed with those presented earlier by Klopman *et al.* (17), which were −0.035, −0.272, and −0.320, respectively. Although the previous investigation had suggested a significant correlation between mutagenicity and Hückel LUMO energies, extension of the Hückel method to the compounds in this report resulted in a markedly poorer correlation for TA98 (F statistic = 8.5, r = −0.57) than that determined by the *ab initio* results (F statistic = 35.9, r = −0.82), as evident in Fig. 5. The lower correlation using the simple Hückel method is probably a reflection of the inadequacies in the parameterization to account for atomic orbital overlap and variations in the molecular geometry (i.e., nitro group orientation).

TABLE 1

Nitroarene LUMO energies and corresponding mutagenicity assays

References for parent aromatic ring structures used in the *ab initio* calculations are included beside the compounds listed below. The assay values were taken from results compiled by Rosenkranz and Mermelstein (2).

Compound	E_{LUMO} STO-3G[a]	E_{LUMO} Hückel[b]	TA98[c]	TA100	TA1537	TA1538
1. 1,3,6-Trinitropyrene (25)	0.09270[d]	−0.142	40,700	7,450	20,100	20,300
2. 2,4,7-Trinitro-9-fluorenone (26)	0.10169[e]	−0.074	2,125	159	572	2,860
3. 1,3-Dinitropyrene (25)	0.11448[d]	−0.217	144,760	42,280	13,400	78,960
4. 1,6-Dinitropyrene (25)	0.11450[d]	−0.182	183,570	12,159	33,000	61,100
5. 1,8-Dinitropyrene (25)	0.11486[d]	−0.182	254,000	55,420	11,800	34,700
6. 2,7-Dinitro-9-fluorenone (26)	0.11694[e]	−0.088	1,459	457	195	1,720
7. 1-Nitrofluoranthene (27)	0.12808[d]	−0.205	544	124		
8. 2-Nitroanthracene (28)	0.12823[f]	−0.297	892	1,133	186	
9. 2,7-Dinitrofluorene (29)	0.13026[f]	−0.289	471	6.0	290	346
10. 3-Nitrofluoranthene (27)	0.13248[d]	−0.219	5,439	2,967	321	1,286
11. 8-Nitrofluoranthene (27)	0.13281[f]	−0.286	11,125	396	124	9,889
12. 1-Hydroxy-3-nitropyrene (25)	0.13665[d]	−0.263	7,370			
13. 3-Nitro-9-fluorenone (30)	0.13835[f]	−0.080	383			
14. 1-Nitropyrene (25)	0.13838[d]	−0.263	453	59	67	80
15. 7-Nitrofluoranthene (27)	0.14792[f]	−0.305	74	989		
16. 2-Nitronaphthalene (23)	0.15969[e]	−0.339	0.2	1.3	0.02	0.4
17. 2-Nitrofluorene (29)	0.16155[f]	−0.348	14	6.0	0.04	7.0
18. 2-Nitrophenanthene (31)	0.16647[f]	−0.347	128	62		145
19. 1-Nitronaphthalene (22)	0.16769[e]	−0.309	0.05	1.0		0.1
20. 5-Nitroacenaphthene (32)	0.18047[d]	−0.385	2.5	1.9	0.30	0.8

[a] STO-3G LUMO energies are given in a.u.
[b] Hückel LUMO energies are given in units of β.
[c] Mutagenicity is expressed as number of revertants/nmol of mutagen.
[d] LUMO energy was calculated using 1,5-dinitronaphthalene NO_2 conformation (22).
[e] LUMO energy was calculated at X-ray structure.
[f] LUMO energy was calculated using 6-amino-2-nitronaphthalene NO_2 conformation (23).

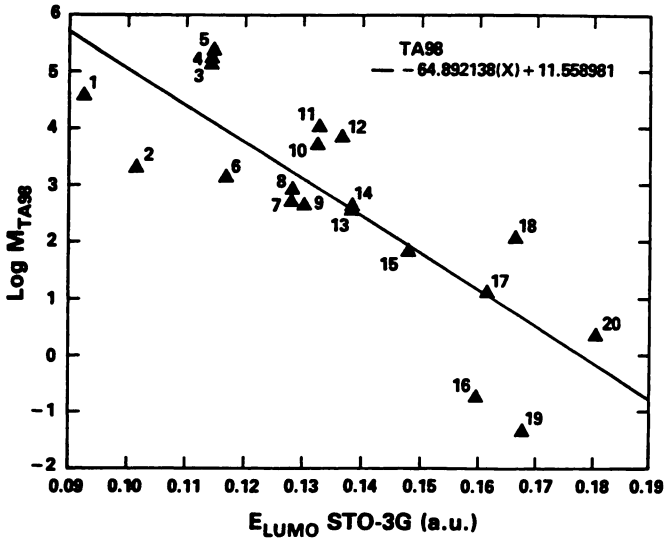

Fig. 1. Correlation between the logarithm of nitroarene mutagenicity in TA98 and STO-3G LUMO energy (r = −0.82, F statistic = 35.9). The numerically labeled points correspond to the compounds listed in Table 1.

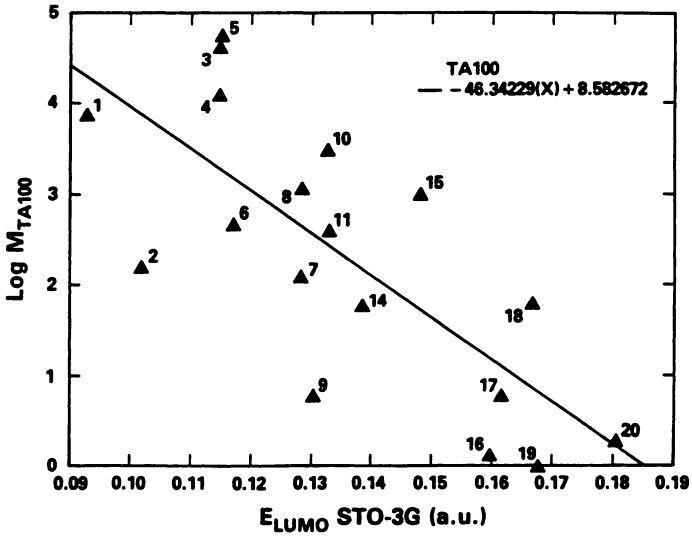

Fig. 2. Correlation between the logarithm of nitroarene mutagenicity in TA100 and STO-3G LUMO energy (r = −0.75, F statistic = 20.5).

Discussion

The basis for using *ab initio* LUMO energies as measures of the electron affinities is given by Koopmans' theorem (33) which states that the electron affinity (EA) of a compound is equal to the negative energy of the virtual orbital to which an additional electron occupies:

$$EA = {}^{N}E_o - {}^{N+1}E^r = -\epsilon_r$$

where ${}^{N}E_o$ is the total electronic energy for the N electron system and ${}^{N+1}E^r$ is the total electronic energy with the addition of the $N + 1$ electron to virtual orbital r. The theorem assumes that the N-electron SCF wave function is unaltered or "frozen" with addition of the $N + 1$ electron to the system. Unrestricted SCF calculations, using a 4–31G (34) basis set for the anions of nitrobenzene and *p*-nitrotoluene confirmed that the highest occupied molecular orbitals (HOMOs) for these anions were indeed in group assignment and similar in electron density distribution to the LUMOs of the neutrals. In practice, Koopmans' theorem (33) generally gives poor electron affinities due to neglect of electronic relaxation and correlation effects, as

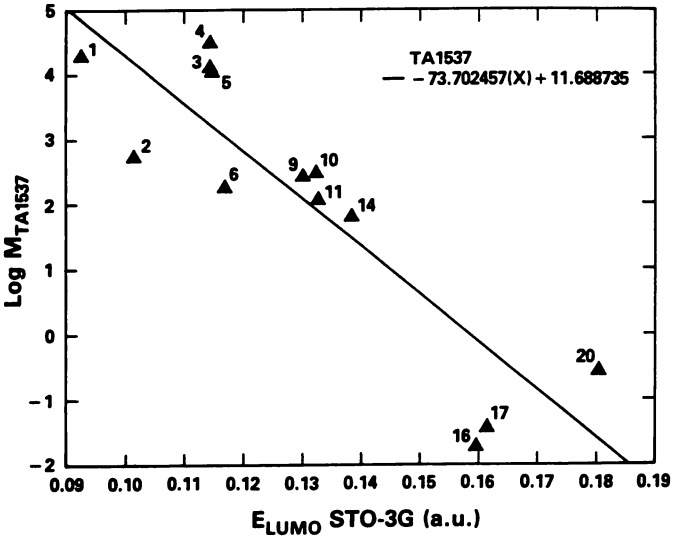

Fig. 3. Correlation between the logarithm of nitroarene mutagenicity in TA1537 and STO-3G LUMO energy ($r = -0.88$, F statistic = 38.1).

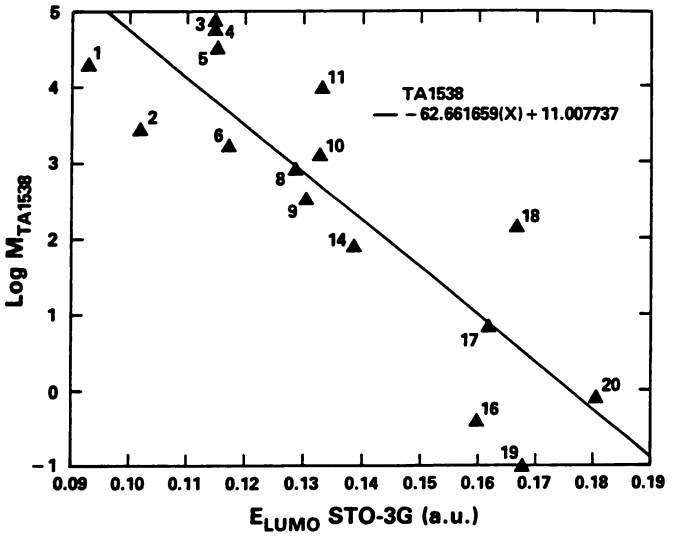

Fig. 4. Correlation between the logarithm of nitroarene mutagenicity in TA1538 and STO-3G LUMO energy ($r = -0.86$, F statistic = 39.6).

seen in the values of Table 2.[2] However, the existence of a linear relationship between the STO-3G LUMO energies and the experimental electron affinities for nitroarenes validated the application of LUMO energies in serving as measures of the relative reduction potentials. This is revealed in Fig. 6, in which the experimental electron affinities for a series of nitrobenzenes are ploted against their corresponding STO-3G LUMO energies. An excellent linear correlation was found to exist with a correlation coefficient of 0.98 and an F statistic of 96.9.

Given the inverse dependence between nitroarene mutagenic activity and LUMO energy, it is important to consider what

[2] A more rigorous theoretical method for determining electron affinities, which involved taking the difference in total energy between the neutral and anion species at their 4–31G optimized geometries, gave excellent agreement with experimental electron affinities for nitrobenzene (EA = 22.4 kcal/mol) (G. Caldwell and P. Kebarle, personal communication to M. Bursey, University of North Carolina) and p-nitrotoluene (EA = 20.9 kcal/mol) (35). Electron affinities determined by this technique were 23.2 and 21.8 kcal/mol for nitrobenzene and p-nitrotoluene, respectively.

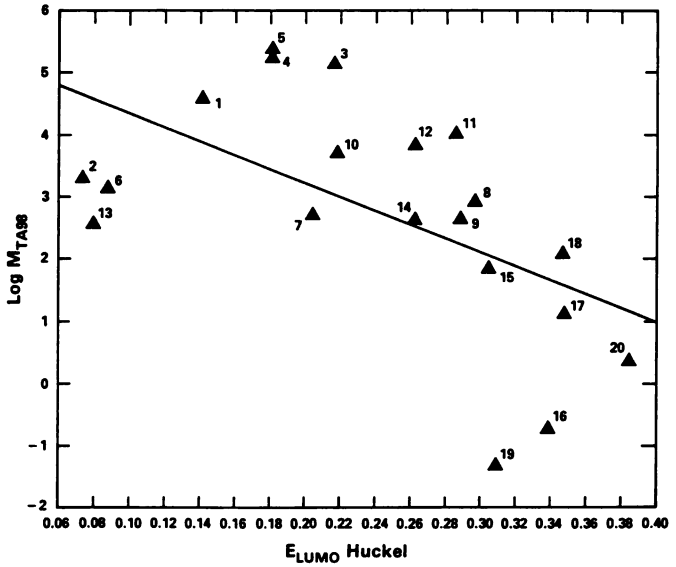

Fig. 5. Correlation between the logarithm of nitroarene mutagenicity in TA98 and Hückel LUMO energy ($r = -0.57$, F statistic = 8.5). The Hückel energies are expressed in units of $-\beta$.

TABLE 2

Experimental electron affinities vs. LUMO energies for nitrobenzene systems

The premise for using the LUMO energy of a compound to estimate its reduction potential is Koopmans' theorem (33), which relates LUMO energy to electron affinity, $EA = -E_{LUMO}$. The theorem, however, makes no provisions for electron relaxation. Moreover, single determinant SCF wavefunctions neglect additional effects of electron correlation. The application of the theorem, as a result, yields poor absolute electron affinities, as seen above. Fig. 6, however, demonstrates that the linear relationship between EA and E_{LUMO} for the substituted nitrobenzene systems below is preserved.

Compound	E_{LUMO}[a]	EA[a] (experimental)
1,4-Dinitrobenzene	75.6	43.5[b]
1,3-Dinitrobenzene	85.5	36.1[b]
p-Chloronitrobenzene	94.7	27.0[b]
3,4-Dimethylnitrobenzene	101.0	20.2[b]
Nitrobenzene	105.1	22.4[c]
p-Methylnitrobenzene	107.5	20.9[b]
2,3-Dimethylnitrobenzene	110.0	19.1[c]

[a] Energies are given in kcal/mol (1 a.u. = 627.51 kcal/mol).
[b] Ref. 35.
[c] G. Caldwell and P. Kebarle, personal communication to M. Bursey, University of North Carolina.

molecular structural features contribute to a lower LUMO energy. A central feature to understanding this dependence is given by examining the molecular orbital contribution of the nitro group to the LUMO. The LUMO for nitrobenzene in Fig. 7 gives a general illustration of the properties for the nitroarenes studied. As expected, it consists of a π-system of atomic orbitals with the majority of the orbital density localized on the C—NO_2 unit. For optimized 4–31G nitrobenzene, 76.6% of the LUMO density is localized here with 33% at the nitrogen center alone, indicating the importance of this region to the reduction process. These orbital densities were obtained by computing

$$\sum_i \sum_j c_i c_j < \alpha_i \mid \alpha_j >$$

where c_i and c_j are the normalized LUMO coefficients associated with atomic orbitals α_i and α_j, and $<\alpha_i \mid \alpha_j>$ is the overlap integral between α_i and α_j. The double summation is over the

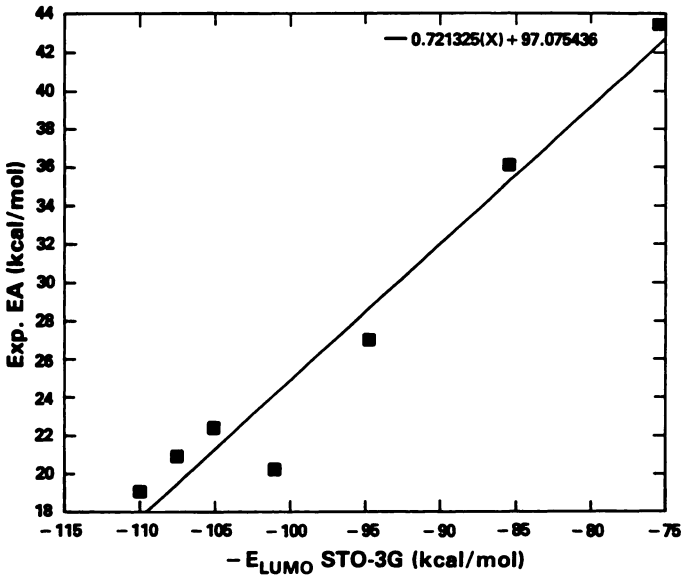

Fig. 6. Experimental electron affinities versus STO-3G LUMO energies, taken from Table 2, for nitrobenzene systems (r = 0.98, F statistic = 96.9). The linear correlation supports the use of STO-3G LUMO energies as relative measures of nitroarene electron affinities.

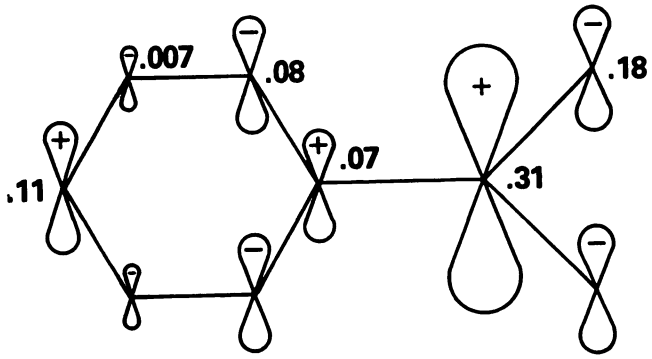

Fig. 7. The 4–31G LUMO of nitrobenzene. Values adjacent to the nuclear centers above correspond to the sum of the LUMO electronic population associated with each center and reflects the electron density distribution of the LUMO. The figure illustrates the tendency of an additional electron to be localized on the nitrogen and the π-overlap about the C—N bond. The figure further reveals preferred localization at the *ortho* and *para* positions relative to the *meta*, as would be predicted from examination of resonance structures.

LUMO atomic orbitals of the nuclei of interest (i.e., the C—NO_2 nuclei).

As is evident from Fig. 7, the existence of significant π-bonding overlap about the C—N bond has important consequences to the LUMO energies of nitroarenes given nitro groups with coplanar or rotated orientations relative to the aromatic ring system. Previous studies have empirically considered the importance of nitro group orientation to mutagenic activity (36, 37) but have neglected to provide a physical basis for relating geometry to electron affinity. Table 3 demonstrates the relationship between the LUMO C—N bond population and the LUMO energy at various nitro group rotations. As conjugation is diminished with rotation, the LUMO energy increases, thus resulting in a lower electron affinity. A correlation coefficient of −0.99 was calculated for the relationship between LUMO energy and LUMO C—N bond population for nitrobenzene. It is important to note that, unless proper provisions are made in the parameterization of nitro groups with

TABLE 3

LUMO energy and LUMO C—N π-bond population of nitrobenzene

LUMO energy and LUMO C—N bond population are correlated with rotation of the nitro group relative to the benzene ring [barrier of rotation calculated = 0.008 a.u. (5 kcal/mol)] A correlation coefficient of −0.99 was calculated between the LUMO C—N bond population and energy.

Nitro group rotational angle[a]	LUMO C—N bond population[b]	ΔE_{LUMO}[c]
degrees		
0.0	1.000	0.0
10.0	0.991	0.00070
15.0	0.979	0.00157
30.0	0.916	0.00601
50.0	0.753	0.01539
70.0	0.475	0.02711
90.0	0.185	0.03503

[a] Angle is defined as the dihedral angle O—N—C—C.

[b] The bond population corresponds to

$$\sum_{I}^{C}\sum_{J}^{N} C_{Ik}C_{Jk}\langle\alpha_{Ik}\,|\,\alpha_{Jk}\rangle$$

where the MO k is the LUMO, summation I is the AOs (α_I) and corresponding coefficients (C_I) of carbon, and likewise the summation J is over nitrogen. The values are scaled to the maximum LUMO bond population at the coplanar conformation.

[c] The LUMO energies have been scaled to the LUMO energy of nitrobenzene at its STO-3G minimized geometry (E_{LUMO} = 0.16753 a.u.).

planar versus nonplanar configurations, significant error could occur in the LUMO energies given by an empirical approach such as the Hückel method.

The trend in increased LUMO energy with nitro rotation is also seen in comparing the LUMO energies of 1- and 2-nitronaphthalene. Crystallographic data (22) indicate that the 1-nitro group is rotated 48° relative to the naphthalene plane. Correspondingly, the *ab initio* calculations predict a higher LUMO energy for 1-nitronaphthalene by 0.008 a.u. Also, a subsequent calculation of 9-nitroanthracene at its crystal structure (38), in which the nitro group is rotated nearly perpendicular to the anthracene plane, gave a LUMO energy 0.006 a.u. higher than planar 2-nitroanthracene. Although a higher LUMO energy was expected, closer analysis of the LUMO revealed that there was no significant orbital density on the nitro group, thus making the calculation suspect. Anthracene and naphthalene with planar nitro groups are more mutagenic than their nonplanar isomers.

Although NMR data suggest that the 1-, 3-, 7-, and 8-nitrofluoranthene isomers are planar (37), comparison of the LUMO energies for these isomers and their corresponding mutagenicities does not follow a predictive trend. Distance calculations on 1- and 7-nitrofluoranthene indicated significant NO_2 contact with the *peri* hydrogen in the bay region (NO—H 1.7Å). An *ab initio* optimization of the rotational angle of the nitro group predicted a rotation of 60° (rotational barrier ≅ 23.9 kcal-mol^{-1}) for a nitro group in the 1- and 7-positions. *Ab initio* calculations were repeated for 1- and 7-nitrofluoranthene using a 60° rotation for the nitro groups and resulted in a LUMO energy of 0.1352 a.u. (0.0071 a.u. increase) for 1-nitrofluoranthene and no significant change for 7-nitrofluoranthene. With this correction, the calculations predicted the trend in the mutagenicity of the isomeric nitrofluoranthene series. The 3-nitro (E_{LUMO} = 0.1325 a.u.) and 8-nitro (E_{LUMO} = 0.1328 a.u.) isomers have similar activity, followed by the lower activity of the 1-nitro isomer, and finally the 7-nitro isomer (E_{LUMO} = 0.1479 a.u.).

Another feature of interest to the chemistry of the nitroar-

enes in this study is the evidence of a region of positive electrostatic potential which exists above and below the C—NO_2 bond, perpendicular to the molecular plane for planar nitroaromatic systems. This is supported by the existence of electropositive atomic charges at both C—N nuclei and, further, by the region of LUMO π-density about the C—N bond as illustrated above. The Mulliken STO-3G atomic charges for the C—N carbon (+0.10) and nitrogen (+0.13) of nitrobenzene, for example, are a reflection of the diminished electron density in this region, given the occupied molecular orbitals of the SCF wavefunction. The large amount of LUMO density above and below the C—N bond also indicates the propensity of an additional electron to be localized in this region, as confirmed by examination of the HOMO in anion calculations. Existence of this positive electrostatic region was in fact confirmed in a study by Politzer *et al.* (39) which involved an examination of the *ab initio* electrostatic potential surface of nitrobenzene. Politzer *et al.* (39) extended this investigation to implicate the C—N bond region as a site for nucleophilic reception as well. It is therefore interesting to note that the nitro function possesses the proper ingredients to facilitate the reduction process. Not only does it contribute to anion stability, but it provides a convenient location to accommodate an electron-donating species in the proximity of the reduction site. The reduction process can be facilitated through favorable electrostatic interaction, as well as frontier orbital intermolecular interactions. The coupling of these properties exist for a variety of nitroarenes and may have implications bearing on the mechanism of reduction. It should also be pointed out that negative regions of electrostatic potential can also exist in the vicinity of the ring carbons, which could lead to significant competing pathways to phenolic metabolites through electrophilic oxygen attack and arene oxide formation. This could complicate any direct correlation of mutagenicity with the reduction potential of the compound.

Although the motivation for seeking a correlation between nitroarene electron affinity and mutagenicity is based on the premise that enzymatic nitroreduction is the primary means of activation, in some cases subsequent *O*-acetylation of the reduced metabolites, leading to aryl hydroamic acid esters, has proven to be equally important (15, 16). Notable is the case of 1,8-dinitropyrene in which a transacetylase has been implicated in the conversion of the reduced metabolites 1-amino-8-nitropyrene and 1,8-diaminopyrene (16) to a hydroamic acid ester (15). It is interesting to note, however, that this step does not appear to be necessary for the activation of all nitroarenes, aryl amines, or aryl *N*-hydroxyl amines. Reduced metabolites such as aryl *N*-hydroxyl amines can be direct-acting through nonenzymatic conversion to nitrenium ions (9, 16, 40).

Why some nitroarenes require biotransformation to aryl hydroxamic acid forms may be partially answered in considering what factors would either facilitate or prevent nitroreduced metabolites from directly acting via nitrenium ion formation. Inspection of 1-nitropyrene and 1,8-dinitropyrene, in which the latter proceeds through conversion to the hydroxamic acid, whereas the former apparently does not, suggest that the additional nitro group of 1,8-dinitropyrene may destabilize nitrenium ion formation. To test this postulate, STO-3G *ab initio* calculations were performed on 1-amino-8-nitropyrene and its corresponding nitrenium form, and likewise for 1-aminopyrene. Optimized NH_2 and NH^+ geometries found for aniline were used in the calculations. The difference in total energy of the amine and its nitrenium counterpart yields a heat of formation which serves as an estimate of the activation energy required in the conversion to the nitrenium ion. Table 4 shows that the additional nitro group in the 8-position destabilizes nitrenium ion formation by 0.0332 a.u. Additional calculations, allowing substituent geometry optimization, on a series of substituted anilines also showed a trend in nitrenium ion stabilization in accord with electronic resonance considerations. The results indicate that the need for *O*-acetylation may simply be due to the acetate group, being a much better leaving group than hydroxyl, significantly diminishing the energy of activation associated with nitrenium ion formation. This step may thus be required for cases in which nitrenium ion formation is destabilized due to substitutent effects. We note that this bears no mechanistic information on whether a true or incipient nitrenium ion is involved in DNA lesion (40). It is also noted that the same substituent effects which stabilize nitrenium ion formation could serve to decrease the electron affinity of the compound, thus reducing the ease of reduction.

Although the correlations demonstrated between nitroarene mutagenicity and LUMO energy suggest a means for predicting the activity of new and untested nitrated PAH systems, two points should be noted. First, extension of this treatment to molecular systems with other chemical functionality is cautioned. Hartman and Hartman (13), for instance, found a lack of correlation between polarographic half-wave reduction potentials and mutagenic activity for a series of nitroheterocyclic compounds. As pointed out above, metabolic modes of action other than nitroreduction may be of key importance in determining the pathway to DNA lesion with introduction of additional functionality. Nonetheless, an important extension of the present investigation would be to incorporate the effects of alkyl substitution, at various ring positions, on the electronic properties of nitroarenes. Nitrated PAH systems present in emission extracts (4, 5) are alkylated to a large degree; however, knowledge of their mutagenic properties is lacking. Recently, Loew *et al.* (18) have investigated properties associated with the mutagenic activity of nitrated toluene systems. Alkyl group contributions of electron density to the aromatic system, as

TABLE 4

Substituent effects on the stability of aryl nitrenium ion formation

The influence of various aromatic ring substituents can have important consequences on the energetics of aryl nitrenium ion formation. As would be predicted from electronic resonance considerations, the nitro group of nitroaniline is destabilizing particularly in the *para* position. Conversely, hydroxyl or methyl substitution stabilizes the positive ion. The nitro group in the 8-position of 1-amino-8-nitropyrene destabilizes nitrenium ion formation by nearly the same extent as the nitro group of *p*-nitroaniline. Energies were obtained at optimized geometries for aromatic ring-functional group distances.

Compound	ΔE_{TOTAL}[a]	Stabilization energy[b]
Aniline/$C_6H_5NH(+1)$	0.8429	0.0
P-Nitroaniline/$C_6H_4NO_2NH(+1)$	0.8775	−0.0346
m-Nitroaniline/$C_6H_4NO_2NH(+1)$	0.8697	−0.0268
p-Methylaniline/$C_7H_7NH(+1)$	0.8301	0.0128
p-Hydroxyaniline/$C_6H_4OHNH(+1)$	0.8210	0.0220
1-Aminopyrene/$C_{16}H_{10}NH(+1)$	0.7916	0.0
1-Amino-8-nitropyrene/$C_{16}H_9NO_2NH(+1)$	0.8248	−0.0332

[a] Ground state energy difference between nitrenium ion and amine, $\Delta E_{TOTAL} \equiv E_{TOTAL}$ (nitrenium)$-E_{TOTAL}$ (amine). Energies are in a.u.

[b] The difference between ΔE_{TOTAL} for compounds with differing substituents using aniline and 1-aminopyrene as references, i.e., Stabilization Energy $x = \Delta E_{TOTAL}$ (aniline/1-aminopyrene) − ΔE_{TOTAL} (with substituent x).

well as the stabilization of resonance forms, could have important consequences to nitroarene activity. Second, the results of this study emphasize the importance of appropriate empirical parameterization for nitrated PAH systems. Strong correlations were found between nitroarene mutagenicity and LUMO energy using *ab initio* methods, whereas the correlation derived from Hückel calculations was poor.

In conclusion, the correlation results of this study support the experimental evidence that the dominating metabolic pathway by which nitroarenes express their bacterial mutagenic activity involves nitroreduction. Moreover, this step is a metabolically distinguishing, if not limiting, process in determining the rate of formation of the DNA-bound adduct. The hypothesized mode of action of these polycyclic aromatic nitro compounds not only involves initial nitroreduction leading to hydroxylamines, but ultimate conversion to electrophilic intermediates, namely, arylnitrenium ions, which interact with key tissue macromolecules. The ease of formation, stability, and reactivity of these nitrenium ions will be of importance (41–43). Whether deviation in the mutagenicity versus LUMO energy correlations suggests other key modes of nitroarene action or the influences of other metabolic processes is unclear, given the variability of the assay data. However, this question could largely be answered by studying the *in vitro* rates of nitroreduction by nitroreductase catalysis and formation of the DNA adduct(s), using methods similar to those of Howard *et al.* (7) and Messier *et al.* (8) in the case of 1-nitropyrene. In an investigation of this type it would be of interest to see how well the rates of nitroreduction or DNA binding are predicted by the LUMO energies in this report, on what time scale reduction and DNA binding coincide, and, if possible, to identify the DNA adduct(s). This would provide direct evidence of the importance of the role of nitroreduction in leading to mutagenic lesion. Correlation of the *in vitro* results with the bacterial mutagenesis rates could further elucidate the effects of other metabolic processes.

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