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A Quantum Mechanical Approach to the Theory of Cancer from Polynuclear Compounds

Metabolic Activation and Carcinogenicity of Extended Anilines and Aminoazo Compounds

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SUMMARY

Calculations have been carried out of the electronic structure and molecular properties in relation to metabolic activation and carcinogenic activities of polycyclic aromatic amines (PAAs). Quantum mechanical molecular orbital method MINDO/3 is employed in the calculations mainly on anilines, extended anilines, and aminoazo and other azo compounds. The calculations, in agreement with findings of Arcos and Argus, indicate that for the highest level of carcinogenic activity obtainable with the dicyclic aromatic amines, the amino substituent must be introduced at the terminal carbon atom of the longest conjugate chain. In the case of monocyclic compounds, in particular, charge distribution of the amino substitution aids in identifying the carcinogenic character of the PAAs. Our results demonstrate that ring hydroxylation leads to detoxification of the compounds. However, the major pathway leading to carcinogenic activity involves transformation to hydroxylamines and subsequently to electrophilic arylnitrenium ions (ANIs). These are in line with findings from experiments. Calculations of certain electronic parameters give expected relative carcinogenic potencies. In all cases the ANIs function as ambient electrophiles which can undergo both electrostatic and covalent binding with nucleophilic centers of proteins and DNA bases.

INTRODUCTION

The induction of cancer in laboratory animals by PAAs[1] has been extensively studied since the initial finding (1) of its very occurrence in man some 200 years ago. Research during the last 20 years in particular have accomplished (2) a unified body of knowledge on the mechanism as well as on the controlling processes of cancer by PAAs. They have uncovered also an increasing list of carcinogens (3) that occur in environment, food, water, air, drugs, and naturally occurring fungi and green plants. All these are subjects of excellent reviews by Miller and Miller (4), Clayson and Garner (2), and Kriek and Westra (5).

The PAAs are important intermediates in the dyestuff and pharmaceutical industries and are known as substrates of cytochrome P-450 mixed function oxidases. Careful studies indicate that skin cancer (6) by these compounds in adult man is mainly derived from epithelial tissues and that environmental PAAs, both natural and man-made, produce cancer in tissues distant from the site of applications, the latter being organs such as bladder, kidney, intestine, liver, breast, ear duct, mammary glands, etc. Exposure to ultraviolet radiation is the main etiologic agent (7) in the case of skin cancer.

In the induction of cancer, chemical carcinogens react directly or indirectly with critical molecules in cells (8). The available data indicate that mainly covalent interaction of the carcinogens or of significant parts of their structures take place with the nucleic acids and proteins of tumor-susceptible tissues *in vivo* and *in vitro* (9). Good correlations have been observed in many instances between the amount of macromolecular binding and the degree of carcinogenic potency. The nature of the binding *in vivo* of chemical carcinogens with tissue components such as proteins and nucleic acids has been very clearly established in the past decade. While no common structural feature appears evident among chemical carcino-

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The abbreviations used are: PAA, polycyclic aromatic amine; MO, molecular orbital; EAAs, extended anilines and aminoazo compounds; ANI, arylnitrenium ion; HOMO and LUMO, highest occupied and lowest unoccupied MOs; NNC,net negative charge; 4-BPA, 4-aminobiphenyl; MRIA, most reactive isomer; AB, aminobenzidine; AS, aminostilbene.

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gens, it is clear (10) that the ultimate reactive form in most of these structures is electrophilic (electron-deficient) agents. The majority of the chemical carcinogens are not very reactive by themselves; they are activated through a series of metabolic transformation (10).

In PAAs, *N*-hydroxylation is generally considered (4) to be the first step in the metabolic activation sequence. This is evident from the fact that aromatic amine and amide carcinogens are also *N*-hydroxylated compounds, and that their carcinogenicity is much higher than that of their parent compounds. While *N*-hydroxylation is a sufficient activation step, in many instances it is suggested that *N*-hydroxy esterification is also necessary (7). This esterification is normally a deactivating step in the metabolism with ring hydroxylation being the major route for it. The phenols which are formed from this hydroxylation undergo further conjugation with either sulfate, glucuronic acid, or phosphate groups in the subsequent stages. As an example, a scheme of metabolic activation of PAAs is shown in Fig. 1. The ultimate electrophilic metabolites of PAAs combine with nucleophilic (electron-rich) groups in proteins and nucleic acids and give rise to covalently bound derivatives. *N*-Hydroxy intermediates are the only enzymatically formed metabolites with sufficient chemical reactivity that react nonenzymatically with cellular constituents, leading to the formation of the covalent bond.

Not all aromatic amines which have been tested (3) in laboratory animals are potent carcinogens. In fact, they form a class of compounds with great diversity in species and tissue specificity. Once the *N*-hydroxy compound is formed, it is subjected to competing metabolic pathways. Both the levels of activating enzymes and of detoxification enzymes work simultaneously, and it is the balance of the two that determines the tissue sensitivity to a particular PAA carcinogen. Clayson and Garner (2) have subdivided the PAAs into five major groups. These are (*a*) anilines, (*b*) extended anilines, (*c*) fused ring amines, (*d*) aminoazo and other azo compounds, and (*e*) heterocyclic amines. Though aminoazo dyes have long been considered as a separate class of carcinogens, the Millers and co-workers (11) have observed that they undergo metabolic activation in rat liver essentially in the same manner as the aromatic amines. Therefore, the aminoazo dyes are in fact inseparable from extended anilines.

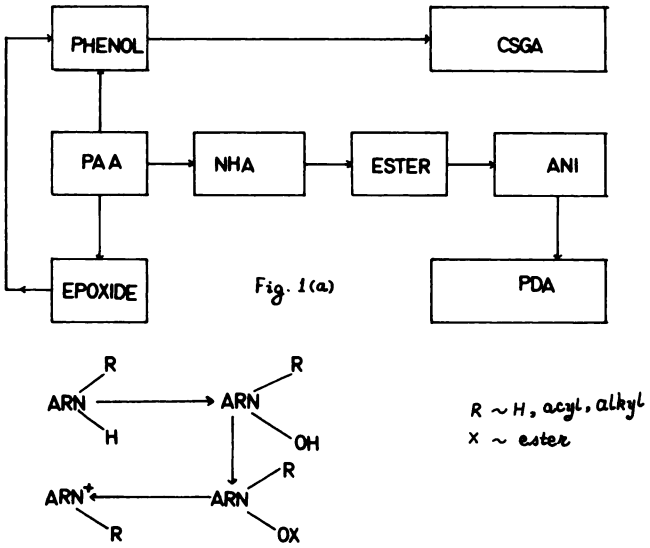

FIG. 1. *Schematic representation of metabolic activation of aromatic amines*

NHA, *N*-hydroxyamine; *PDA*, PAA-DNA adduct; *CSGA*, conjugation with sulfate, glucuronic acid, etc.; *AR*, aryl.

Our aim in this investigation is to use all valence electron semi-empirical MO MINDO/3 method (12) to identify and calculate the electronic structure and molecular properties of a series of extended anilines and aminoazo compounds. The compounds together are called EAAs. Certain parameters are calculated to predict the major metabolites of them, and to determine the stability and electrophilicity of their ANIs that are hypothesized to be the ultimate carcinogens. Animal experiments have proved the existence of a very large number of carcinogenic aromatic amines (5). Among them, EAAs are comprised mainly of systems like aminobiphenyl, benzidine, aminostilbene, methylenedianiline, phenylazoaniline, etc. In the present report, these molecules and some of their derivatives are considered as EAA representative. Some of them are displayed schematically in Fig. 2.

RESULTS AND DISCUSSION

In an earlier investigation (13) (hereafter referred to as I) on the carcinogenicity and metabolic activation of polycyclic aromatic hydrocarbons we optimized the molecular geometry of benzene. The semi-empirical MINDO/3 method was used in the calculations. It was observed that our geometry for benzene is identical to that of Bingham *et al.* (28) and quite close to one obtained from experiment (28). For various EAAs, the same benzene ring is used except that the torsional angle variations of the nitrogen substituents as well as bond angles and bond lengths in the vicinity of the nitrogen were further optimized. The molecular coordinate system in the rings correspond to the *x*-*y* plane, making the p_z atomic orbital on the nitrogen most relevent to the stability and electrophilicity of ANIs. In order to evaluate the relative electrophilicity of arylhydroxyl amines and ANIs, electronic parameters involving both s- and p-electrons of the nitrogen and ring carbon atoms are calculated. Table 1 presents the calculated values of parameters which are assumed to be measure of the carcinogenic reactivity of positional isomers of EAAs. This table can be used to predict the relative susceptibility to *N*-hydroxylation while the *N*-substitution occurs at different carbon centes of EAAs. In recent years, there has been a growing interest in the frontier orbitals (14), viz., HOMO and LUMO of the electrophiles as chemical tools applicable to predictions of intermolecular reactivity. The eigenvalues corresponding to both HOMO and LUMO are included in Table 1. In most cases, the energy of the HOMO is the least negative, and that of the LUMO the least positive. Occasionally, as will be seen in the case of 3,3′-dichloro-4,4′-diaminostilbene, 3,3′-dichlorobenzedine, and 4,4′-methylenedianiline, the residual energy of the LUMO is also negative. As a general criterion, we denote energy of an MO (say, *n*th MO) with

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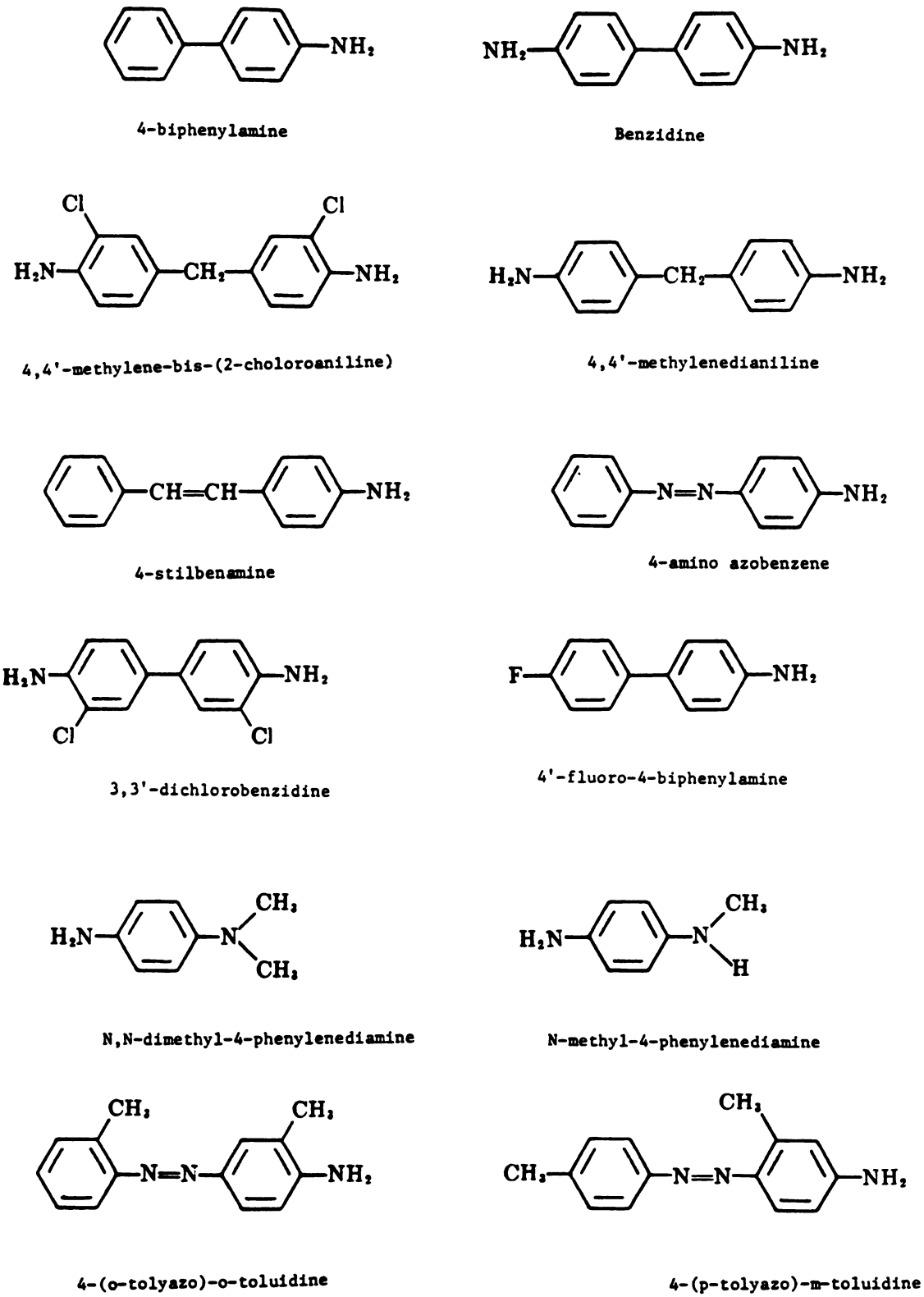

FIG. 2. *Molecular structure of various extended anilines and aminoazo and other azo compounds.*

the lowest negative value as E_{H1}, E_{L1} is the energy corresponding to the $(n + 1)$th MO.

Most reactive isomer. The majority of biological reactions in an electron-donating species are liable to take place at the position and in the direction where the HOMO predominates in the interaction; in similar cases in an electron-accepting reaction, the LUMO is at its maximum. In Table 1, the EAAs with amino group in the extended *para*-positions all have least negative value of E_{H1}, and most positive value of E_{L1}. In electrophilic reactions, an electron is removed from occupied MOs, preferably from the HOMO of the reactant. In nucleo-

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TABLE 1

Calculated molecular quantities of extended anilines and aminoazo compounds

Compound	E_{H1}	E_{L1}	$-E_{CN}$
3,3′-Dichloro-2-benzedine	−0.363	0.303	17.593
3,3′-Dichloro-3-benzedine	−0.375	0.291	16.927
3,3′-Dichloro-4-benzedine	−0.309	0.370	16.188
2-Aminobiphenyl	−7.508	0.607	17.750
3-Aminobiphenyl	−7.647	0.606	17.663
4-Aminobiphenyl	−7.406	0.768	17.798
2,4′-Dimethylene-bis-(2-chloroaniline)	−0.686	0.291	15.709
3,4′-Dimethylene-bis-(2-chloroaniline)	−0.497	0.491	15.518
4,4′-Dimethylene-bis-(2-chloroaniline)	−0.187	0.617	11.398
2,4′-Methylenedianiline	−0.220	1.125	16.696
3,4′-Methylenedianiline	−0.210	1.121	16.738
4,4′-Methylenedianiline	−0.121	1.220	17.353
2-Aminostilbene	−7.409	0.417	17.743
3-Aminostilbene	−7.611	0.386	17.745
4-Aminostilbene	−7.381	0.555	17.794
2-Methyl-2-Stilbeneamine	−7.513	0.387	17.550
2-Methyl-3-Stilbeneamine	−7.558	0.341	17.524
2-Methyl-4-Stilbeneamine	−7.371	0.521	17.715
4′-Fluoro-2-stilbeneamine	−7.512	0.350	17.746
4′-Fluoro-3-stilbeneamine	−7.687	0.270	17.717
4′-Fluoro-4-stilbeneamine	−7.448	0.437	17.770
2-Aminobenzidine	−7.088	0.837	17.334
3-Aminobenzidine	−7.349	0.780	17.518
4-Aminobenzidine	−6.981	0.693	17.729
N-Methyl-4-phenylenediamine	−6.941	1.213	17.282
N-Methyl-3-phenylenediamine	−7.432	1.143	17.281
N-Methyl-2-phenylenediamine	−7.209	1.111	17.221
2,4′-Diaminostilbene	−7.097	0.638	17.700
3,4′-Diaminostilbene	−7.351	0.620	17.731
4,4′-Diaminostilbene	−7.071	0.789	17.648
2-Amino-4′-fluorobiphenyl	−7.597	0.470	17.392
3-Amino-4′-fluorobiphenyl	−7.766	0.387	17.551
4-Amino-4′-fluorobiphenyl	−7.470	0.613	17.812
N,N′-Dimethyl-2-phenylenediamine	−7.166	0.689	15.240
N,N′-Dimethyl-3-phenylenediamine	−7.383	0.766	15.824
N,N′-Dimethyl-4-phenylenediamine	−6.926	0.827	15.495
2,2′-Dichloro-2,4′-diaminostilbene	−0.033	0.464	17.270
2,2′-Dichloro-3,4′-diaminostilbene	−7.535	0.010	17.349
2,2′-Dichloro-4,4′-diaminostilbene	−0.016	0.560	17.387
3,3′-Dichloro-2,4′-diaminostilbene	−0.090	0.016	16.176
3,3′-Dichloro-3,4′-diaminostilbene	−0.292	0.047	16.254
3,3′-Dichloro-4,4′-diaminostilbene	−0.497	0.197	15.966
4-(Phenylazo)aniline	−5.959	0.110	18.034
3-(Phenylazo)aniline	−6.099	0.733	17.458
2-(Phenylazo)aniline	−6.026	0.063	17.929
2-(*o*-Tolyazo)-*o*-toluidine	−6.125	0.797	17.583
3-(*o*-Tolyazo)-*o*-toluidine	−6.087	0.772	17.470
4-(*o*-Tolyazo)-*o*-toluidine	−6.019	0.050	17.668
2-(*p*-Tolyazo)*m*-toluidine	−6.084	0.855	17.663
3-(*p*-Tolyazo)*m*-toluidine	−6.051	0.766	17.428
4-(*p*-Tolyazo)*m*-toluidine	−6.011	0.660	17.678

philic reactions, electrons are transferred into unoccupied MOs, most dominantly into the LUMO of the electrophile. The lower value of E_{H1} indicates that an electron can be knocked out with ease from the HOMO of an EAA. High positive value of E_{L1} conforms to the possibility of nucleophilic reaction which involves large charge transfer from occupied MOs of DNA bases to the unoccupied MOs of EAA metabolite. All these are consistent with predictions by Klopman's generalized perturbation equation (15). According to the latter, a so-called hard nucleophile will react with an electrophile at that position carrying the greatest positive charge, while a soft nucleophile will be directed to the position at which the LUMO coefficient of it (e.g., electrophile) has its greatest numerical value. The charge transfer to the electrophile brings about an isolation of the nitrogen with respect to the neighboring atoms and causes the stretching of the bond between nitrogen of EAA metabolite and one of the DNA bases whose charge density provides a major contribution to the formation of covalent adduct. An destabilization of the bonding MOs and stabilization of the antibonding MOs follow from the loosening of the bond in the bond region concerned. Further, the charge transfer causes the elevation of the HOMO and the lowering of the LUMO, which in the case of EAAs with an amino substituent at the terminal carbon atom of the longest conjugated chain are the maximum. Thus, most strikingly, our finding agrees with the experimental observation (6) of Arcos and Argus: "for the highest level of carcinogenic activity obtainable with the di- and tricyclic aromatic amines, the amino substituent must be introduced at the terminal carbon atom of the longest conjugted chain." We have considered only monocyclic and bicyclic amines, and all of them with an amino group in the *ortho* and *meta* positions are either inactive or much less reactive as compared to those stated above.

E_{CN} is the two-center energy (16) between skeleton carbon atom and amino group nitrogen atom, one directly bonded to the other. This is presented in column 4 of Table 1. As postulated before (17), the highest reactivity of a C-N bond corresponds to the lowest value of E_{CN} for it. E_{CN} values in Table 1 show that EAA activities are indeed quite dependent on the site of NH_2 substitution. The substitution in the extended *para* position greatly enhances the activities. While in the *ortho* positions, it, however, adversely affects the activities. This is mainly due to steric hindrance from the central carbon and/or nitrogen atoms of the same ring or from the carbon and hydrogen atoms of the adjacent ring.

Metabolic activation. Calculted atomic charge distributions of some representative EAAs and of their respective ANIs are depicted in Fig. 3. In an earlier investigation (18), we have formulated the bond reactivity index $R_{AB}(\pi)$. The index represents the susceptibility of the A-B bond to reaction with nucleophilic centers of DNA bases. Based on these charge distributions and $R_{AB}(\pi)$, a mechanistic approach leading to biological events, viz., metabolic transformations, competing ring hydroxylation and epoxidation, the electrophilic behaviors of ANIs, and possibly others, can be realistically outlined. It would necessitate these hypotheses. 1) All unsubstituted ring positions carrying NNC and having no electronegative group or atom that is substituted in the neighboring position(s) or no neighboring skeleton carbon that exhibits NNC are susceptible to ring hydroxylation. Obviously, the higher the value of NNC, the higher would be the likelihood of the hydroxylation. 2)An A-B bond with higher (e.g., less negative) value of $R_{AB}(\pi)$ is more susceptible to epoxidation. EAAs for which

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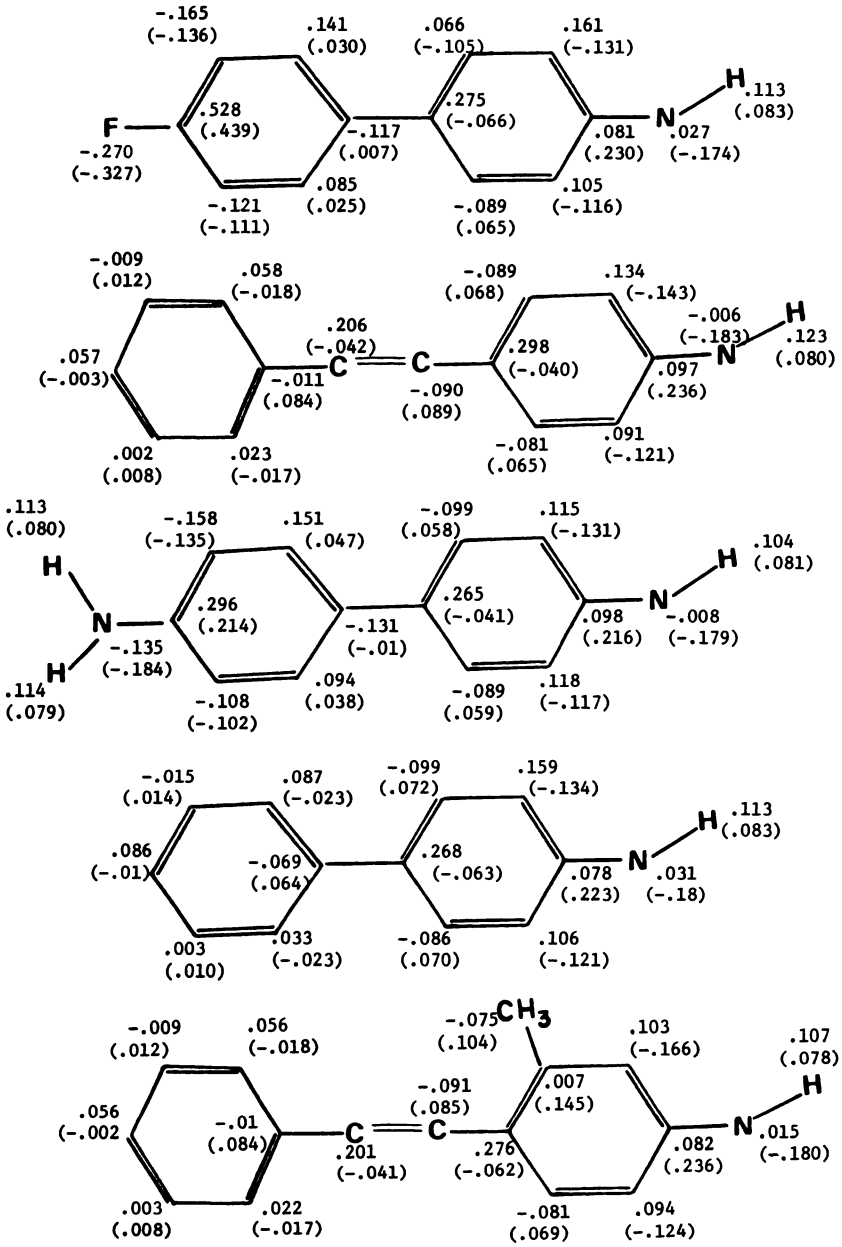

FIG. 3. *Net atomic charges on the atoms of some representative arylnitrenium ions*
The entries in parentheses correspond to similar charges of the respective parent EAAs. The charge against CH_3 is the charge of the methyl group as a whole.

$R_{AB}(\pi)$ corresponding to all peripheral unsaturated bonds are almost identical and are very close to −0.10 would lack a preferred site of epoxidation. By this we mean the bond that would readily rearrange to give rise to an electrophilic species capable of binding to DNA, RNA, and protein. 3) An NH_2 substitution would undergo *N*-hydroxylation provided that the NNC of the N atom is quite high (see Fig. 3).

On the basis of our hypothesis, hydroxylation can hardly occur at positions *ortho* to the amino groups. Formation of phenol at these positions are hampered if not inhibited due to repulsive interactions with atoms of the NH_2 substitution. Thus, positions 2′ and 4′ are the most appropriate phenolic sites in 4-BPA. That *N*-hydroxylation is a major metabolic route for the compound is evident from very high NNC of the N atom of the NH_2 group. Generally, ring hydroxylation produces compounds which are less carcinogenic (19, 20) than those by *N*-hydroxylation. Phenolization is thus a detoxification route (21), the carcinogenicity being dependent on how predominantly it affects the activation of the compound. 4-BPA induces bladder tumors in rabbits in a period of 2 to 5 years; it is weakly carcinogenic (22). The weakness is rooted in the detoxificaion of the compound through the 2′- and/or 4′-position. When detoxification is weakened due to blocking position 4′ by an F atom, the compound acquires much higher carcinogenicity, which is comparable to that of benzidine. In the latter,

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TABLE 2

Calculated values of $R_{AB}(\pi)$ corresponding to various peripheral A-B bonds of some representative EAAs

EAA	Bond A-B					
	1–2	1′–2′	2–3	2′–3′	3–4	3′–4′
4-Aminobiphenyl	−0.1169	−0.1133	−0.1100	−0.1089	−0.1065	−0.1064
4-Aminostilbene	−0.1143	−0.1151	−0.1089	−0.1197	−0.1076	−0.1081
(4-Amino)benzidine	−0.1149	−0.1149	−0.1170	−0.1170	−0.1179	−0.1179
4,4′-Methylenedianiline	−0.1145	−0.1145	−0.1166	−0.1166	−0.1169	−0.1169
4-Phenyazoaniline	−0.1134	−0.1146	−0.1173	−0.1168	−0.1150	−0.1153
4′-Fluoro-4-aminobiphenyl	−0.1072	−0.1070	−0.1125	−0.1137	−0.1048	−0.1040

position 4′ is blocked by an amino group. The net charges at the 4′-position in 2-, and 3-BPAs are −0.09 and −0.12, respectively. The phenol formation which is favored by this charge distribution is highly detoxifying to neutralize almost entirely the *N*-hydroxylation mediated electrophilicity of 2-, and 3-BPAs. Our calculations demonstrate that positions 4′ and 2′ are the most important phenolic sites in 4-aminostilbene (4-AS). These sites are not changed due to methylation of position 2 of the compound. The derivatives of BPA and AS illustrate some of the apparent generalizations that apply to other two-ring aminoazo compounds and extended anilines.

Calculated values of $R_{AB}(\pi)$ corresponding to unsaturated peripheral bonds of EAAs appear identical and are close to −0.10 (Table 2). As mentioned in the above hypothesis, this stems from inability of the bond to undergo a preferred epoxidation. Ring epoxidation of EAAs occurs by direct addition of the electrophilic oxygen across the ring C-C bond, and perpendicular to it. The endoplasmic reticulum membranes that are responsible for epoxidation necessitate stronger overlap of π-orbitals of the combining carbon atoms together with a supplement of $NADPH_2$ for its mixed function oxidases. Orbital charge distributions of EAAs are not asymmetric enough to facilitate direct insertion of the atmospheric oxygen into certain prefered C-C bond(s).

Relative carcinogenic potencies. Loew *et al.* (23) employed π-superdelocalizability indices in order to compute the mutagenic potencies of a series of fused ring PAAs. Unfortunately, these data from experiments are not available for EAAs. Nevertheless, we define a new parameter $\rho_N(\pi)$ as a measure of reltive carcinogenicities of the compounds, which are given by

$$\rho_N(\pi) = \sum_{j=1}^{n} c_j^N(\pi)$$

where n is the sum of all occupied MOs, and $c_j^N(\pi)$ is that part of the charge density which is associated with the π-orbital nitrogen atom of the substituted amino group. It may be noted that $\rho_N(\pi)$ is not a superdelocalizability index, but a measure of the resultant electronic occupancy of the π-orbitals of the N atom. In order to be susceptible to reactions with negative centers of DNA bases, an electrophile should exhibit lower value of this occupancy. Since we consider only the occupied MOs, this would be represented sufficiently by $\rho_{AN}(\pi)$, the absolute value of $\rho_N(\pi)$. The values of $\rho_N(\pi)$ of various EAAs are listed in Table 3. These values correspond to MRIA as determined by E_{H1} and E_{L1}, respectively. In an earlier investigation,[2] we observed that the values of E_{L1} nicely correlate with the relative carcinogenic potencies of methylbenzo(*a*)pyrenes. The values of E_{L1} of the MRIA are included in Table 1 to see if similar correlations hold also in the case of EAAs. Based on the assumptions that higher value of E_{L1} and/or higher absolute value of $\rho_N(\pi)$ [for instance, $\rho_{AN}(\pi)$] corresponds to lower carcinogenicity of EAAs, 4-aminobiphenyl, 4,4′-methylenedianiline, etc. are weakly carcinogenic. 4,4′-Methylenedianiline is hepatotoxic but there is no evidence that it is truely carcinogenic. When injected into rat, neither *N*-methyl-4-phenylenediamine nor *N,N*′-dimethyl-4-phenylenediamine induces tumors (2). The values of both $\rho_{AN}(\pi)$ and E_{L1} for these compounds are quite large. 4-(*o*-Tolyazo)-*o*-toluidine is highly carcinogenic in hamsters, dogs, and possibly rabbits (2). It is more carcinogenic in mice than in rats, leading to hepatomas, pulmonary adenomas, and hemangio-endotheliomas in several tissues. Very high activity of the compound is represented by quite small values of $\rho_{AN}(\pi)$ and E_{L1}. Five positional isomers of 4-(*o*-tolyazo)-*o*-toluidine were tested for carcinogenecity in various groups of rats

TABLE 3

Relative carcinogenic potencies of various EAAs

EAA	ρ_N	E_{L1} (*eV*)
4-Aminobiphenyl	1.6587	0.768
4-Aminostilbene	0.2817	0.555
4′-Fluoro-4-aminobiphenyl	0.3019	0.613
4,4′-Diaminostilbene	−1.8999	0.789
4′-Fluoro-4-aminostilbene	−0.0548	0.437
2,2′-Dichloro-4,4′-diaminostilbene	0.4364	0.560
3,3′-Dichloro-4,4′-diaminostilbene	0.1471	0.197
2-Methyl-4-aminostilbene	−0.5714	0.521
	−0.5140	0.491
3-Methyl-4-aminostilbene(4-amino)-benzidine	−0.5805	0.993
3,3′-Dichloro-(4-amino)benzidine	0.9660	0.370
2,2′-Dichloro-(4-amino)-benzidine	0.5553	0.344
4,4′-Methylenedianiline	−0.9698	1.220
4,4′-Dimethylene-bis(2-chloroaniline)	0.1189	0.617
N-methyl-4-phenylenediamine	−0.5511	1.213
N,N′-Dimethyl-4-phenylenediamine	0.6455	0.827
4-(*o*-Tolyazo)-*o*-toluidine	−0.1398	0.050
4-Phenylazoaniline	−0.1315	0.110
2-(*o*-Tolyazo)-*p*-toluidine	−0.2712	0.233
4-(*p*-Tolyazo)-*m*-Toluidine	0.7757	0.660

[2] S. Noor Mohammad, unpublished data.

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and mice. Only 2-(*o*-tolyazo)-*p*-toluidine was carcinogenic to the liver of the animals while 4-(*p*-tolyazo)-*m*-toluidine produced hepatomas in mice. Comparatively larger values of $\rho_{AN}(\pi)$ and E_{L1} for the latter suggest that it is perhaps weakly carcinogenic. The amino substitution does not belong to the extended *para* position in 2-(*o*-tolyazo)-*p*-toluidine. We calculated $\rho_{AN}(\pi)$ and E_{L1} for this compound and found that they belong to the intermediate range (see Table 2). This confirms the experimental observations (2) that the compound is moderately carcinogenic. 4-Phenylazoaniline is not yet proved to be carcinogenic. Surprising, both $\rho_{AN}(\pi)$ and E_{L1} for this compound are quite low. These are indications that the compound should be highly reactive. As shown in Fig. 1, phenol formation belongs to the detoxification route of EAA metabolism. This detoxification occurs through aromatic ring positions, particularly position 4′ of the compounds. Substitution of F or an NH_2 group at this 4′ or at neighboring positions blocks the detoxification. Obviously, the blockade is better provided by the more electronegative F atom than by the NH_2 group. This is evident from the results from $\rho_{AN}(\pi)$ and E_{L1}. These results demonstrate that 4′-F-4-BPA is more carcinogenic than (4-amino)benzidine (AB). Due to further blockade of M and M′ (M=M′ = 2,3) positions by Cl atom M,M′-dichloro-AB is more carcinogenic than AB itself. The presence of a Cl atom both at 3- and 3′-positions accompanies repulsive interactions with the neighboring NH_2 groups of 4- and 4′-positions, respectively. The interaction leads to reduction in electronegativity both of the Cl atom and of the NH_2 group. This justifies why 3,3′-dichloro-AB is less reactive than 2,2′-dichloro-AB. The reactivity of the latter, of course, is not very high as compared to that of AB. Positions 2 and 2′ of AB do not correspond to active phenolic sites. Any blockade of these sites thus adds nothing to the activation of AB. Carcinogenicity of AB and its derivatives is in line with predictions by values of E_{L1} and $\rho_{AN}(\pi)$. 4-AS causes cancers in breast and in ear duct (2). Our calculations predict that it is moderately carcinogenic. Fluorine substitution at position 4′ or methyl substitution at position 2 or 3 leads to an increase of its activity. Very high values of calculated parameters seem to indicate that 4,4′-diaminostilbene is weakly carcinogenic. A less convincing preliminary report (2) describes it to induce tumors in liver. We notice that substitutions of Cl atoms at the 2- and 2′- or at 3- and 3′-positions increase its activity; the increase in the case of 2, 2′ substitution is more than in the case of 3, 3′ substitution. The NH_2 substituent at position 4′ is weakly electronegative and a charge distribution from the neighboring 3′-Cl atom assists it to block more effectively the detoxification.

4-Phenylenediamine and *N*,*N*′-dimethyl-4-phenylenediamine are single ring aminoazo compounds. Tests with chemicals like hydrochlorides of aniline or *m*-toluidine indicate that they are incapable (2) of inducing tumor in experimental animals. The lack of carcinogenic potency of these compounds can be explained in the light of charge distributions in their corresponding ANIs. When these distributions are examined, the net charges in their NH_2 group nitrogen atoms are found slightly positive. The net electrophilicities possessed by them are, therefore, marginally favorable for binding to DNA, RNA or protein.

Interaction between EAA and DNA. The LUMO of various EAAs is a delocalized π-orbital with insignificant charge density on the nitrogen atoms. In ANIs, these atoms all have quite low positive or negligibly negative net atomic charges. Interestingly, the charge negativity of the nitrogen is in general higher in systems with a larger ring system. We assume that among these systems those with less electrophilic nitrogen exhibit the lower carcinogenic activities. The assumption is consistent with experimental observations (24) that electrophiles which result from bond-breaking mechanisms in chemical carcinogens are capable of forming covalently bound carcinogen residues through substitution reactions. There is no experimental evidence of other forms of stabilization though the possibility that the tight noncovalent bindings may be as important as covalent reactions cannot be ruled out.

The results given in Fig. 3 show that the carbon atom directly attached to the nitrogen and the two ring carbon atoms which form single and double bonds with the former have a much higher positive charge than the nitrogen atom itself. These carbon atoms should therefore be involved in covalent interaction with the nucleophilic centers of DNA bases. Experimental observations indicate that nucleophilic centers are indeed available in both the nucleic acids and proteins, and both the nucleic acid- and protein-bound derivatives are formed through interactions of the regions of large negative potential above and in the planes of the former with the positive ring carbon atoms and with the nitrogen of EAAs. The ANIs thus function as ambient electrophiles which can undergo both electrostatic and covalent binding with nucleophilic centers of proteins and DNA bases. Our conclusion is in line with *ab initio* predictions (25) that, for guanine and adenine electrostatic potential maps, there are regions of large negative potential available around the base pairs.

CONCLUSIONS

Scribner *et al.* (26) and Loew *et al.* (23) attempted to correlate qualitatively hydroxylamine ester reactivity and nitrenium ion stability with carcinogenicity and mutagenicity of a number of PAAs. Recent calculations by Hartman and Schlegel (27) indicate that the reactivity of the hydroxylamine ester as given by the stability of the singlet nitrenium ion versus that of the parent amine does not correlate with the observed carcinogenic potential. These calculations, however, revealed that hydroxylamine esters arising from nonmutagenic amines are less reactive than those from genotoxic amines. In the present calculations, we have attempted to find out the EAA isomer NH_2 substitution which leads to induction of cancer. The finding agrees remarkably well with experiments. There is no experiment to justify accuracy of the predicted relative carcinogenicities of the compounds. However, calculated data closely follow the physicochemical and biological reasonings of relative carci-

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nogenicities of the compounds. This following is a reflection perhaps of a realistic formulation of the addition-rearrangement of the electrophilic oxygen as a possible mechanism for *N*-hydroxylation.

The earlier calculations are based primarily on the assumption that *N*-hydroxylation represents the major metabolic path that leads to EAA activation to carcinogenic ANIs. Our investigation rigorously establishes it in terms of charge densities of the atoms and in the framework of postulated hypothesis put forth in the previous section. According to our theory, *N*-hydroxylation and ring hydroxylation correspond respectively to the activating and deactivating paths, and the carcinogenecity of the compound depends on the predominance of the *N*-hydroxylation over the ring hydroxylations. These are largely in line with the experimental findings.

In our report, calculated data correspond to optimized geometry of benzene as described above. However, since the present investigation aims at developing relative carcinogenic properties of EAAs, it is practically irrelevent if we use optimized or unoptimized geometries in the calculations, and if our optimized geometries compare remarkably with the experiment. The theory requires only that the geometries must all be constructed in a consistent manner. Our results should be the same with any reasonable choice of bond lengths for the benzene fragment. However, we used MINDO/3-optimized geometries to ensure improved consistency of EAA results determined by the same method.

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