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Relative Preferences of Carcinogenic Aromatic Nitrenium Ions for Adduct Formation with Different DNA Base Sites: Theoretical Elucidations

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Abstract. The ultimate carcinogenic form of aromatic amines, the nitrenium ions, interact with DNA bases in order to exert their carcinogenic effects.

Experimental findings have indicated the general trend to produce N (deoxy guanin-8-yl) as the major adduct and the minor adduct involving the extra cyclic N^2 of guanine and the carbon in a β-position of the amine nitrogen.

The adduct formation with DNA bases by aromatic amines has been studied with several contributing factors in view. The theoretically computed values of these factors serve as clues to rationalize experimentally observed findings concerning this adduct formation.

Key words: Nitrenium ions, DNA base sites, interaction energy, no calculation.

1. Introduction

Aromatic amines have the potential of eliciting a variety of mutagenic and carcinogenic responses [1]. All aromatic amines require metabolic activation into a strong electrophilic reactant to be able to bind chemically to the nucleophilic sites in the target [2]. N-Oxidation is considered to be the initial activation step in carcinogenesis. Aryl nitrenium ions have been postulated as the ultimate carcinogens of aromatic amines [3].

One of the best studied interactions of carcinogens with macro molecules is that of covalent attachment of the reactive metabolites of aromatic amines (aryl nitrenium ions) to different sites of DNA bases [4].

The major product, in general, in the case of carcinogenic aromatic amines were detected as N (deoxyguanosin-8-yl) and the lesser amount of adduct was identified where the ring carbon adjacent to the nitrogen of aryl nitrenium ion is covalently bonded at N^2-guanine position of guanine [5].

Theoretical calculations have been done previously on activation of aromatic amines and their interaction with DNA bases [6–8].

In the present study DNA modification by the ultimate carcinogenic form of 2-naphthyl amine (2NA), 4-aminobiphenyl (4AB), benzidine (BZ), 2-acetyl-

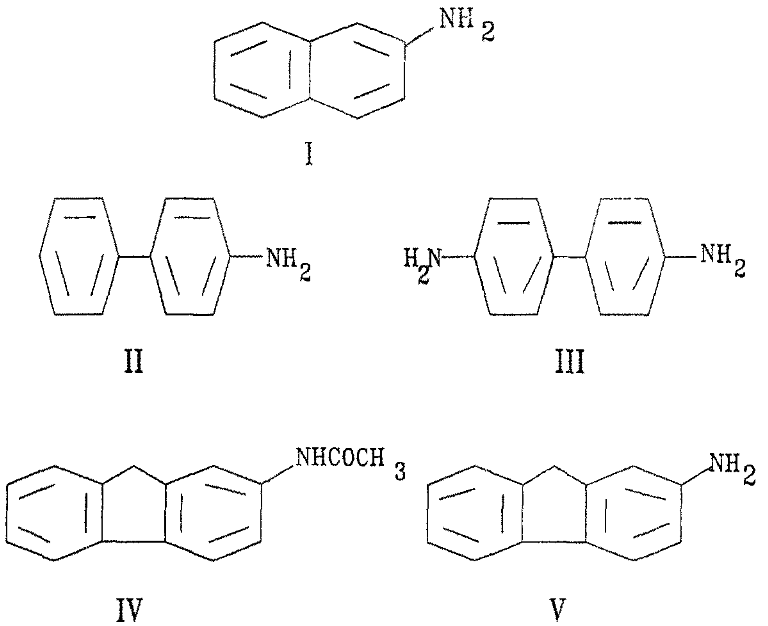

Fig. 1. Different aromatic amines studied: 2-naphthylamine (I), 4-aminobiphenyl (II), benzidine (III), 2-acetylaminofluorene (IV), 2-aminofluorene (V).

aminofluorene (2AAF), 2-aminofluorene (2AF) (Fig. 1), where the carcinogenicity of the species is well established [9], was studied using the semiempirical MNDO SCF–MO methodology, focusing on carcinogen–DNA reactivity in the isolated reactants.

Reactive sites on the ultimate carcinogen included the nitrenium N-site as well as the carbon just proximate to the C–N bond. It was found that while nitrogen–nitrogen or carbon–carbon affinity were not the best favoured, carbon–nitrogen and nitrogen–carbon affinity was appreciably high.

2. Theoretical Procedures

Semiempirical MNDO [10,11] calculations were performed using MOPAC Package [12] subjecting all molecular species to full geometry optimization with no constraint. In the case of amines and nitrenium ions the trial geometries are calculated from standard bond lengths and angles [13].

2.1. INTERACTION ENERGY BETWEEN NITRENIUM IONS AND DNA BASE SITES

Electrophilic attack of nitrenium ions to DNA bases is conventionally treated by Pearson's principle of hard and soft acids and bases [14]. The approach used here to calculate non-bonded affinities between the electrophile and the nucleophile stems from the approach of Klopman [15]. The concepts of charge and frontier-controlled aspects of reactivity were introduced.

In the simplified form of the energy of interaction E_{ab} between an electrophile A and a nucleophile B may be expressed as a sum of an electrostatic term E_{es} and a covalent term E_{fo}:

$$E_{ab} = E_{es} + E_{fo} \tag{1}$$

Electrophile A here corresponds to the UC site, and the nucleophile B corresponds to the DNA base site.

$$E_{fo} = -FK \tag{2}$$

where,

$$F = \sum_{m}^{A} \sum_{n}^{B} (2C_n C_m)^2 / E_a - E_b \tag{3}$$

C_m and C_n being coefficients of atomic orbitals m and n for atoms in A and B, E_a, E_b refers to the energies of appropriate LUMO and HOMO orbitals in A and B respectively. It is assumed that E_{fo} would depend on the value of F; K is a constant. And

$$E_{es} = \sum_{a}^{A} \sum_{b}^{B} Q_a Q_b / D R_{ab} \tag{4}$$

Where Q_a and Q_b are the point charges on atoms a and b summed over molecules A and B respectively, separated by a distance R_{ab} (taken constant $3A$ for all the cases), D being the dielectric constant. Vacuum phase is assumed here for simplicity in the absence of precise knowledge of D for biological media.

2.2. INDICES CONCERNING REACTIVITY

Charge on relevant atoms (Q); net positive charge including hydrogen attached (as this collective charge would make itself felt keenly upon the nucleophilic sites by the reason of proximity); energy of the highest occupied molecular orbital (E_{HOMO}) and energy of the lowest unoccupied molecular orbital (E_{LUMO}) involving relevant atoms and frontier electron density Q_f which is given by the equation:

$$Q_f = C_s^2 + C_{px}^2 + C_{py}^2 + C_{pz}^2 \tag{5}$$

TABLE Ia. MNDO calculated FMO and ED indices for aromatic nitrenium ions (N-site of amines)

Species	Q_N	Q_{NH}	Q_f	E_N^{LUMO}
2NA$^+$	−0.033	0.102	0.139	−7.16
4AB$^+$	−0.046	0.094	0.138	−6.91
BZ$^+$	−0.090	0.039	0.092	−6.15
AF(2)$^+$	−0.063	0.073	0.114	−6.76
AAF(2)$^+$	−0.164	–	0.187	−6.97

TABLE Ib. MNDO calculated FMO and ED indices for C-sites (adjacent to amine group attached) of carcinogenic aromatic nitrenium ions

Species	Q_C	Q_{CH}	Q_f	E_C^{LUMO}
2NA$^+$	0.188	0.288	0.310	−7.16
4AB$^+$	0.153	0.273	0.286	−4.14
BZ$^+$	0.103	0.213	0.271	−3.29
AF(2)$^+$	0.153	0.272	0.241	−3.98
AAF(2)$^+$	0.161	0.276	0.271	−4.18

C's are the coefficients of atomic orbitals.

2.3. THE MOLECULAR ELECTROSTATIC POTENTIAL MINIMA ASSOCIATED WITH THE DNA-BASE SITES

Pullman *et al.* [16] have computed tha values, in descending order of magnitude: C8-G > N^2-G. The NH_2 group is generally considered as secondary site for electrophilic attacks, where the energy minima associated with the isolated base guanine corresponds to a repulsion.

2.4. THE STERIC ACCESSIBILITIES ASSOCIATED WITH THE DNA-BASE SITES

Using a water molecule probe, steric accessibilities have been calculated by Lavery *et al.* [17], the order of magnitude being again C8-G > N^2-G.

3. Results and Discussion

Tables 1a and 1b present the MNDO values of electron distribution (ED) and frontier molecular orbital (FMO) indices for N-site and C-site (adjacent to the amine

TABLE II. MNDO calculated FMO and ED indices and steric accessibility (double helix) for different sites of DNA bases

Site	Q_b	E^{HOMO}	C^{HOMO}	SA
oxygen site				
O^6-G	−0.313	−11.541	0.587	2.6
O^4-T	−0.330	−11.357	0.641	2.2
O^2-C	−0.333	−11.626	0.508	0.0
nitrogen site				
N7-G	−0.146	−11.421	0.370	4.1
N^2-G	−0.251	−11.822	0.622	0.0
N3-A	−0.287	−12.774	0.525	0.7
N^6-A	−0.249	−11.344	0.501	0.0
carbon site				
C8-G	0.052	−8.853	0.450	1.0
C8-A	0.096	−8.963	0.404	0.9

nitrogen) of different aromatic nitrenium ions. A good demarcation is afforded by the FMO and ED indices for electrophilicity of these two sites.

The order of electrophilic reactivity is in descending order of magnitude: C-site > N-site for all aromatic nitrenium ions ($\mathrm{ArNH^+}$) taken into account.

Electrophilicity is closely linked with hardness of acidity. So the Hard–Soft Acid–Base (HSAB) argument shows the C-site of $\mathrm{ArNH^+}$ is harder acid than that of the N-site.

Table II gives MNDO values for the Mulliken atomic charge Q_b, the coefficient of the HOMO C_{HOMO}, appropriate HOMO energy level E_{HOMO} and steric accessibility (SA, in double helical structure) for three sets of O-sites (O^6-G, O^4-T, O^2-C), four sets of N-sites (N7-G, N^2-G, N^6-A, N3-A) and two sets of C-sites (C8-G, C8-A) of DNA bases (Fig. 3). The Q_b and Q_f (eq.5) indices for nucleophilicity as well as hardness reveal that the order of magnitude is, O-sites > N-sites > C-sites.

C–N bond distance for all the $\mathrm{ArNH^+}$ studied are lower (1.3 A) than the usual C–N single bond distance (1.4 A), pointed out by Ford [18], i.e. nitrenium ions can exist as imine form also (Fig. 2) which may take part in N^2-G adduct formation.

Klopman's equation predicts that a so-called hard nucleophile will add to an electrophilic site at the position carrying the greatest positive charge, while a soft nucleophile will be directed to the position at which the LUMO coefficient has its greatest numerical value. For alkylation it has been noted [19] that those agents that lead to higher proportions of O-alkylated products are those for which smaller

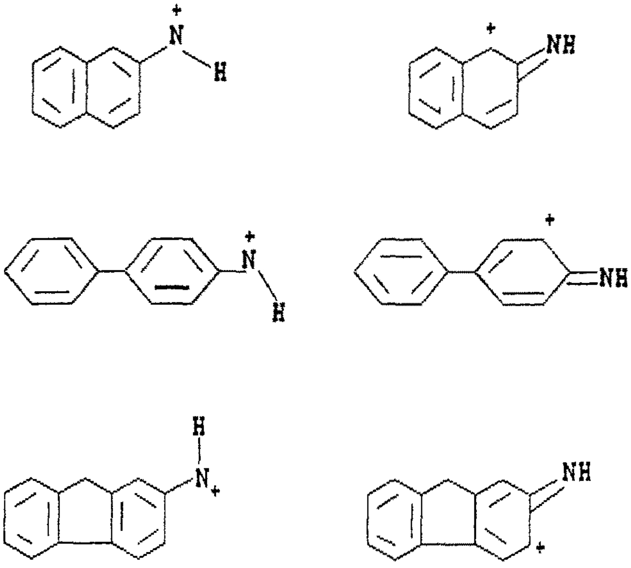

Fig. 2. Nitrenium ions (ultimate carcinogenic form) and their imine forms for different aromatic amines studied.

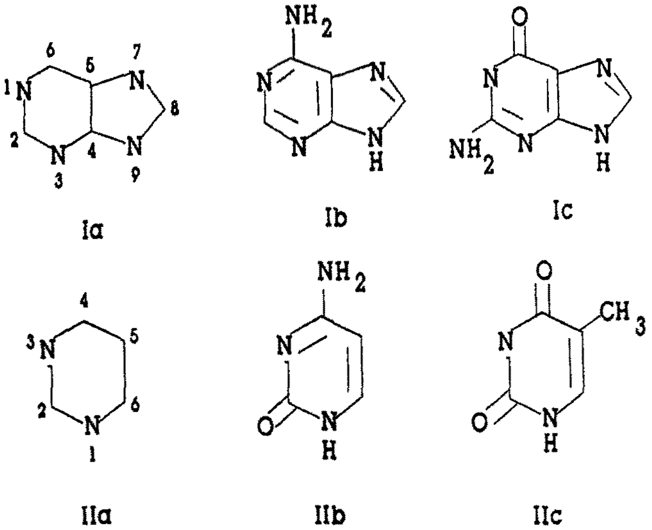

Fig. 3. Atomic numbering system for purine (Ia) and pyrimidine (IIa) bases and structure of adenine (Ib), guanine (Ic), Cytosine (IIb) and Thymine (IIc).

Swain–Scott substrate constants [20] have been measured. This is traditionally been taken to imply that these reactions are more 'S_N1-like'. The degree of S_N1 character for the DNA alkylation reaction is of consequence for *O*-specificity [21]. Experimental model reaction for aromatic amine and DNA-base adduct formation shows that reaction proceeds *via* S_N2 mechanism [22]. So the

TABLE IIIa. F terms (in a.u.) for C-sites of carcinogen with different sites of DNA bases

C-Sites	N^2-G	N7-G	N3-A	N6-A	C8-G	C8-A
2NA	5.47	0.73	1.42	1.71	2.59	0.81
4AB	1.94	0.49	0.84	0.91	0.89	0.55
BZ	2.49	0.42	0.72	0.97	0.72	0.91
AF(2)	1.68	0.41	0.70	0.75	0.73	0.92
AAF(2)	1.74	0.47	0.80	0.87	0.86	1.08

TABLE IIIb. F terms (in a.u.) for N-sites of carcinogen with different sites of DNA bases

N-Sites	N^2-G	N7-G	N3-A	N6-A	C8-G	C8-A
2NA	1.06	0.39	0.61	0.76	1.53	1.17
4AB	1.00	0.38	0.59	0.71	1.32	1.01
BZ	0.42	0.21	0.35	0.41	0.64	0.49
AF(2)	0.80	0.30	0.48	0.47	1.02	0.78
AAF(2)	1.36	0.54	1.56	0.99	1.86	1.42

affinity towards O-sites is not favoured by aromatic nitrenium ions. This inference fits in very well with the observations regarding adduct formation by aromatic nitrenium ions.

Comparison of the values of all these indices reveals that N-sites of $ArNH^+$ should have preference towards the C-site of DNA bases, whereas the C-site of $ArNH^+$ should have affinity to the N-sites of DNA bases (as the hard–soft acid–base argument would imply).

Tables IIIa and IIIb give the covalent interaction energy (F term in a.u., eq. 3) of N- and C-sites of $ArNH^+$ (Figs. 4, 5) with different N- and C-sites of DNA bases, showing the N-C8 guanine and C-N^2 guanine interactions are the best favoured.

TABLE IVa. E_{es} terms (in kcal) for C-sites of carcinogen with different sites of DNA bases

C-sites	N^2-G	N7-G	N3-A	N6-A	C8-G	C8-A
2NA	−7.56	−2.86	−5.68	−4.88	3.11	1.89
4AB	−7.18	−2.33	−4.58	−3.97	2.96	1.54
BZ	−5.58	−1.57	−3.09	−2.68	2.30	1.03
AF(2)	−7.15	−2.34	−4.59	−3.98	2.94	1.54
AAF(2)	−7.24	−2.45	−4.81	−4.17	2.98	1.61

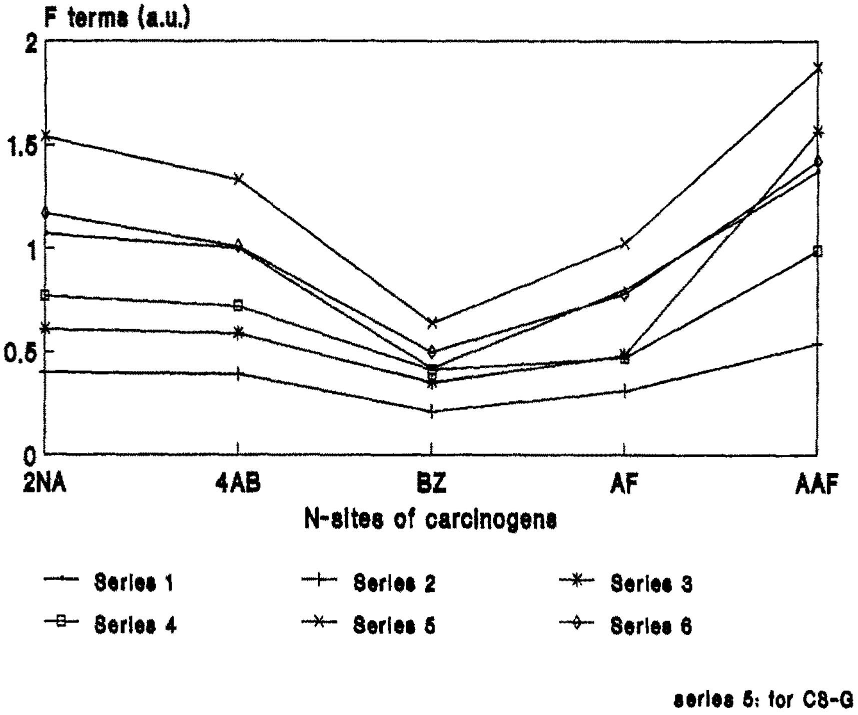

Fig. 4. Comparison of F terms (in a.u.) for N-site of carcinogens with different DNA-base sites.

TABLE IVb. E_{es} terms (in kcal) for N-sites of carcinogen with different sites of DNA bases

N-sites	N^2-G	N7-G	N3-A	N6-A	C8-G	C8-A
2NA	1.04	0.60	1.19	1.03	−0.40	−0.21
4AB	1.43	0.83	1.63	1.42	−0.55	−0.29
BZ	2.83	1.64	3.22	2.79	−1.04	−0.58
AF(2)	1.98	1.15	2.26	1.96	−0.55	−0.41
AAF(2)	5.17	3.00	5.90	5.11	−1.98	−1.06

Tables IVa and IVb present the electrostatic (E_{es} terms, in kcal, eq. 4) interactions which manifest that the energy associated with the C–C and N–N interactions correspond to a repulsion.

All these theoretical indicators point towards the specificity of N-site of $ArNH^+$ toward C8-G as well as the specificity of the C-site of $ArNH^+$ toward the N^2-G site of DNA and thus can help to furnish good rationalisations for experimental findings.

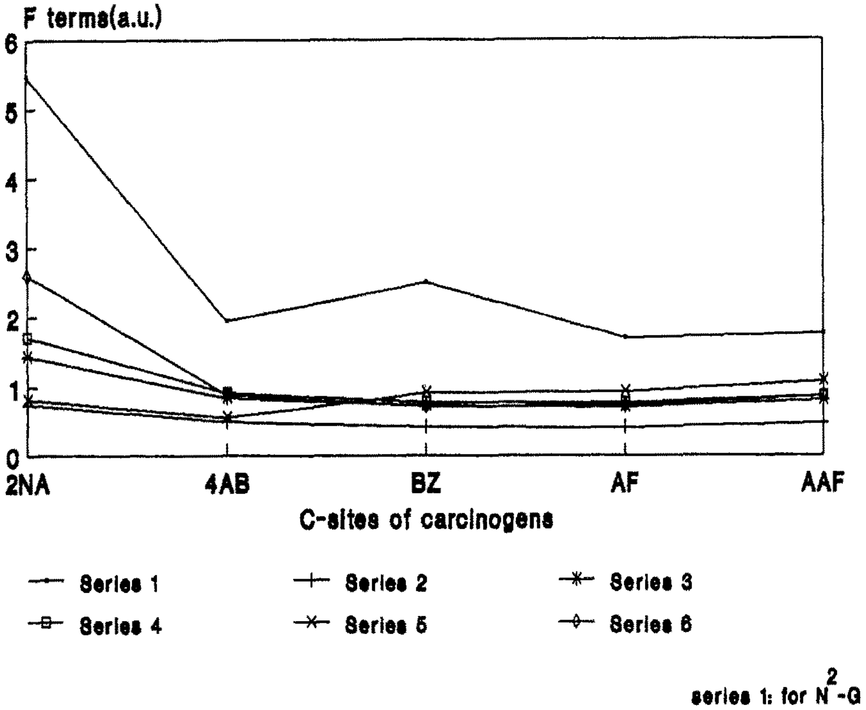

Fig. 5. Comparison of F terms (in a.u.) for C-site of carcinogens with different DNA-base sites.

4. Conclusion

Upon considering the results of this study the N(C8-G) and the C(N^2-G) adducts are explained on the basis of comparative assessment of the hard and soft contributions to reactivity.

Theoretical treatments of product distribution from the view point of the interaction energies point towards establishment of the N(C8-G) and C(N^2-G) interactions.

The above predictions arrived at by these calculations are in good accord with experimental observation.

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