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Mutagenicity of Dimethyl Heteroaromatic Triazenes in the Ames Test: The Role of Hydrophobicity and Electronic Effects

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SUMMARY

The mutagenicities of five heterocyclic 3,3-dimethyltriazenes have been evaluated in the Ames test. The octanol-water partition coefficients (*P*) for these triazenes have been measured, and their electron distributions and molecular orbital energies were calculated using the MNDO semiempirical molecular orbital method. Molecular structures of three triazenes have been determined using X-ray crystallography. The mutagenicities of these five triazenes, which range from nearly inactive to very highly mutagenic, are well predicted by quantitative structure-activity relationships that had been derived previously for the mutagenicity of aryltriazenes. The form of these equations indicates that more hydrophobic and more electron-rich triazenes are more active in the Ames test. This supports the hypothesis that the ease of initial triazene activation by cytochrome P-450 governs the mutagenicity of these compounds.

An essential step in understanding the causes of cancer is elucidating the chemical mechanisms by which mutagenic chemicals interact with DNA. The Ames test for mutagenicity in *Salmonella typhimurium* gives reproducible and quantitative estimates of relative mutagenic activity, making the test an excellent model system for studying the chemical mechanisms underlying the mutation process. Because the Ames test is also fairly inexpensive, it has become the *de facto* standard method for screening chemicals for their mutagenic activity.

Recently, we have shown that QSARs can be formulated for the mutagenic action of several families of chemicals in the Ames test (1–3). The QSARs are useful for identifying the structural features of a chemical that control its mutagenicity and, thus, give insight into the chemical mechanism of mutagenesis. These same QSARs are also valuable because they can predict the mutagenicity of untested chemicals before their introduction into the laboratory or marketplace.

Venger *et al.* (1) recently measured the mutagenicity of 18 substituted 1-methyl-1-alkyl-3-phenyltriazenes (compounds **1-18**) in the Ames bacterium *S. typhimurium* strain TA92 to which the S9 microsome preparation had been added. They found that the mutagenicities of 17 of these phenyltriazenes (compounds **2-18**) obey the QSAR given by Eq. 1.

$$\log 1/C = 1.04\ (\pm 0.17) \log P - 1.63\ (\pm 0.35)\ \sigma^{+} + 3.06 \quad (1)$$

$n = 17$; $r = 0.974$; and $s = 0.315$.

In this equation, C is the molar concentration of triazene that causes 30 mutations above background/10^8 TA92 bacteria, P is the octanol-water partition coefficient of the triazene, and σ^{+} is the Hammett substituent constant for reactions involving through-resonance. The statistical parameters associated with these equations are n, the number of data points; r, the correlation coefficient; and s, the standard deviation. Figures in parenthesis are for construction of the 95% confidence intervals.

More recently, we (4) have calculated the electronic structures of these same triazenes using the semiempirical MNDO method (5, 6). Our calculations yielded two parameters that, when combined with log P, give the QSARs shown in Eqs. 2 and 3.[2] The Hammett constant, σ^{+}, is replaced by either ϵ_{HOMO}, the energy of the triazene's HOMO in eV, or by q_{HOMO}, the electron density on N_1 in the HOMO.

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[2] Eqs. 2 and 3 differ slightly from Eqs. 3 and 4 of Ref. 4 due to the recalculation of the electronic structure of 1,1-dimethyl-3-(4-sulfonamidophenyl)triazene using MNDO 3.01 parameters for sulfur (6).

ABBREVIATIONS: QSAR, quantitative structure-activity relationship; DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; HOMO, highest occupied molecular orbital; MNDO, minimum neglect of differential overlap; P, octanol-water partition coefficient; q_{HOMO}, electron density on N_1 of triazene in the highest occupied molecular orbital; ϵ_{HOMO}, energy of the highest occupied molecular orbital; σ^{+}, Hammett substituent constant for through-resonance with a cationic center.

$$\log 1/C = 0.95\ (\pm 0.32)\log P + 1.91\ (\pm 0.89)\ \epsilon_{HOMO} + 19.85 \quad (2)$$

$n = 17$; $r = 0.912$; and $s = 0.571$.

$$\log 1/C = 0.92\ (\pm 0.36)\log P - 6.90\ (\pm 3.96)\ q_{HOMO} + 5.70 \quad (3)$$

$n = 17$; $r = 0.887$; and $s = 0.641$

Eqs. 1–3 describe highly similar relationships between phenyltriazene structure and mutagenicity. Namely, each QSAR shows that activity is enhanced for more hydrophobic and electron-rich triazenes. A mechanistic interpretation of these QSARs can be obtained by referring to the scheme in Fig. 1, the generally accepted mechanism for triazene activation and mutagenesis. In this scheme, initial hydroxylation of the *N*-methyl group by cytochrome P-450 is followed by spontaneous tautomerization and hydrolysis reactions leading to the formation of a reactive carbocation, R^+. Even though it is R^+ that actually reacts with DNA, Eqs. 1–3 do not reveal any correlation between the structure/stability of R^+ and triazene mutagenicity. The two factors that do correlate with increased mutagenicity, i.e., hydrophobicity and electron-donating substituents, are the same factors that favor triazene hydroxylation by cytochrome P-450 (7). Thus, the relative activity of the phenyltriazenes correlates with their initial activation rate.

The three QSARs do differ in their quality of fit and in their potential scope. Eq. 1, the σ^+ QSAR, gives a better correlation of phenyltriazene action than do either of the quantum chemical QSARs. The σ^+ QSAR, unfortunately, can only be applied to phenyltriazenes and, therefore, can only treat a limited range of mutagen structure and activity. The quantum chemical QSARs, on the other hand, should apply to a wider range of triazenes. Indeed, we have shown that Eqs. 2 and 3 accurately predict the mutagenicity of the antitumor drug DTIC (compound **19**) (4). This triazene not only contains an imidazole ring, it also is significantly more hydrophilic and less mutagenic than any of the phenyltriazenes, suggesting that Eqs. 2 and 3 can be extrapolated over a wide range of triazene structures and activities.

The successful prediction of the activity of DTIC suggested to us a broader study of triazene mutagenicity. We now report syntheses, partition coefficients, MNDO calculations, and mutagenic activities for five new heteroaromatic triazenes (Fig. 2) in S. *typhimurium* TA92 as a more general test of Eqs. 2 and 3. We also report X-ray crystallographic structures for three of the triazenes.

Materials and Methods

General. 2-Aminothiazole, 3-amino-1,2,4-triazole, and 2-aminobenzimidazole were obtained from Aldrich Chemical Co. (Milwaukee, WI). 5-Aminoindazole was purchased from Pfaltz & Bauer, Inc. (Waterbury, CT). Dimethylamine was obtained as a 40% aqueous solution from Sigma Chemical Co. (St. Louis, MO) and used without modification. 2-Nitrodibenzofuran (8) and 2-(3,3-dimethyl-1-triazeno)-1,3-thiazolidine (9) were prepared according to literature procedures. ^{1}H NMR spectra were obtained on a General Electric QE-300 spectrometer using dimethyl sulfoxide-d_6 as a solvent. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane. Elemental analyses were performed by Desert Analytics (Tucson, AZ).

2-(3,3-Dimethyl-1-triazeno)dibenzofuran (compound 21). Reduction of 2-nitrodibenzofuran with hydrogen over a Pd-C (5%) catalyst gave 2-aminodibenzofuran, m.p. 128–129°; literature m.p. 128° (10).

An ice-cold solution of sodium nitrite (1.0 g, 14 mmol) in 3 ml of water was added dropwise to a cold (−5°) suspension of 2-aminodibenzofuran (1.8 g, 9.8 mmol) in 8 ml of concentrated HCl and 10 ml of water. The resultant solution was added in portions to a stirred cold (−5°) solution of dimethylamine (5 ml of 40% solution, 40 mmol) and 5 g of sodium hydroxide dissolved in 20 ml of water. After stirring for 1 hr, the yellow product was washed with water and recrystallized (dilute ethanol) to give 0.9 g (40%) of triazene, m.p. 49–50°. Analysis: found (%) C, 70.17; H, 5.39; N, 17.44; calculated (%) C, 70.29; H, 5.44; N, 17.57. The structure of this triazene was verified by X-ray crystallography.[3]

3-(3,3-Dimethyl-1-triazeno)-1,2,4-triazole (compound 22). An ice-cold solution of sodium nitrite (1.72 g, 25 mmol) in 3 ml of water

[3] X-ray crystal structures of compounds **20–22** will be published elsewhere.

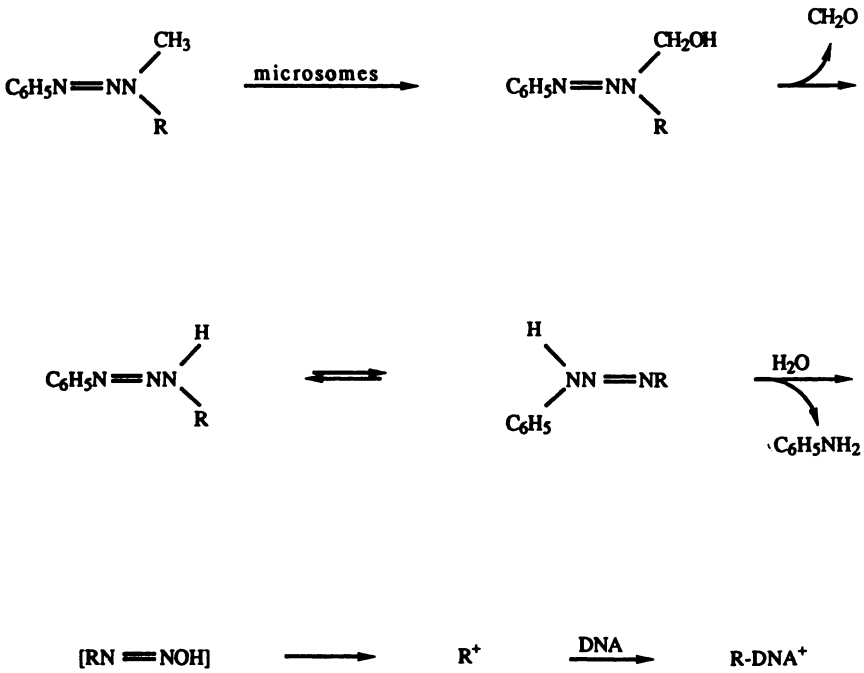
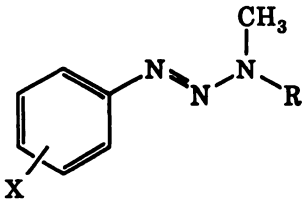
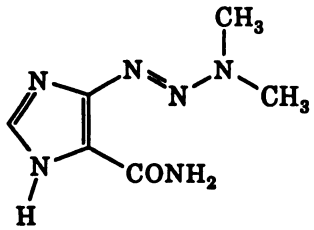


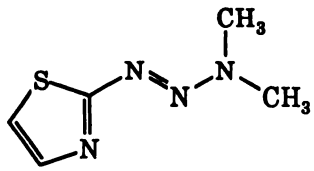
Fig. 1. Mechanism of triazene activation and mutagenesis.



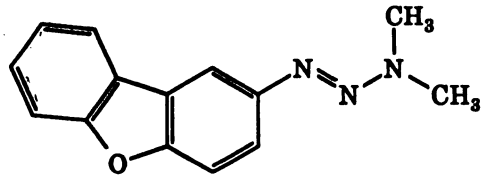
Compounds 1-18



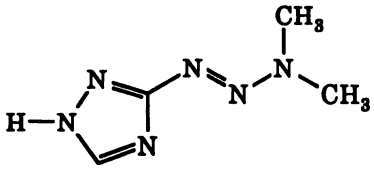
Compound 19



Compound 20

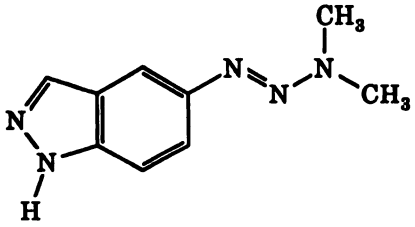


Compound 21

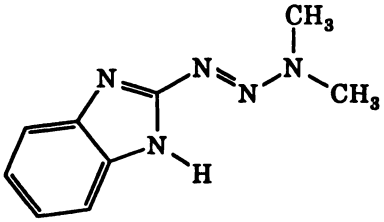


Compound 22

Fig. 2. Structures of phenyl and heterocyclic triazenes.



Compound 23



Compound 24

was added with stirring to a cold (−5°) solution of 3-amino-1,2,4-triazole (2.1 g, 25 mmol) in 15 ml of concentrated H_2SO_4 and 5 ml of water. The resultant solution was stirred for 30 min and then added dropwise to a stirred mixture of dimethylamine (15 ml of 40% solution, 130 mmol) and 10 g of ice. After the cold solution was stirred for 1 hr, it was made alkaline with ammonium hydroxide, furnishing a precipitate. This solid was washed with water and recrystallized (ethyl acetate) to give 1.2 g (34%) of triazene, m.p. 135–136°. Analysis: found (%) C, 34.33; H, 5.57; N, 60.28; calculated (%) C, 34.29; H, 5.71; N, 60.00. The structure of this triazene was verified by X-ray crystallography.[3]

5-(3,3-Dimethyl-1-triazeno)indazole (compound 23). This compound was prepared by the same method used for 3-(3,3-dimethyl-1-triazeno)-1,2,4-triazole, starting instead with 5-aminoindazole (2.66 g, 20 mmol). Recrystallization of the product (benzene/petroleum ether) gave 2.1 g (56%) of triazene, m.p. 144–145°. Analysis: found (%) C, 57.35; H, 5.91; N, 36.92; calculated (%) C, 57.14; H, 5.82; N, 37.03. ^{1}H NMR (δ): 3.28 (br s, 6 H, NCH_3), 7.50 (m, 2 H, ArH), 7.62 (s, 1 H, ArH), 8.03 (s, 1 H, N—CH), and 13.01 (br s, 1 H, NH).

2-(3,3-Dimethyl-1-triazeno)benzimidazole (compound 24). An ice-cold solution of sodium nitrite (1.38 g, 20 mmol) in 3 ml of water was added dropwise with stirring to a cold (−5°) solution of 2-aminobenzimidazole (2.66 g, 20 mmol) in 20 ml of concentrated HCl and 10 ml of water. After stirring for 30 min, a yellow solid appeared, which was dissolved in a minimum quantity of ice-water. The resulting solution was slowly added to a stirred ice-cold solution of dimethylamine (3 ml of 40% solution, 30 mmol) and 10 g of sodium carbonate in 40 ml of water. The solid obtained after the cold solution was stirred for 30 min was washed with water and recrystallized (dilute ethanol) to give 0.7 g (20%) of triazene, m.p. 210° (exploded with decomposition). Analysis: found (%) C, 57.20; H, 5.91; calculated (%) C, 57.14; H, 5.82. ^{1}H NMR (δ): 3.27 (s, 3 H, NCH_3), 3.62 (s, 3 H, NCH_3), 7.08 (m, 2 H, ArH), 7.40 (br s, 2 H, ArH), 12.00 (br s, 1 H, NH).

Triazene mutagenicity. The mutagenicity of the heterocyclic triazenes, compounds **20-24**, was evaluated in *S. typhimurium* strain TA92 containing the S9 fraction by Microbiological Associates (Bethesda, MD), according to the procedure of Venger *et al.* (1). The S9 fraction used in these tests was isolated from the livers of male Sprague-Dawley rats that had been injected with Aroclor 1254, according to the procedure of Venger *et al.* (1).

S. typhimurium strain TA92 is an experimental strain developed by

Bruce N. Ames (University of California, Berkeley) and contains histidine mutation hisG46 and R factor plasmid pKM101. TA92 differs from the more widely used tester strain TA100 in having an intact lipopolysaccharide barrier on the bacterial surface and an intact DNA excision repair system. (11).

Octanol-water partition coefficients. Log P values for the heterocyclic triazenes were determined experimentally (12).

MNDO calculations. MNDO calculations (5, 6) were performed using the AMPAC program (MNDO version 3.01) running on the IBM 4341 (Quantum Chemistry Program Exchange No. 523). Atomic coordinates were either fixed or optimized with respect to energy using routines contained in the AMPAC program.

Heavy atom positions for compounds **20–22** were fixed using crystallographically determined bond lengths and bond angles subject to the constraint that all these atoms be coplanar.[3] The crystal structure of compound **22** shows two independent triazenes within each unit cell. MNDO calculations were performed for each of the two geometries (referred to as molecule A and molecule B). Heavy atom positions in compounds **23** and **24** were determined subject to the constraint that all these atoms be coplanar. Ring atom positions were completely optimized while the five heavy atoms of the N=N—N(CH_3)$_2$ group were positioned using the same bond lengths and angles that had been employed for the calculations on the phenyltriazenes (4).

Hydrogen atoms in all of the compounds were located using standard C—H bond lengths in combination with standard bond angles (CH_3) (13) or optimized bond angles (ring CH). Hydrogen atoms bonded to heteroatoms were completely optimized.

Results

Triazene syntheses and properties. The new triazenes were conveniently prepared from the corresponding heterocyclic amines via the diazonium salts. The molecular structures of three of the triazenes (compounds **20-22**) were determined by X-ray crystallography and show normal bond lengths and angles (Table 1).[3] Two independent molecules are found in the unit cell of the triazolyltriazene (molecules A and B, compound **22**); however, the bond lengths and angles for these two compounds are nearly identical.

The hydrophobicity and electronic structures of the heterocyclic triazenes are strongly dependent on the nature of the heterocyclic ring (Table 2). Log P for the six heterocyclic triazenes varies from −0.44 (triazole, compound **22**) to 4.55 (dibenzofuran, compound **21**), compared with a range of 0.98 to 4.40 for the phenyltriazenes.

ϵ_{HOMO} and q_{HOMO} values for the heterocyclic triazenes also span a wider range than is calculated for the phenyltriazenes. Using ϵ_{HOMO} as an index of electron donation by the ring, we find that electron donation increases in the order 3-triazolyl (compound **22**) < 2-thiazolyl (compound **20**) < 5-(4-carboxamide)-imidazolyl (compound **19**) < 2-dibenzofuranyl (compound **21**) < 2-benzimidazolyl (compound **24**) < 5-indazolyl (compound **23**) (Table 2). The same order is obtained using q_{HOMO}, except that compounds **23** and **24** are reversed, the latter being more electron-rich (Table 2). Separate MNDO calculations at the two crystallographic geometries of the triazole (compound **22**, molecules A and B, in Table 2) show that the electronic structure of the triazole is not affected by the geometry differences between the two molecules.

Triazene mutagenicity. Five heterocyclic triazenes (compounds **20-24**) were tested for mutagenicity in *S. typhimurium* TA92 containing the S9 fraction. The procedure used to measure mutagenicity was identical to the one previously employed by Venger *et al.* (1). As a check on the reproducibility of this procedure, one previously tested phenyltriazene (compound **14**) was also tested. The new log $1/C$ measured for compound **14**, 5.96, was in good agreement with the previously reported value of 5.99 (1).

Triazene mutagenicities are given in Table 2. The structure of the heterocyclic ring greatly affects activity and either renders the triazene inactive (triazole, compound **22**) or extremely mutagenic (dibenzofuran, compound **21**). The dibenzofuran derivative (log $1/C$ 8.55) is the most mutagenic triazene we have studied and is nearly as mutagenic as aflatoxin B_1 (log $1/C$ 9.48), which is one of the most carcinogenic compounds known (1). The dibenzofuran derivative is 355,000 times more active than DTIC, the triazene with the weakest measurable activity in this study.

Eqs. 4 and 5 describe QSARs for the full set of triazenes (compounds **2-21**, **23**, and **24**). The new QSARs differ little from Eqs. 2 and 3 and demonstrate that ϵ_{HOMO} and q_{HOMO} are useful as electronic indices for phenyl and heteroaromatic triazenes simultaneously.

$$\log 1/C = 0.95\ (\pm 0.25) \log P + 2.22\ (\pm 0.88)\ \epsilon_{HOMO} + 22.69 \quad (4)$$

$n = 21$; $r = 0.919$; and $s = 0.631$.

$$\log 1/C = 0.97\ (\pm 0.24) \log P - 7.76\ (\pm 2.73)\ q_{HOMO} + 5.96 \quad (5)$$

$n = 21$; $r = 0.931$; and $s = 0.585$.

One triazene that could not be included in the QSARs is the inactive triazole (compound **22**). Treatment of TA92 with 0.468 mM triazole gives 12 mutations above background/10^8 bacteria, which is not a statistically significant number of mutations. Lower concentrations of triazole do not give any mutations and neither do higher concentrations, due to the cytotoxicity of the triazole. Consequently, we were unable to include the triazole in the derivation of Eqs. 4 and 5. The predicted activity of the triazole (Table 2) is roughly 6 to 70 × 10^6 times less than that measured for the dibenzofuran compound.

Two other triazenes, compounds **1** and **20**, were also excluded from the data set used to construct Eqs. 4 and 5. The

TABLE 1
Crystallographic bond lengths and angles for 3,3-dimethyltriazenes

Compound	Bond lengths			
	r(C—N_1)	r(C—N_3)	r(N=N)	r(N—N)
	Å			
8[a]	1.442, 1.445	1.418	1.270	1.316
19[b]	1.446, 1.449	1.378	1.288	1.305
20[c]	1.439, 1.444	1.387	1.271	1.294
21[c]	1.443, 1.435	1.443	1.270	1.328
22[c] (molecule A)	1.447, 1.455	1.400	1.285	1.304
22[c] (molecule B)	1.447, 1.447	1.403	1.285	1.307
DTPC[d]	1.45, 1.46	1.429	1.281	1.309

Compound	Bond Angles		
	∠(C—N—N)	∠(C—N=N)	∠(N=N—N)
8[a]	122.7°, 115.9°	111.62°	114.68°
19[b]	121.8°, 116.7°	112.0°	113.2°
20[c]	121.8°, 118.4°	111.1°	115.5°
21[c]	122.8°, 115.4°	112.0°	113.3°
22[c] (molecule A)	122.8°, 116.4°	110.6°	114.9°
22[c] (molecule B)	123.1°, 116.9°	111.2°	114.1°
DTPC[d]	123.8°, 115.6°	111.7°	113.3°

[a] From Ref. 16.
[b] Hydrochloride salt. From Ref. 17.
[c] X-ray crystal structures of compounds **20–22** will be published elsewhere.
[d] 2-(3,3-Dimethyl-1-triazeno)phenyl-1-carboxamide. From Ref. 18.

TABLE 2

Parameters used in the formulation of Eqs. 2–5 for the mutagenic activity of triazenes in *S. typhimurium* TA92

	Compound		log 1/C							
			Eq. 4			Eq. 5		ϵ_{HOMO}[b]	q_{HOMO}[c]	log P[a]
	X-Phenyl	R=	Observed[a]	Calculated	Residual	Calculated	Residual			
1	4-$CONH_2$	*t*-Butyl	3.83	5.54	−1.71	5.02	−1.19	−8.854	0.446	2.61
2	3,5-CN	CH_3	3.46	4.05	−0.59	4.32	−0.86	−9.341	0.483	2.18
3	4-SO_2NH_2	CH_3	3.49	2.57	0.92	2.90	0.59	−9.499	0.516	0.98
4	3-$CONH_2$	CH_3	3.51	4.16	−0.65	3.68	−0.17	−8.879	0.444	1.21
5	4-$CONH_2$	CH_3	4.04	3.92	0.12	3.88	0.16	−8.981	0.417	1.20
6	4-$CONH_2$	Allyl	4.16	4.93	−0.77	4.67	−0.51	−8.905	0.426	2.09
7	3-$NHCONH_2$	CH_3	4.19	4.97	−0.78	5.08	−0.89	−8.547	0.274	1.29
8	4-CN	CH_3	4.43	4.93	−0.50	5.08	−0.65	−9.035	0.410	2.39
9	4-$COCH_3$	CH_3	4.47	5.01	−0.54	4.97	−0.50	−8.945	0.409	2.27
10	H	CH_3	5.32	5.97	−0.65	5.46	−0.14	−8.652	0.386	2.59
11	4-$CONH_2$	*n*-Butyl	5.41	5.29	0.12	5.04	0.37	−8.903	0.425	2.46
12	4-$NHCONH_2$	CH_3	5.59	5.41	0.18	5.15	0.44	−8.331	0.260	1.25
13	4-$NHCOCH_3$	CH_3	5.83	5.70	0.13	5.45	0.38	−8.324	0.257	1.54
14	4-CF_3	CH_3	5.99	5.61	0.38	5.79	0.20	−9.287	0.482	3.70
15	3-CH_3	CH_3	6.44	6.22	0.22	5.76	0.68	−8.649	0.380	2.85
16	4-Cl	CH_3	6.48	6.16	0.32	6.21	0.27	−8.881	0.382	3.33
17	4-CH_3	CH_3	7.00	6.36	0.64	5.98	1.02	−8.620	0.362	2.93
18	4-C_6H_5	CH_3	7.67	8.32	−0.65	8.64	−0.97	−8.363	0.202	4.40
19	DTIC		3.00	2.91	0.09	3.19	−0.19	−8.824	0.327	−0.24
20	2-1′,3′-Thiazolidine		5.90	3.71	2.19	4.52	1.38	−9.105	0.344	1.27
21	2-Dibenzofuran		8.55	7.88	0.67	8.25	0.30	−8.625	0.271	4.55
22	3-1′,2′,4′-triazole									
	Molecule A[d]		Inactive	1.80		0.76		−9.238	0.615	−0.44
	Molecule B[d]		Inactive	1.74		0.62		−9.268	0.633	−0.44
23	5-Indazole		6.53	6.51	0.02	6.31	0.22	−8.308	0.249	2.36
24	2-Benzimidazole		6.48	5.17	1.31	6.21	0.27	−8.494	0.139	1.38

[a] Compounds **1–19** from Ref. 1.
[b] MNDO HOMO energy in eV.
[c] MNDO HOMO electron density on N_1 (maximum value = 2).
[d] Separate MNDO calculations were carried out for the two crystallographically independent forms of triazene **22**.

bulky *tert*-butyl triazene, compound **1**, is significantly less active than is predicted. This low activity has been attributed to steric hindrance by the *tert*-butyl group of cytochrome P-450-catalyzed methyl hydroxylation (1, 4).

The worst fit triazene is the thiazolidine, compound **20**. This compound is significantly more active than either Eq. 4 or Eq. 5 predicts. The observed activity listed in Tables 2 for this compound is actually the average of two separate measurements (log 1/C 5.94 and 5.86). The high activity of the thiazolidine may be due to an additional mechanism for inducing mutations. On the other hand, it may also be possible that the MNDO method does not give an accurate description of sulfur in this oxidation state.

Discussion

Triazene mutagenicity in *S. typhimurium* TA92, as modeled by Eqs. 1–5, is determined by the hydrophobicity and electronic properties of the triazene. The positive coefficients with log *P* indicate that increased mutagenicity is correlated with increased lipophilicity. The positive coefficients with ϵ_{HOMO} and the negative coefficients with q_{HOMO} indicate that increased mutagenicity is also correlated with increased electron donation from the ring to the triazene moiety and, thereby, with increased ease of triazene oxidation. Because these are the same correlations that would be expected for cytochrome P-450-catalyzed hydroxylation (7), it would appear that mutagenicity is determined by the rate of initial triazene activation and not by the rate of DNA alkylation by R^+ (Fig. 1). A similar type of situation appears to govern the mutagenic activity of nitropolycyclic aromatic hydrocarbons in the Ames test (3). Here again, mutagenic activity appears to be determined by the rate of mutagen activation. In this case, the initial activation step involves reduction of the nitro group.

Perhaps the most exceptional aspect of Eqs. 4 and 5 is the extremely wide range of triazene activities and structures that is covered. Structurally diverse triazenes, with few exceptions, all appear to cause mutations by the same mechanism. This suggests that by the proper manipulation of physicochemical parameters one can either eliminate triazene mutagenicity or raise it to the highest level. This ability to predict triazene behavior using Eqs. 4 and 5 may be of use in the design of less toxic drugs or pesticides.

The triazole compound (compound **22**) probably represents the lower end of the triazene mutagenicity scale. Its lack of activity appears to be a combination of its hydrophilicity and the electron-withdrawing properties of the 1,2,4-triazole ring. The upper end of the mutagenicity scale is currently represented by the dibenzofuran derivative (compound **21**). The dibenzofuran ring is both hydrophobic and relatively electron-donating. It should be possible to raise triazene mutagenicity to even greater levels by substituting the triazene with more hydrophobic groups. Although most of our attention to date has been focused on the aromatic ring, variations in the *N*-alkyl substituent play an important role by contributing to the overall hydrophobicity (compounds **6** and **11**).

Despite the central role played by the initial activation step, the details of the hydroxylation mechanism are still obscure. The two most likely alternatives are shown in Fig. 3. One

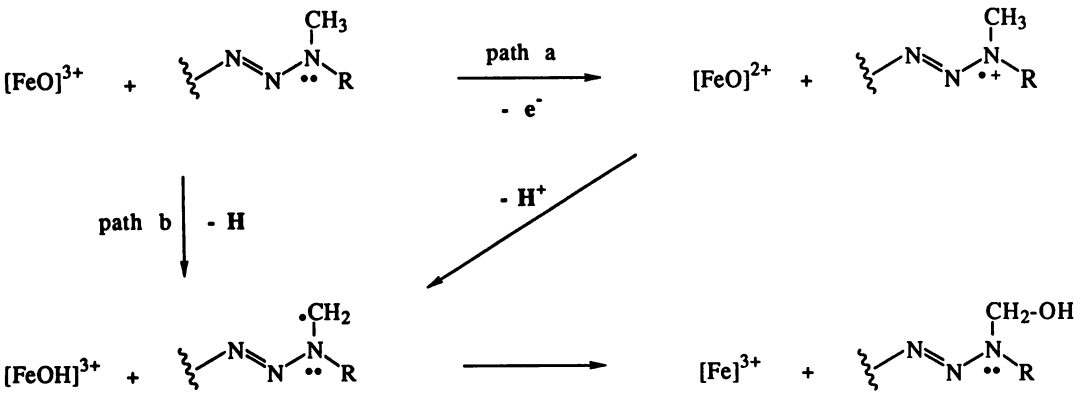


Fig. 3. Proposed mechanisms of triazene hydroxylation.

possibility involves electron transfer from the triazene to the enzyme (Fig. 3, *path a*). This path, which is also the path favored for hydroxylation of tertiary amines (14), yields a radical cation, which can then lose a proton to give a triazene-substituted methyl radical. Another way the radical might form is via hydrogen atom abstraction (Fig. 3, *path b*). Recombination of the radical with hydroxyl radical would give the hydroxylated intermediate.

Eqs. 4 and 5 both predict that electron-rich substrates will be more active and, therefore, do not distinguish between paths a and b. Electron-donating rings are expected to stabilize a radical cation intermediate but might also accelerate hydrogen atom abstraction when the hydrogen atom is being transferred to an electrophilic radical, such as the heme-bound oxygen atom of cytochrome P-450. We are currently investigating the properties of the different reaction intermediates shown in the scheme in Fig. 3, in an effort to learn more about the detailed mechanism of triazene activation.

The correlation of activity with both ϵ_{HOMO} and q_{HOMO} raises an interesting point regarding the relative superiority of Eqs. 4 and 5. ϵ_{HOMO} provides a statistically superior correlation of phenyltriazene mutagenicity (Eqs. 2 and 3) but an inferior correlation when the heterocyclic triazenes are included (Eqs. 4 and 5). Based on the expected relationship between ϵ_{HOMO} and oxidation potential, one might have expected ϵ_{HOMO} to be the sole parameter capable of correlating with mutagenic activity. In fact, our study of phenyltriazenes showed that it is common for several quantum chemical parameters to be correlated for a set of similar molecular structures (4). Furthermore, perturbation theory shows that changes in molecular orbital composition and energy are mathematically related when electron-electron repulsion is neglected (15). Thus, varying a substituent or ring can cause simultaneous and correlated changes in several electronic indices, any of which might give a statistically significant treatment of biological activity. The electronic parameter that gives the best fit of the data may not be the best parameter for understanding the mechanism of the electronic effect.

Conclusion

The mutagenicity of aryldialkyltriazenes in *S. typhimurium* TA92 containing added S9 fraction is highly dependent on the structure of the triazene substituents. Variations in triazene structure, as reflected by triazene hydrophobicity and electronic characteristics, can be used to predict mutagenic activity quantitatively over a 10^{6-7}-fold range of activity. The conformity of nearly all of the triazenes to the same quantitative relationship suggests that similar mechanistic factors control mutagenicity over this entire structure and activity range. The form of the quantitative relationship, on the other hand, suggests that the initial rate of triazene activation by cytochrome P-450 controls mutagenic activity.

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