Conformation, Electronic Structure, and Biological Activity of Antitumour Triazenes

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The MNDO molecular orbital method has been used to calculate the preferred conformation and values of electronic indices for some antitumour triazenes. The computed rotation barrier about the N(2)–N(3) bond shows a good correlation with the Hammett σ substituent constant, and increases as the ability of the substituent to withdraw electrons increases. Analysis of the stretching force constant for the N(3)–Me bond indicates that the demethylation of triazenes observed *in vitro* should occur more readily in the planar conformation, which is the most stable. On the other hand, the antitumour activity shows a high correlation with the LUMO orbital energy, suggesting a charge-transfer mechanism with electron-acceptor triazenes. Finally, with respect to toxicity, no important correlation was found with the electronic indices considered.

It is well established that analogues of 3,3-dimethyl-1-phenyl triazenes have antitumour activity against Sarcoma-180 and L1210 leukemia in mice.[1–3] Dunn *et al.*[2a] have shown that the activity is a function of the electronic nature of the phenyl substituent, basing their conclusion on the correlation between the activity (pC_{130}) and the Hammett σ constant found to be given by equation (1). They have also found a good correlation

$$pC_{130} = 3.41 - 0.69\sigma \qquad (1)$$

between antitumour activity and hydrolysis rate constant. Kolar and Preussmann[4] have studied the hydrolysis of the triazenes and have suggested two different mechanisms for their carcinogenic activity. Audette *et al.*[5] have pointed out that the presence of at least one methyl group seems to be necessary for antitumour activity. Thus, we considered that an investigation of the way the electronic structure of the triazenes depends on the substituents might yield an explanation for their different activities.

Molecular orbital methods have produced valuable contributions to the understanding of the nature of structure–activity relations, charge-transfer phenomena involving drugs and biologically important molecules, and other, related problems.[6] Since *ab initio* methods are very expensive in such cases, semiempirical calculations have been usually used. However, the validity and the interpretation of the results thus obtained has been impaired sometimes by the nature of the approximations employed in the calculations. One of the most successful semiempirical methods for the calculation of molecular properties is the MNDO molecular orbital method,[7] and here we have used it to study both the conformations and the electronic structures of derivatives of the anti-tumour triazenes. We hoped to understand not only how the substituents affect the activity of these molecules, but also the mechanism of action, which is not well established.[2]

Results and Discussion

Conformation and Rotation Barrier.—The most stable conformation of the 3,3-dimethyl-1-phenyltriazenes calculated by the MNDO method corresponds to the planar structure shown in Figure 1 for the NO_2-substituted species. The geometrical parameters for the methyl and the phenyl groups, which are not considered to change much along the triazene series, were taken from energy-optimized MNDO calculations for CH_4 and C_6H_6, respectively.[7] For the triazene molecules

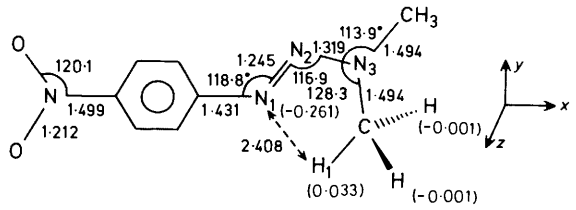

Figure 1. Optimized geometrical parameters for 3,3-dimethyl-1-(4-nitrophenyl)triazene, obtained by the MNDO method, and the Cartesian co-ordinate system used; atomic net charges are given in parentheses

optimization was thus restricted to the substituent and the N_3 moiety.

There are at least two factors which contribute to stabilize the conformation in Figure 1. First, the attractive interaction between the in-plane hydrogen atom of the methyl group and the nitrogen N(1), favouring a planar five-membered ring structure. The net charges on these atoms are given in parentheses in Figure 1. In particular, we note the differences in net charges of the methyl-group hydrogen atoms which arise from this interaction. Secondly, non-planar structures decrease the π-electron delocalization over the molecule. This can be illustrated by the change in the net charge on N(3) between θ = 0° and 90°, where θ is the torsion angle between $(CH_3)N(3)N(2)$ and N(1)N(2)N(3). When θ = 0° the lone pair on N(3) is oriented along the *z* axis and can undergo conjugation, in contrast to when θ = 90°. Thus, when θ = 0° the N(3) net charge depends on the nature of the substituent on the phenyl group. To illustrate the problem two distinct cases are considered. With X = 4-NO_2 (electron-acceptor substituent) and X = 4-NH_2 (electron-donor substituent) the net charges on N(3) for θ = 0° are −0.361e and −0.408e, respectively, whereas for θ = 90° the net charges are both approximately equal at −0.494e. This larger value means there is more localization than in the planar structure.

Since we are discussing electronic effects of rotation about the N(2)–N(3) bond, which seems of some importance for the activity of the triazenes,[4] we decided to analyse the influence of the substituent on the magnitude of this rotation barrier. The rotation barriers were computed for each rotamer with re-optimization of bond angles and lengths of the N_3 moiety. Our calculations indicate that the height of the rotation barrier about the N(2)–N(3) bond increases with increasing ability of the substituent to withdraw electrons. This is shown for X = 4-

Table 1. Experimental and calculated antitumour activities, Hammett σ constants, LUMO energies, and rotation barriers for 3,3-dimethyl-1-phenyltriazene derivatives

X	pC_{130} [a]	$(pC_{130})_{calc.}$ [b]	$(pC_{130})_{calc.}$ [c]	σ	ε_{LUMO} [d]	Δε(90° − 0°) [d]
H	3.42	3.41	3.43	0.00	−0.108	0.423
3-CH_3	3.37	3.46	3.43	−0.07	−0.109	0.422
4-CN	2.91	2.96	2.97	0.66	−0.748	0.463
3-Cl	3.16	3.16	3.21	0.37	−0.418	0.444
3-CF_3	3.18	3.11	3.00	0.43	−0.712	0.459
3-CO_2H	3.01	3.16	3.19	0.37	−0.448	0.435
3-SCH_3	3.33	3.31	3.33	0.15	−0.254	0.433
4-F	3.24	3.37	3.20	0.06	−0.437	0.438
4-n-C_3H_7	3.56	3.50	3.44	−0.13	−0.101	0.415
3-$NHCOCH_3$	3.45	3.27	3.41	0.21	−0.136	0.429
4-COC_6H_5	3.16	3.11		0.43		
4-C_6H_5	3.43	3.52		−0.01		
4-CH=$CHCO_2H$	2.79	2.79		0.90		
4-NO_2		2.87	2.58	0.78	−1.302	0.501
4-NH_2		3.86	3.63	−0.66	0.170	0.382

[a] Ref. 2; $pC_{130} = -\log C_{130}$. [b] From equation (1). [c] From equation (3). [d] In eV.

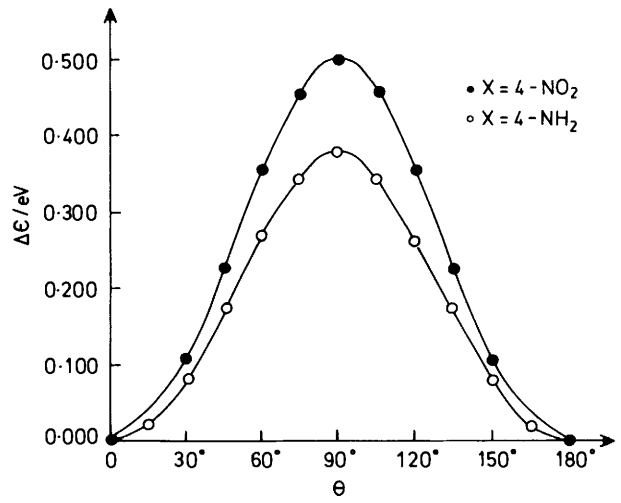

Figure 2. Rotation barriers about the N(2)–N(3) bond

NO_2 and X = 4-NH_2 in Figure 2, and is substantiated by the relationship involving the height of the barrier and the Hammett σ substituent constant [equation (2); r is the

$$\Delta\varepsilon(90° - 0°) = 0.08\sigma + 0.42 \quad (2)$$
$$r = 0.94$$

correlation coefficient]. The equation is significant at >95% (F = 73.33; $F_{\alpha=0.05} = 4.96$; $n = 12$) and the values of Δε(90° − 0°) and σ for the various substituents are given in Table 1. Thus, the more positive is σ (electron-acceptor substituent) the higher is the rotation barrier. Calculations for 4-COC_6H_5, 4-C_6H_5, and 4-CH=$CHCO_2H$ were not carried out because of the limited capability of our computing facilities.

Bond Lengths and Force Constants.—The strengths of the N(3)–CH_3 and N(2)–N(3) bonds are of special interest for the triazenes under study. The former is of interest because of the enzymic monodemethylation observed *in vitro*, which might be responsible for activity, according to Audette *et al.*[5] and Shealy.[8] The latter is susceptible to hydrolytic cleavage[4] to form the diazonium cation, thus implying another possible mechanism for the action of the triazenes.[2a,4] As is well known, the magnitudes of the vibrational force constants are closely related to the nature of the chemical bonding;[9] several correlations exist between force constants and bond parameters such as bond energies and bond lengths.[10,11] It seemed therefore worth examining the influence of rotation about the N(2)–N(3) bond on the stretching force constants at the rotation site.

Table 2. Bond lengths and stretching force constants of the N(2)–N(3) bonds for θ = 0° and θ = 90° (in parentheses)

	4-NO_2	4-NH_2
r[N(3)–Me] Å	1.494 (1.479)	1.489 (1.477)
F[N(3)–Me] mdyn $Å^{-1}$	6.937 (7.460)	7.064 (7.528)
r[N(2)–N(3)] Å	1.319 (1.366)	1.329 (1.369)
F[N(2)–N(3)] mdyn $Å^{-1}$	11.539 (10.419)	11.071 (10.409)

As we go from θ = 0° to θ = 90° the length of the N(3)–CH_3 bond decreases, and its stretching force constant correspondingly increases. In Table 2 we show the results for X = 4-NO_2 and X = 4-NH_2. This indicates[12] that the bond becomes strongest for θ = 90°. Thus, we may expect the demethylation observed *in vitro* for these triazenes[5,8] to occur more readily in the planar conformation. On the other hand, the length of the N(2)–N(3) bond increases and its stretching force constant decreases (see Table 2 for X = 4-NO_2 and X = 4-NH_2) from θ = 0° to θ = 90°. This indicates that the bond becomes weakest for θ = 90° but the corresponding conformation is energetically less stable than that for θ = 0°. If we assume that hydrolytic cleavage of the N(2)–N(3) bond does take place, then these results do not point to a definite conclusion about the preferred conformation for cleavage.

Structure–Activity.—To elucidate the electronic effect of the substituent on the antitumour activity and the toxicity of the triazenes, we calculated several electronic indices selected on the basis of chemical intuition and literature reports, using the MNDO method. These indices were determined for the most stable conformation of ten members of the 3,3-dimethyl-1-phenyltriazene series; then a regression analysis was carried out with the biological data and these indices. The correlation coefficients obtained from the simple regression are given in Table 3, together with the indices used in the regression. The symbols have the following meanings: $\Sigma Q_{\pi,C}$ is the summation of the π charge densities on the phenyl group; Σq is the summation of net charges on the substituent; $\Sigma Q_{\pi,N}$ is the summation of π charge densities on N(1), N(2), and N(3); q_α is

Table 3. Correlation between biological data and electronic indices calculated by MNDO

Indices	pC_{130}	pLD_{50}
$\Sigma Q_{\pi,C}$	0.43	0.31
Σq_X	0.64	0.38
$\Sigma Q_{\pi,N}$	−0.72	−0.28
$q_{N(1)}$	0.52	0.41
$q_{N(2)}$	−0.49	−0.52
$q_{N(3)}$	−0.79	−0.42
$q_{H(1)}$	−0.74	−0.51
μ_x	−0.45	−0.19
μ_y	−0.25	−0.01
μ	−0.50	−0.31
ε_{HOMO}	0.81	0.37
ε_{LUMO}	0.87	0.46
$\Delta\varepsilon$(H − L)	0.58	0.46
$\Delta\varepsilon(90° - 0°)$	−0.82	−0.49

the net charge on the α atom; μ_τ is the component of the dipole moment along the τ (t = x or y) axis; and $\Delta\varepsilon$(H − L) is the difference between the HOMO and LUMO orbital energies.

From Table 3 we can draw some important conclusions. First, the most important index for explaining antitumour activity is the LUMO (π) orbital energy. This suggests that the mechanism of action of the triazenes involves charge transfer, (electron-acceptor molecules). The activity and the LUMO orbital energy are related through equation (3), and the

$$pC_{130} = 0.173\varepsilon_{LUMO} + 3.510 \quad (3)$$
$$r = 0.87$$

equation is significant at >95% ($F = 24.92$; $F_{\alpha=0.05} = 5.32$; $n = 10$). According to equation (3), the antitumour activity increases as the LUMO energy becomes more negative. This is essential for an electron-acceptor molecule, since it decreases the energy gap with the donor molecule, thus favouring the transfer of an electron. This electron-acceptor behaviour is in accord with the fact that the activity increases with increasing ability of the substituent to withdraw electrons, since the more positive σ, the more negative is the LUMO energy. The activities calculated from equations (1) and (3), the experimental activities, and the LUMO orbital energies are given in Table 1.

Further information can be drawn from two other indices, which present smaller correlation coefficients with antitumour activity. They are the height of the rotation barrier about the N(2)–N(3) bond [$\Delta\varepsilon(90° - 0°)$] and the HOMO orbital energy (ε_{HOMO}). This can be rationalized as follows. A substituent that confers a large activity on the molecule tends to increase the rotation barrier [see equations (1) and (2)] and thus decrease the probability that the molecule will shift to a non-planar structure. On the other hand, the more negative is the HOMO orbital energy, the greater is the antitumour activity. This situation is obviously unfavourable for an electron-donor molecule, which should have a high-lying HOMO energy to decrease the energy gap with the acceptor molecule. Consequently, these results indicate that the triazenes must behave as electron-acceptor molecules, interacting with the receptor in a planar structure.

Multiple regression analysis was also carried out with the electronic indices considered, but no more significant correlations were found. With respect to toxicity, simple and multiple regressions were carried out but no significant result was obtained. In Table 1 we show the results obtained from simple regression analysis. Finally, as antitumour and toxic activities seem to be difficult to separate,[2a] further investigation would be welcome. In particular, molecular orbital studies including solvent effects would be highly desirable, in view of the high correlation between antitumour activity and hydrolysis rate constant.

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