Ab initio Calculations Relevant to the Mechanism of Chemical Carcinogenesis by Nitrosamines. Part 5.[1] The Role of Diazomethane

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Recent work suggested that the role of diazomethane in carcinogenesis by nitrosamines should be re-investigated. *Ab initio* self-consistent-field gradient calculations using a 4-21G basis set have shown that on energetic grounds the involvement of diazomethane cannot be excluded. Diazomethane may be formed as a minor product in the presence of methanediazohydroxide and base. The transition structure for the direct formation of diazomethane from methanediazohydroxide is reported; the second-order Møller–Plesset (MP2) energy barrier suggests that this exothermic reaction may proceed slowly.

Nitrosamines were first discovered to be carcinogenic by Barnes and Magee in 1956.[2] Since then there has been an enormous amount of work on nitrosamines, but despite this many features of their formation and metabolism are still not fully understood. In particular, the nature of the ultimate carcinogen which alkylates DNA has not been determined. Experimental studies have been hampered by the very short lifetimes of the metabolic intermediates involved; consequently theoretical studies have been initiated both in this laboratory and elsewhere in an attempt to unravel these problems. These theoretical studies have been equally inconclusive. Thus, in recent articles, methanediazohydroxide,[3] diazonium ions,[4] and carbocations[5,6] have been discussed as alkylating agents. We now extend the discussion to include diazomethane.

The diazoalkane was in fact suggested as the alkylating agent in some of the earliest work, independently by both Rose and Schoental.[7] This idea may have arisen because diazomethane can be prepared by the action of base on a nitrosourea.[8] (Nitrosoureas, unlike nitrosamines, are directly acting carcinogens, and are already oxidized in the α-position. The corresponding metabolically activated nitrosamine is the α-hydroxy nitrosamine,[1] which is highly unstable;[9,10] the possibility of the formation of diazomethane from the α-hydroxy nitrosamine has consequently not been investigated.) Lijinsky's mass spectrometric studies on the DNA and RNA hydrosylates from rats treated with $(CD_3)_2NNO$ found that the main alkylation product showed *m/z* of 168, corresponding to 7-trideuteriomethylguanine; this implied that diazomethane was not the main alkylating agent. Smaller peaks for 7-methylguanine and 7-dideuteriomethylguanine were also found, but Lijinsky's main conclusion was that other alkylating agents besides diazoalkanes need to be considered, and carbocations were suggested.[11] Similar results[12] were found when $(C_2D_5)_2NNO$ was employed.

Although these results were taken to be definitive, ruling out the diazoalkane, the work was done on *N*(7)-methylguanine, when N-7 of guanine was known to be the major alkylation site of DNA and RNA. However, the work was carried out before it was found that formation *N*(7)-methylguanine did not correlate with tumour production,[13,14] and in the days when DNA isolation techniques destroyed *O*^{6}-alkylguanine. Loveless observed the formation of *O*^{6}-alkylguanine,[13] and suggested that alkylation at O^6 rather than at N-7 is the cause of mutations. This has been substantiated, partly by studies of the relative abundance of O^6 and N-7 alkylation products producted by methylating and ethylating agents.[15,16] (Ethylating agents tend to attack oxygen atoms in DNA bases and tend to be more carcinogenic, whereas methylating agents tend to attack nitrogen atoms.[17])

There is evidence that different nitrosamine-derived alkylating agents are involved in alkylation at O^6 and at N-7. Thus, *in vivo* studies show that *N*-nitrosodi-n-propylamine alkylates without rearrangement[18,19] at N-7, but with rearrangement[19] at O^6. Park *et al.* concluded that carbocations were not involved[18] (and chose to implicate diazonium ions), whereas Scribner *et al.* concluded that the alkylation proceeds *via* a loose transition structure (they did not choose to identify the alkylating agent).[19]

It is concluded, therefore, that the experimental evidence does not rule out the involvement of the diazoalkane in the important alkylation at O^6 of guanine. Recent evidence implies that diazomethane may even be involved in the alkylation at N-7 of guanine, as it has been shown that deuterium labelling may force a change in the mechanism through the kinetic isotope effect.[20] For these reasons our theoretical investigations on the formation and metabolism of nitrosamines have included a study of the formation of diazomethane.

The reactions of methanediazohydroxide in the presence of acid have been considered previously [equation (1)].[21,22] In this

$$CH_3NNOH \xrightarrow{H^+} CH_3NN^+ + OH^- \qquad (1)$$

article the effects of base, represented by reactions (2) and (3), and internal rearrangements, reaction (4), are considered.

$$CH_3NNOH \longrightarrow CH_3NNO^- + H^+ \qquad (2)$$

$$CH_3NNOH \longrightarrow CH_2NNOH^- + H^+ \qquad (3)$$

$$CH_3NNOH \longrightarrow CH_2N_2{\cdot}H_2O \qquad (4)$$

Reaction (2) represents the formation of diazoates. Although these are unlikely to play a major part in the metabolism of nitrosamines, they may well prove useful experimental tools in elucidating the reactions of the diazohydroxide. Moss has found that the *trans*-diazoate is more stable than the *cis*-isomer, and that in certain circumstances the hydrolysis of diazoates leads to the formation of diazomethane.[23] Lown *et al.* have shown that in CD_3OD the *cis*-isomer rapidly forms diazomethane, whereas the *trans*-isomer is stable for up to 12 h.[3] These observations suggest that both base catalysis and internal rearrangements may be involved in the possible formation of diazomethane from methanediazohydroxide.

Methods

Ab initio Hartree–Fock (HF) self-consistent-field gradient techniques using a split-valence 4-21G basis set have been used,

as in our previous work on nitrosamines.[1,21,22,24–26] Electron correlation has been calculated for some species using second-order Møller—Plesset theory.[27] All stationary points have been fully optimised, unless otherwise stated, using the algorithm of Schlegel.[28] Transition structures were obtained from an initial guess at the geometry and Hessian, which was obtained by a full optimisation using a sub-minimal (STO-2G)[29] basis set; this optimisation employed analytical second derivatives[30] at every point. While the calculation of second derivatives is normally very expensive, this is not necessarily the case with a sub-minimal basis set, and the extra cost involved is offset by a more rapid quasi-Newton optimisation to the saddle point once a good starting point has been obtained. This procedure is very useful for five- and six-membered-ring transition structures, which have rather flat potential energy surfaces.

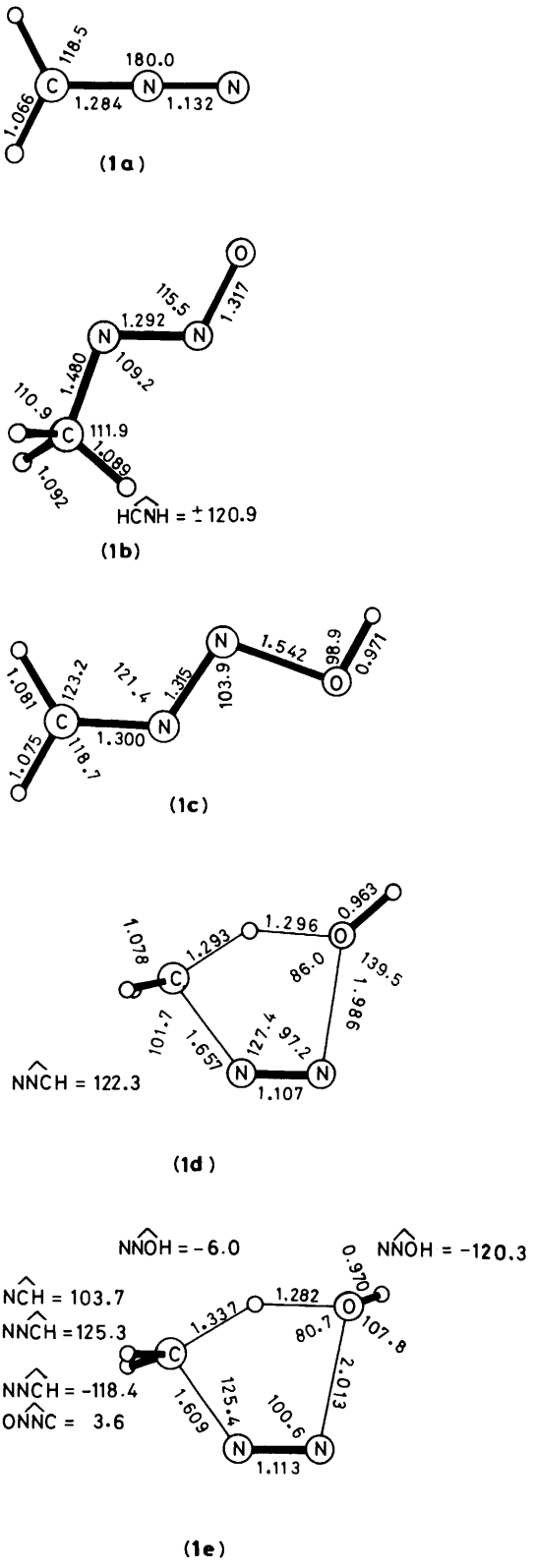


Figure 1. Structures involved in the formation of diazomethane

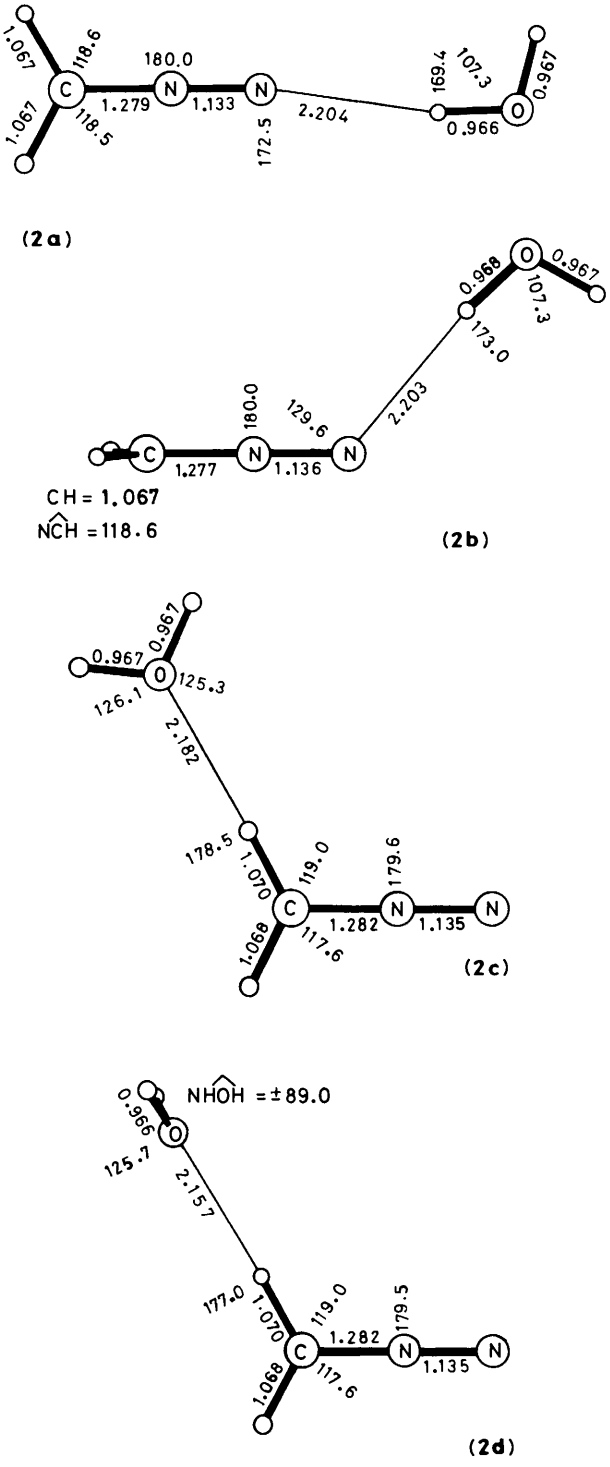


Figure 2. Structures of monohydrated diazomethane

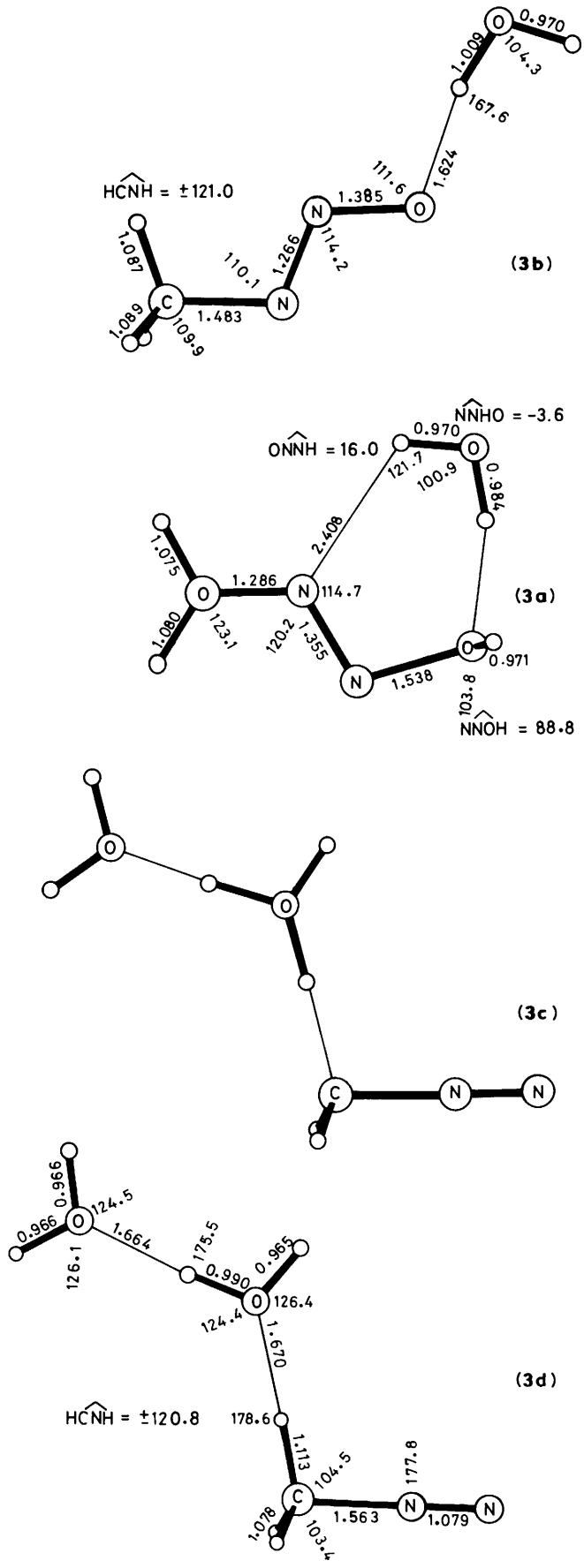
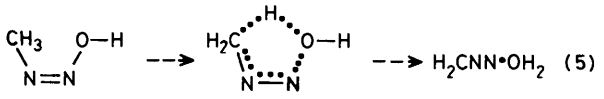


Figure 3. Hydrated anions (**3a** and **b**) and hydrated cations (**3c** and **d**)

Results and Discussion

The energies of all species studied are given in the Table, and the structures are shown in Figures 1—3. The energies of the different conformations of methanediazohydroxide have been discussed elsewhere.[3,22,24]

Monohydrated Diazomethane.—Monohydrated diazomethane is formally related to methyldiazohydroxide by a porton shift [reaction (5)]. The various conformations of monohydrated diazomethane are shown in Figure 2. The actual arrangement that would result from reaction (5) (with the O of



the H_2O bound to the terminal nitrogen atom in a linear arrangement) is repulsive; the H_2O prefers to bind to the terminal nitrogen through one of its hydrogen atoms, as in (**2a** and **b**). The most favourable arrangement is with the H_2O binding to one of the protons in a perpendicular fashion, as in (**2d**); in this binding mode ΔE for reaction (5) is -14 kJ mol^{-1}. Although this energy difference is not great, and the figure may not be very reliable at this level of theory (RHF/4-21G), because $H_2CNN{\cdot}H_2O$ is lower in energy than CH_3NNOH reaction (5) cannot be excluded on energetic grounds. Moreover, the Generalised Valence Bond study of diazomethane by Walch and Goddard showed that diazomethane has diradical character.[31] Diazomethane also has an unrestricted Hartree–Fock (UHF) wavefunction which is lower in energy (by about 2 kJ mol^{-1}) than the restricted Hartree–Fock (RHF) wavefunction.[32,33] Thus, in a full CI study the relative energy difference between CH_3NNOH and $H_2O{\cdot}H_2CNN$ may be increased further, though it is possible that CH_3NNOH, and other nitrosamine derivatives, may also have diradical character.

The Effects of Acid and Base.—The proton shift in reaction (5) could be effected in any of several ways; these could include base catalysis or an internal rearrangement. Reaction (2) is more likely than reaction (3), as CH_3NNO^- (**1b**) is more stable than CH_2NNOH^- (**1c**) (see Figure 1) by 45 kJ mol^{-1}. The effect

Table. Energies (in atomic units) of molecules at RHF/4-21G optimised geometry

Molecule	HF/4-21G energy	MP2/4-21G energy
OH^-	−75.104 378	−75.215 869
H_2O	−75.821 207	−75.943.959
N_2	−108.666 310	
CH_2NN (**1a**)	−147.493 865	−147.819 402
CH_2NN (UFH singlet)	−147.494 629	
CH_2NNOH^- (**1b**)	−222.677 640	
CH_3NNO^- (**1c**)	−222.694 884	
T.S. (2) (**1d**)	−223.236 862	−223.697 254
T.S. (1) (**1e**)	−223.242 488	−223.706 497
t,t-CH_3NNOH	−233.315 778	
c,t-CH_3NNOH	−223.318 655	−223.759 091
$CH_2NN + H_2O$	−223.315 072	−223.763 361
$CH_2NN{\cdot}HOH$ (**2a**)	−223.320 649	
$CH_2NN{\cdot}HOH$ (**2b**)	−223.321 526	
$H_2O{\cdot}CH_2NN$ (**2c**)	−223.323 713	
$H_2O{\cdot}CH_2NN$ (**2d**)	−223.324 111	
$CH_2NNOH{\cdot}HOH^-$ (**3a**)	−298.542 331	
$CH_3NNO{\cdot}HOH^-$ (**3b**)	−298.558 279	
$H_2O{\cdot}H_3O{\cdot}CH_2NN^+$ (**3c**)	−299.546 028	
$H_2O{\cdot}H_2O{\cdot}CH_3NN^+$ (**3d**)	−299.574 069	

of monohydration on reactions (2) and (3) can be represented by reactions (6) and (7). Again $CH_3NNO^-\cdot HOH$ [(**3b**) in Figure 3] is more stable than $CH_2NNOH\cdot HOH^-$ (**3a**), by 42 kJ mol^{-1}. These results suggest that although reprotonation of CH_2NNOH^- (**1b**) could lead to the formation of diazomethane, it is more likely that the effect of base will lead to the formation of CH_3NNO^- (**1c**). The energy difference between (**1b**) and (**1c**) or (**3a**) and (**3b**) is, however, small. While reactions (6) and (7) both proceed without an energy barrier in the gas phase, this may not be the case in aqueous solution.

$$CH_3NNOH + OH^- \longrightarrow CH_3NNO^-\cdot HOH \quad (6)$$

$$CH_3NNOH + OH^- \longrightarrow CH_2NNOH^-\cdot HOH \quad (7)$$

Elsewhere[22] it has been demonstrated that $H_2O\cdot HCH_2NN^+$ is the only minimum geometry for monohydrated H_3CNN^+. A linear hydration chain could lead to the formation of $H_2O\cdot H_3O\cdot CH_2NN^+$ (**3c**). However, (**3c**) is not a stationary point and lies about 74 kJ mol^{-1} above $H_2O\cdot H_2O\cdot HCH_2NN^+$ (**3d**). These results may be compared with the effect of a linear hydration chain on $ON\cdot H_2O^+$, where a proton is removed and HONO is formed.[26] However, in the formation of (**3c**) from (**3d**) it may be that electron correlation plays an important part; electron correlation is clearly important in diazomethane as already shown.

The endothermicities of the reactions presented here are not very large; this leaves open the possibility that acid (and more probably base) catalysis may lead to the formation of diazomethane. However, it would be difficult to determine the transition structures and energy barriers for such reactions in a system that models the conditions in aqueous solution.

Internal Rearrangements.—The experimental evidence suggests that *cis*-diazoates are more reactive than *trans*-diazoates.[3,23] This implies that internal rearrangements as in reaction (4) are more likely than base-catalysed pathways [reactions (2) and (3)]. Therefore the formation of diazomethane by a concerted mechanism has also been investigated. The transition structure for reaction (5) is shown in Figure 1. The structure (**1d**) is a second-order transition structure with two negative eigenvalues (as confirmed by analytical second-derivative calculations[30]); one negative eigenvalue corresponds to a proton shift, and one to inversion at the oxygen atom. The structure (**1e**) is the true transition structure, and lies 15 kJ mol^{-1} below (**1d**) on the Restricted Hartree–Fock 4-21G surface. Perhaps the most interesting feature of these transition structures is the long C–N bond; this is due to an antibonding situation in the highest occupied π-orbital. The RHF/4-21G energy barrier is 200 kJ mol^{-1}; the MP2/4-21G barrier is 138 kJ mol^{-1}. In order for this reaction to proceed we would expect an energy barrier of less than 100 kJ mol^{-1} (ref. 22). Clearly, electron correlation results in a decrease in this barrier. Moreover, the effect of solvent assistance in this reaction has not been investigated. While these results suggest that the reaction is unlikely to proceed, further refinement of the method to include more of the correlation energy, and a study of the possibility of solvent assistance may result in a significant lowering of this energy barrier.

Conclusion

For over a decade it has been accepted that diazomethane is not involved in carcinogenesis by nitrosamines. Recent work however has opened up the possibility that diazomethane may indeed be involved. The theoretical results presented here also suggest that the involvement of diazomethane cannot be excluded, even though it may be produced only in small amounts. The initial conclusion is that Lijinsky's original labelling experiments should be repeated on the O^6-alkyl derivative, even though deuterium labelling may force a change in the mechanism.[20]

One of the failings of many theoretical studies on the nature of the alkylating agent is that suggestions have been made solely on the basis of indices of reactivity. Perhaps one of the most interesting theoretical studies on this problem was carried out by Ford and Scribner, who actually determined the transition structures for the interaction of methanediazonium ions and ethanediazonium ions with various N- and O-containing nucleophiles.[4] Certainly major breakthroughs were made as a result of the experimental study of the behaviour of various ethylating and methylating agents towards *N*- and *O*-alkylation sites in DNA. Similar breakthroughs may come from systematically studying the transition structures for the interaction of all possible methylating and ethylating agents with *N*- and *O*-centred nucleophiles, ideally guanine. There are many disparities in the literature in this field between results from semiempirical and *ab initio* methods; semiempirical methods should be used with care. The results presented here suggest that diazomethane (and diazoethane) should be included in such a study.

Acknowledgements

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