Theor Chim Acta (1994) 88: 111–116

Theoretica Chimica Acta

An ab initio study of the attack of a nitrosoiminium ion on formamide as a model for DNA bases

Anne-Marie Sapse[1], Danielle S. Sapse[2], and Duli C. Jain[3]

[1] City University of New York, Graduate Center and John Jay College, 445 West 59 Street, New York, NY 10019 and Rockefeller University, New York, NY 10021, USA
[2] Nightingale-Bamford School, New York, NY 10029, USA
[3] York College of the City University of New York, Jamaica, NY 11451, USA

Received November 4, 1992/Accepted July 7, 1993

Summary. The attack of a nitrosoiminium ion (an intermediate formed in the decomposition of nitrosamines) on formamide is studied by ab initio methods at Hartree–Fock level and with the Moller–Plesset (MP2) method. The results show that the complex thus formed is lower in energy than the reactants.

Key words: Nitrosoiminium ion – Formamide

1 Introduction

The discovery of Magee and Barnes [1] that nitrosamines are carcinogenic to a variety of animals has stimulated an extensive research into their structure, occurrence, and biological effects.

It has been established [2, 3] that the carcinogenic amines require an oxidative mechanism, mediated by cytochrome P450 enzymes to convert them into a precursor of the ultimate carcinogen which is the diazohydroxide ion or its kinetical equivalent the diazonium ion [4, 5]. The same species are thought to be the ultimate alkylating agent in the decomposition of nitrosoureas [6], which are anticancer drugs used clinically against a number of forms of the disease. Methyl and ethyl nitrosoureas feature carcinogenic properties as well as limited carcinostatic activity [7], while haloethylnitrosoureas (Fig. 1) show mainly carcinostatic activity. It is apparent that this activity is due to the cross-linking of the DNA strands, a lethal event for the cell. The preferential cytotoxicity to the cancer cell as opposed to the normal cells is thought to be due to the lack in the former of a repair mechanism present in the latter, which removes the alkyl groups from alkylated DNA bases before it can form an adduct with the other strand [8].

While the nitrosoureas decompose spontaneously into diazohydroxides and isocyanate [9], the electrophilic species generated by enzymatic action from nitrosamines comprise also the nitrosoiminium ions, which have been proposed as intermediates in the nucleophylic displacement of ester conjugates of α-hydroxyalkylnitrosamines [10] (Fig. 2). This study investigates the possibility that the nitrosoiminium ions attack directly the DNA bases and propose a mechanism for this reaction.

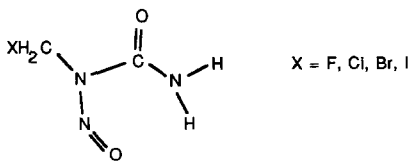

Fig. 1. Haloethylnitrosoureas

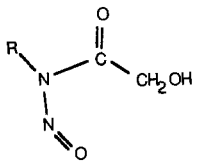

Fig. 2. α-Hydroxyalkylnitrosamines

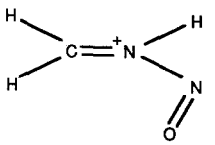

Fig. 3. Nitrosoiminium ion

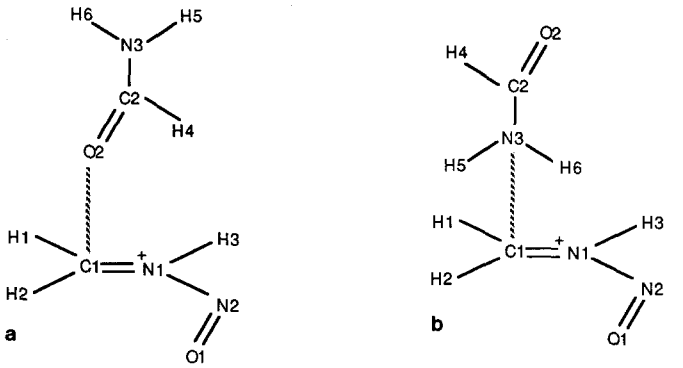

Fig. 4a. Approach of formamide's oxygen to C_1. b. Approach of formamide's nitrogen to C_1

2 Method and results

The reaction between the nitrosoiminium ion, depicted in Fig. 3, and formamide, used as a model for the DNA bases, has been investigated using ab initio quantum chemical calculations at the Hartree–Fock level with the 6-31G* basis set (which sets d polarization functions on the non-hydrogen atoms), as implemented by the Gaussian-90 computer program [1]. Electron correlation effects were taken into consideration by calculating single-point energies with the Moller–Plesset perturbation method of second order (MP2), using the Hartree–Fock optimized geometries.

Two possible reactions were considered: the opening of the C_1N_1 double bond by the attack on the oxygen of formamide and the opening of the C_1N_1 bond by the attack on the formamide's nitrogen, as shown in Figs. 4a and b. It is thus presumed

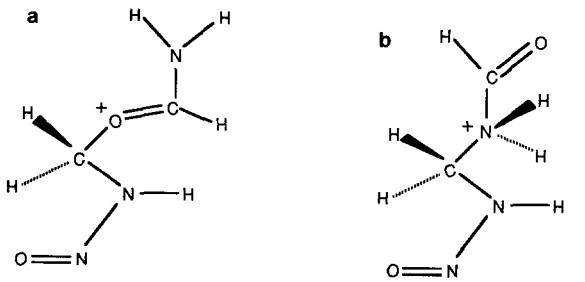

Fig. 5a. Complex formed by the attack of formamide's oxygen on the nitrosoiminium ion. **b.** Complex formed by the attack of formamide's nitrogen on the nitrosoiminium ion

Table 1. Mechanism **A**

C_1O_2 distance	4.0	3.0	2.225 (TS)	1.460 (Opt)
C_1N_1	1.263	1.261	1.280	1.411
N_1N_2	1.516	1.481	1.459	1.319
N_2O_1	1.135	1.141	1.144	1.186
C_2O_2	1.211	1.215	1.225	1.269
C_2N_3	1.326	1.322	1.321	1.284
$O_2C_1N_1$	25.3	61.2	112.3	109.9
$C_2O_2C_1$	147.0	163.3	142.4	120.9
$N_3C_2O_2$	124.4	125.1	124.3	119.8
$O_2C_1N_1N_2$	180.3	180.1	272.5	291.9
$N_3C_2O_2C_1$	179.4	179.5	188.6	184.7
$H_1C_1N_1N_2$	180.0	180.5	179.9	177.3
$H_2C_1N_1N_2$	0.0	0.0	5.8	51.7
		Mechanism **B**		
C_1N_3 distance	4.0	3.0	2.590 (TS)	1.540 (Opt)
C_1N_1	1.275	1.258	1.272	1.411
N_1N_2	1.466	1.553	1.486	1.334
N_2O_1	1.142	1.125	1.137	1.179
C_2O_3	1.214	1.195	1.188	1.164
C_2N_3	1.324	1.366	1.387	1.500
$N_3C_1N_1$	123.6	107.5	114.8	110.2
$C_2N_3C_1$	54.0	87.7	92.3	112.6
$O_2C_2C_1$	124.8	122.9	121.7	118.7
$N_3C_1N_1N_2$	102.7	89.5	64.1	64.7
$C_2N_3C_1N_1$	− 46.8	− 45.4	− 167.7	176.7
$O_2C_2N_3C_1$	6.1	51.0	55.4	78.4
$H_5N_3C_2C_1$	180.0	141.0	136.5	122.6
$H_6N_3C_2C_1$	0.0	− 80.7	− 93.9	− 118.5

that a possible alkylation of the DNA bases by the nitrosoiminium ions would proceed via the opening of the double bond of the ions and it would lead to the formation of complexes shown in Figs. 5a and b.

The C_1O_2 distance and the C_1N_3 distance were used as reaction coordinate for mechanism **A** and mechanism **B**, respectively. They have been kept fixed at values of 4 Å and 3 Å, and the rest of the parameters of the system were optimized. The

Table 2. Energies of intermediate and optimized structures of mechanism **A** and mechanism **B**

	HF/6-31G* (au)	ΔE (kcal/mol)	MP2/6-31G*//HF/6-31G* (au)	ΔE (kcal/mol)
		Mechanism **A**		
C_1O_2				
4.0	− 391.95466	14.2	− 393.03307	13.0
3.0	− 391.95964	11.6	− 393.03526	11.6
2.225	− 391.95274	15.4	− 393.02715	16.7
1.460	− 391.97723	0.0	− 393.05371	0.0
		Mechanism **B**		
C_1N_1				
4.0	− 391.95059	− 5.6	− 393.02403	3.5
3.0	− 391.93284	5.5	− 393.01520	9.1
2.60	− 391.92827	8.3	− 393.00650	14.5
1.54	− 391.94153	0.0	− 393.02963	0.0

Table 3. Energies of the reactants (au)

Structure	HF/6-31G*	MP2/6-31G*//HF/6-31G*
Nitrosoiminium ion	− 222.98532	− 223.60247
Formamide	− 168.93064	− 169.39177

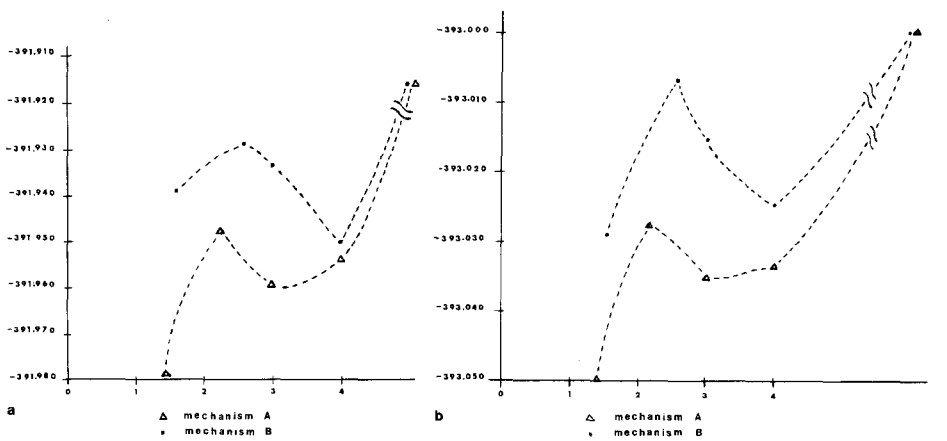

Fig. 6a. Dependence of energy on C_1O_2 distance (mechanism **A**) and C_1N_3 distance (mechanism **B**) for HF calculations. **b.** Dependence of energy on C_1O_2 distance (mechanism **A**) and C_1N_3 distance (mechanism **B**) for MP2 calculations

results are shown in Table 1. Since it was found that transition states are around 2.5 Å, optimization to transition state was performed for both mechanisms, and results are also shown Table 1, together with the complexes optimized for an energy minimum.

The energies corresponding to the geometries displayed in Table 1, together with the energy differences between optimum configurations and the others, are shown in Table 2. Table 3 shows the energies of the reactants. The dependence of energy on the C_1O_2 distance (mechanism **A**) and C_1N_3 distance (mechanism **B**) is shown in Fig. 6a for HF calculations and in Fig. 6b for MP2 calculations.

3 Discussion of results

As stated before, the mechanism proposed here implies an attack of the formamide molecule at the double bond of the nitrosoiminium ion, resulting in the opening of the double bond and formation the complexes 5a and 5b. In the mechanism **A**, as the formamide approaches the C_1, leading to the formation of a CO bond, the C atom changes its hybridization from sp^2 to sp^3. In addition in the **B** mechanism, the N_3 atom changes from a partial sp^2 character present in formamide to an sp^3 character and acquires a positive charge. These changes result in the lengthening of the C_1N_1 bond, the shortening of the N_1N_2 bond and the lengthening of the N_2O_1 bond. Thus the π electrons from the N_2O_1 bond are somewhat delocalized on the N_1N_2 bond. These changes occur in both mechanism **A** and mechanism **B**, as seen from Table 1.

The mechanism **A** features also a shortening of the C_2N_3 bond, while this bond is lengthened in mechanism **B**.

As the complexes 5a and 5b are formed, H_1 and H_2 become positioned above and below the $C_1N_1N_2$ plane. In mechanism **B**, the H_5 and H_6 atoms become positioned above and below $N_3C_2C_1$ plane.

As the formamide approaches the nitrosoiminium ion, according to the mechanism **A**, the energy of the complex becomes lower than the sum of the energies of the reactants. At a 4 Å C_1O_2 distance, the energy is lower by 24.2 kcal/mol. The binding energy increases further at a C_1O_2 distance of 3 Å, becoming lower than the sum of the reactants by 27.4 kcal/mol. This drop in energy is due to an ion-dipole energy minimum. When the C_1O_2 distance becomes 2.2 Å, a transition state is observed, featuring a negative eigenvalue of the hessian matrix. This state is higher in energy than the optimized structure which features a C_1O_2 distance of 1.460 Å, by 15.4 kcal/mol. However, the activated complex is still lower than the sum of the energies of the reactants by 23 kcal/mol.

The values obtained by calculating the correlation energy effects, via the use of the MP2/6-31G*//HF/6-31G* term are similar to the above values, as seen in Table 2.

The approach of N_3 to C_1 is characterized by a drop of energy at C_1N_3 distance of 4 Å (21.6 kcal/mol below the sum of the energies of the reactants). The C_1N_3 distance of 3 Å starts already the ascent toward the transition state, which is found at a C_1N_3 distance of 2.6 Å. It is evident thus, that the O_2 approach presents a ion-dipole energy minimum at a shorter distance than the N_3 approach.

The energy of the optimized complex 5a is much lower than the one of the complex 5b, so it is clear that the attack at the oxygen is more favorable than the attack at the nitrogen. This result is opposite to the attack of diazohydroxides or diazonium ions on guanine, where the N_7 is alkylated preferentially to O_6 [12]. It is found [12] that the O_6 lesions are more damaging than the N_7 lesions. This result might explain the increased carcinogenicity of nitrosamines, in comparison with nitrosoureas which do not feature nitrosoiminium ions as decomposition intermediates. It is possible that the complexes 5a and 5b might be

stable and decompose slowly. As such, the attack of nitrosoiminium ions on the DNA bases, leading to the formation of complexes similar mainly to 5a, might develop lesions pertaining to cancer. Other possibilities include the decomposition of the complexes via hydride transfers, leading to the formation of other carcinogenic agents. Therefore, the presence of nitrosoiminium ions as decomposition intermediates of nitrosamines may contribute to their carcinogenicity.

Further work is in progress, concerning the mechanisms of decomposition of the complexes 5a and 5b.

Acknowledgements. We would like to thank Dr. R. Osman and Dr. K. Raghavachari for useful discussions. AMS also wants to thank Mr. Janni Ravantti for computational help. We also thank the City University of New York for computer time and for Grant No. 668161.

References

1. (a) Magee PN, Barnes JM (1967) Adv Cancer Res 10:163; (b) Magee PN, Barnes JM (1956) Brit J Cancer 10:14
2. Magee PN, Montesaro R, Preussman R (1970) in: Searle CE (ed) Chemical carcinogenesis ACS monograph 173. Am Chem Soc Wash DC, pp 491–625
3. Druckrey HR, Preussman R, Schmahl D, Muller M (1961) Naturwissenschaften 48:134
4. (a) Mizrahi IJ, Emmelot D (1962) Cancer Res 22:339; (b) Margison GP, O'Connor PJ (1979) in: Grover PLD (ed) Chemical carcinogens and DNA. Vol 1, CRC Press, Boca Raton, p 111
5. (a) Singer B (1981) in: Lemontt J, Generoso RM (eds) Molecular and cellular mechanism of mutagenesis. Plenum, NY; (b) Singer B (1976) Nature (London) 264:333; (c) Singer B (1979) J Natl Cancer Inst 62:1329; (d) Lawley PD (1976) in: Searle CE (ed) Chemical carcinogenesis ACS monograph 173. Am Chem Soc Wash DC, pp 83–244
6. (a) Ludlum DB (1975) in: Becker FF (ed) Mechanism of action of 2-haloethylnitrosoureas. Plenum, NY vol 5, p 285; (b) Wheeler GP (1978) A review of studies on the mechanism of action of nitrosoureas, ACS Symp Series 30 Am Chem Soc, Wash DC, p 87; (c) (1976) Proc 7th New Drug Symp on Nitrosoureas, Cancer Treat Rep 60:651; (d) Lown JW, McLaughlin LW, Chang YM (1978) Bioorg Chem 7:47; (e) Kohn KM (1977) Cancer Res 37:1450
7. Montgomery JA, Johnston TP, Shealy YF (1970) in: Burger A (ed) Medicinal chemistry. 4th edn. Wiley, NY
8. Kohn KW, Erickson LC, Laurent G, Ducore J, Sharkey N, Ewig KA (1981) Nitrosoureas: current status and new developments. Academic, NY, chap 6, pp 69–82
9. Lown JW (1982) in: Bardos TJ, Kalman II (eds) Synthesis and properties of new antileukemic 2-haloethylnitrosoureas based on the reactions with DNA. Elsevier, NY, p 61
10. Demeunynck M, Lown JW, Sapse AM (1989) Can J Chem 67:625
11. Gaussian 90, Revision J (1990) Frisch MJ, Head-Gordon M, Trucks GW, Foresman JB, Schlegel HB, Raghavachari K, Robb M, Binkley JS, Gonzalez C, DeFrees DJ, Fox DJ, Whiteside RA, Seegar R, Melius CF, Baker J, Martin RL, Kahn LR, Stewart JJP, Topiol S, Pople JA, Gaussian-90 Inc, Pittsburgh PA
12. Kohn KW, Erickson LC, Laurent G, Ducore J, Sharkey N, Ewig KA (1981) Nitrosoureas: Current status and new developments. Academic, NY