Use of Unrestricted Hartree Fock Computational Theory in the Investigation of the Activation Pathway of Dimethylnitrosamine.

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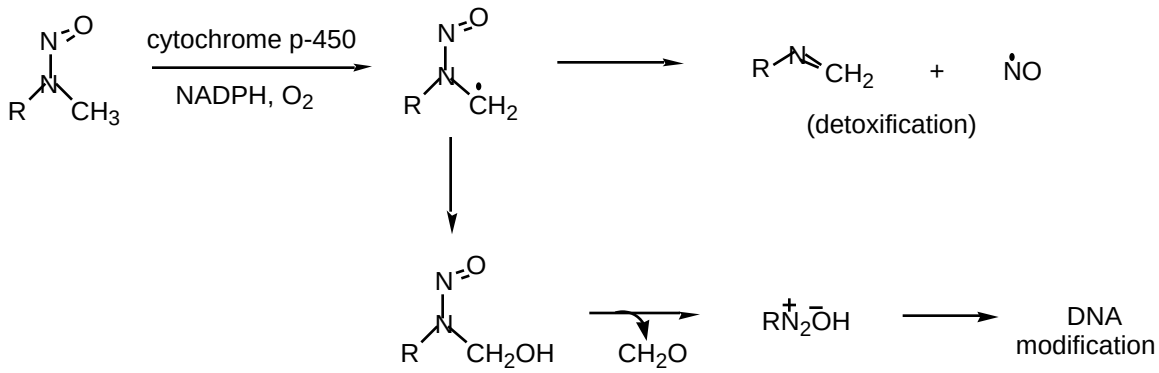
Introduction

Carcinogenisis will always be an important area of study for biochemists, in health conscious societies, such as the one in which we live. Since there is currently *no cure for cancer*, a great deal of emphasis is placed on research in this area. A very important region of study is that which involves the understanding of how compounds are activated *in vivo*, to either modify DNA, or become harmless.

A particularly potent class of ubiquitous carcinogens are nitrosamines, compounds containing the N-N=O functional group. A great deal of progress has been made, in recent years, in understanding their mechanism of activation under biological conditions. The nitrosamine undergoes a single electron transfer to one the various isozymes of the enzyme cytochrome p-450, resulting in a radical α to the N-nitroso group. This reactive intermediate decomposes in one of two ways. Either via the α-hyrdoxynitrosamine, to the diazonium ion[1] which can react at a nucleophilic site on one of the bases of DNA, or a detoxification pathway, involving loss of nitric oxide (NO) and formation of the imine (Scheme 1)[2,3].



Scheme 1

N,N-dimethylnitrosamine (NDMA) is the simplest member of the dialkylnitrosamine family. It is highly hepatotoxic, mutenagenic and carcinogenic with the ability to methylate cellular macromolecules.[4] The major metabolic pathway begins in the liver, with oxidation of NDMA to the α-radical species, by cytochrome p-450 2E1. As described in scheme 1, the radical either undergoes α-hydroxylation resulting in DNA methylation, or loses NO and forms methylimine. This detoxification pathway accounts for 14-20% of the NDMA consumed. The exact geometry of the nitrosamine during the detoxification is unknown. Thus, the goal of this paper is to propose a method for the determination of such geometry.

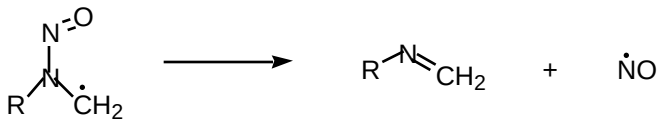
Goals and Objective

The nitrosamine N-N bond is quite rigid, with a relatively high bond rotation energy, and therefore can exist in both a cis and trans isomeric form (bond rotation energy determined experimentally as 23 kcal/mol[5], and calculated as 30.3kcal/mol[6]). Our initial goal is to determine which isomer of the α-radical nitrosamine intermediate is more energetically stable (Figure 1).

cis isomer trans isomer

Figure 1

The most important goal of this proposal is to determine which of the geometric isomers give rise to the lowest activation pathway for formation of the imine (Scheme 2).



Scheme 2

Such information would provide a valuable insight into the mechanism of detoxification of the potent carcinogen, N,N-dimethylnitrosamine. Knowledge of the mechanism of biological reactions, alternative to those that result in DNA mutations, is potentially useful in preventing biological damage. It is likely that other dialkylnitroamines react in a similar manor, so the results of these computations may shed light on much of the field of nitrosamine detoxification.

Computational theory appears to be the only solution to the problem of geometric elucidation of an unstable radical such as the above.

Research Proposal

a) Choice of Methodology

The Roothaan-Hall equations are not applicable to open-shell systems which contain one or more unpaired electrons. Two approaches have been devised to treat open-shell systems, the restricted-spin Hartree Fock (RHF) and unrestricted-spin-Hartree Fock (UHF) methods. RHF uses combinations of singly and doubly occupied molecular orbitals. UHF uses two distinct sets of nuclear orbitals, one for electrons with α spin and the other for electrons with β spins. UHF is the most appropriate way to deal with problems such as molecules near the dissociation limit. Since we are looking at the dimethylnitrosamine free radical, which is an open-shell system, we decided to choose UHF as the theoretical method for our project. There are many reasons for us not to choose semi empirical methods. CNDO, INDO and NDDO were the originally devised semi empirical methods and are now little used. The results produced are not particularly accurate as they were parameterized upon the results of low-level ab initio calculations. They are also limited to small classes of molecules and often require a good experimental geometry to be supplied as input. MINDO/3 was not so much a significant change in the theory being based on INDO. MINDO/3 has several limitations, including errors in heats of formation and in calculated bond angles. More importantly, both MINDO and INDO deal poorly with systems involving lone pairs of electrons making them inappropriate for the nitrosamine radical, which has no less than 4 lone pairs. Large errors in the MNDO heats of formation are encountered in molecules containing NO bonds because no

molecules with NO bonds were used during parametization. Also, heats of formation for neutral radicals in MNDO are largely deviated from experimental results.

The NDMA free radical is a highly polarized molecule with lone pairs on both the oxygen and nitrogen atoms. The basis sets STO-*n*G, 3-21G*, 6-31G* and 6-31G**, are all unable to deal with species containing lone pairs which have a significant amount of electron density away from the nuclear centers. The most appropriate basis set for this problem, in which time and money are not an issue, is 6-31++G**, in which highly diffuse functions are added to the basis set to remedy the deficiency of the basis sets mentioned above. As the molecules we are looking at are relatively small (with only 5 heavy atoms) we are not limited, by complexity, to using lower computational levels.

We are aware that there are limits to the use of Hartree-Fock calculations, the most significant of which is its inability to adequately represent electron correlation, resulting in the calculated energy being higher than the actual energy. When looking at systems involving radicals, spin contamination can also be a problem, resulting in property errors.

b) Scope of Project

We propose the use of the Gaussian 94 program (for example on shiva accounts such as we have used in this class) to run a UHF 6-311++G** geometry optimization of the cis and trans NDMA free radicals. The key data obtained from these calculations will be the optimized geometry for both isomers, and the energy of each. Comparing these energies, we will be able to determine which more stable, and therefore which of the two will be the more dominant isomer. The Z-matrix input for the geometry optimization for the molecules are listed below:

Cis N,N-dimethylnitrosamine radical

```
0  2
N
O  1  NO
N  1  NN     2  NNO
C  3  C4N    1  C4NN    2  C4NNO
C  3  C5N    1  C5NN    2  C5NNO
H  4  H6C4   3  H6C4N   1  H6C4NN
H  4  H6C4   3  H6C4N   1  H7C4NN
H  5  H8C5   3  H8C5N   1  H8C5NN
H  5  H8C5   3  H8C5N   1  H9C5NN
H  5  H8C5   3  H8C5N   1  -H9C5NN
```

NO=1.2, NN=1.3, NNO=125., C4N=1.4, C4NN=115., C5NN=115., C5N=1.45, C4NNO=5., C5NNO=170., H6C4=1.05 ,H6C4N=120., H6C4NN=170., H7C4NN=-5., H8C5=1.1 , H8C5N=110., H8C5NN=0., H9C5NN=120.

Trans N,N-dimethylnitrosamine radical
0 2
N
O 1 NO
N 1 NN 2 NNO
C 3 C4N 1 C4NN 2 C4NNO
C 3 C5N 1 C5NN 2 C5NNO
H 4 H6C4 3 H6C4N 1 H6C4NN
H 4 H6C4 3 H6C4N 1 H7C4NN
H 5 H8C5 3 H8C5N 1 H8C5NN
H 5 H8C5 3 H8C5N 1 H9C5NN
H 5 H8C5 3 H8C5N 1 -H9C5NN

NO=1.2, NN=1.3, NNO=125., C4N=1.4, C4NN=115., C5NN=115., C5N=1.45, C4NNO=-175., C5NNO=-10., H6C4=1.05, H6C4N=120., H6C4NN=170., H7C4NN=-5., H8C5=1.1, H8C5N=110., H8C5NN=0., H9C5NN=120.

The most stable radical may not be the conformer that gives rise to the lowest energy pathway to the products ($CH_3N=CH_2$ + NO). To determine the energy for each reaction, the transition state energy for both the cis and trans NDMA radicals must be calculated. This can be accomplished by carrying out a gradient norm optimization, using the Gaussian 94 program. The suspected geometry of the transition state must be described to the computer in terms of a Z-matrix, with the command *UHF/6-31++G** opt=TS*. It seems logical to assume that the transition state will be much like the NDMA radical, but with a much longer N-N bond (~0.4Å), since this is the bond that breaks during the reaction. The success of this method can be tested by looking at the frequency output. The true transition state will be a first order saddle point with one negative frequency. No more, and no less.

If this approach is unsuccessful, the method of coordinate driving can be used to determine to transition state. This involves carrying out an optimization on the compound at increasing N-N bond lengths. Again, assuming that the N-N bond length increases during the process of the reaction, the geometry can be determined at bond lengths increasing by 0.1 Å, until a energy maximum is observed. This should correspond to the transition state.

c) Interpretation

By comparison of the energy output for the cis and trans N,N-dimethylnitrosamine radicals, the most stable conformer can be identified (this is the one lower in energy). Subtracting the energy of each starting radical from the transition state energy, the energy of activation for the reaction can be calculated:

$$\text{Activation Energy}_{trans} = \text{Transition State Energy}_{trans} - \text{Ground State Energy}_{trans}$$

$$\text{Activation Energy}_{cis} = \text{Transition State Energy}_{cis} - \text{Ground State Energy}_{cis}$$

It is most probable that the reaction with the lowest activation energy is the one most likely to proceed, under thermodynamic conditions.

Since NDMA radicals are easily transformed to NO and imine, we believe that the N-N and C4N bond lengths should be longer than those in NDMA. We also assume the cis and trans NDMA radicals to be almost planar. From optimized geometries of the cis and trans NDMA radicals we can make a judgment about our assumptions.

It is assumed that the energies calculated are in fact the minimum possible in each of the situations.

d) Facilities and Feasibility

A set up like our shiva accounts can be used successfully for this problem. This involves a PC interface connected to a minisupercomputer.

e) Timelines

Despite the large basis set we have chosen to work this problem, due to it's small size, these calculations will not take a considerably long period of time. A week or two is likely to be sufficient.

Group Dynamics

During our first meeting, we decided to talk with our advisor, Dr. Loeppky, who gave us some very interesting ideas for a topic, relevant to our area of research. During this period of course 433 we learned a lot about various theoretical methods, their applications and shortcomings. After a careful analysis of our problem we decided to use UHF to do our calculations on our second group meeting. With the goals in our mind, we discussed the details of the calculations on our third group meeting. Finally each of us wrote part of the report. Each of these formal meetings lasted between 30 minutes and one hour. However, since we share a lab, we frequently discussed our ideas, in short, informal meetings.

As mentioned above, we wanted to design a problem that would be beneficial to our field of study. Sadly, it would appear that very few computations have been carried out on N-nitroso compounds (our search was yielded only one paper). Therefore our literature search has been limited to broadening our knowledge of NDMA and the reaction in question.

Since the topic is very closely related to the research in our group, and itself very important and interesting, we had a lot of fun in the preparation of this project. The research we have carried out, although at times difficult has proved rewarding. We are excited about hearing comments on our proposal, and then carrying out the calculations we have proposed.

Working in a group helps greatly in the understanding of ideas, which is very useful in a complex subject area such as this.

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