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Notes

Molecular Orbital Calculations on Suspected Intermediates in Oxidative Amine Metabolism†

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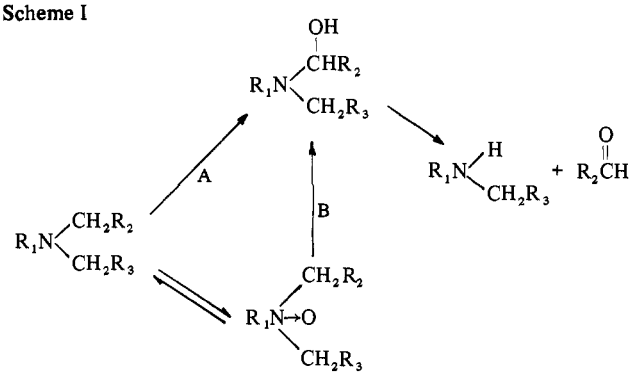
Many biologically important amines such as nicotine,[1] morphine,[2] and others[3] undergo oxidative N-dealkylation and N-oxidation reactions which often represent primary metabolic pathways for such compounds. These metabolic processes have been the subject of a tremendous amount of research in the last 10 years. (For reviews, see ref 4.) One area of continuing interest and debate is the oxidative N-dealkylation of a variety of *tert*-*N*-alkyl compounds, possibly with the exception of *tert*-butyl tertiary amines,[5] with regard to the intermediacy or nonintermediacy of *N*-oxides.[6] The postulated nondetailed mechanisms are shown in Scheme I. The major question is whether pathway A and/or B is viable *in vivo* and, if so, to what extent.

Scheme I



There is evidence supporting both mechanisms and the dispute is still unresolved although most evidence favors path A with some recent work by Bickel[7] indicating both pathways may be operative in certain cases. If the *N*-oxide is a true intermediate, the rearrangement to a carbinolamine must involve an intramolecular migration of the *N*-oxide oxygen atoms to a neighboring carbon atom since the product aldehyde contains ^{18}O when the initial source of oxygen is $^{18}O_2$.[8] *N*-Oxides have been synthesized and are fairly stable unless heated near their melting point, at which time they decompose into radicals where one of the possible recombination products is a carbinolamine.[9] Most acyclic carbinolamines spontaneously break down into an amine and an aldehyde. This surprising reactivity of carbinolamines has never been well explained. It was hoped that some insight into the process of N-dealkylation might be gained by looking into the electronic distributions and relative stabilities of postulated intermediates and products using molecular orbital methods.

We performed CNDO/2 calculations[10] on trimethylamine *N*-oxide, *N*,*N*-dimethylcarbinolamine, the isomeric *N*-methyl-*N*-ethylhydroxylamine, and formaldehyde plus dimethylamine (Figure 1). Hydroxylamines were included in the calculations since certain secondary and primary amines are known to be metabolized to hydroxylamines,[11] and because we wished to compare the energies of the various C_3H_9NO isomers. The conformation and bond distances for trimethylamine *N*-oxide were taken from crystal structure data;[12] for the hydroxylamines, the O-H was taken to be cis to the nitrogen lone pair, as found by Radom, *et al.*,[13] and Giguere and Liu[14]; for the carbinolamines, the completely staggered conformation, with the OH trans to the nitrogen lone pair, was found to be the lowest energy. The Mulliken atomic populations[15] calculated are relatively insensitive to conformational changes involving rotation around single bonds. As can be seen the *N*-oxide was found to be some 41 kcal/mol less stable than the corresponding carbinolamine. The *N*-oxide has a high electron density on the oxygen atom (Mulliken population on O ≡ ζ(O) = 8.504) with the positive charge smeared primarily over the nitrogen and hydrogens of the rest of the molecule. Since the carbon atoms bear little of the positive charge, $\Delta H^{\ddagger}$ for the intramolecular transfer of oxygen from the nitrogen to the carbon atom would probably be significant. Thus, from a kinetic point of view, there would appear to be no *a priori* tendency for intramolecular rearrangement. However, perturbing the system could alter the situation and a mechanism whereby the oxygen with its excess negative charge would polarize one of the C-N bonds during attack on the carbon is conceivable. As the oxygen approached the carbon, the carbon would gradually transfer charge to the nitrogen, becoming more electrophilic and facilitating C-O bond formation. On the other hand, in the thermodynamically more stable carbinolamine, the carbinol carbon is very electron deficient which could render this intermediate quite reactive toward fragmentation to formaldehyde and dimethylamine, a fact

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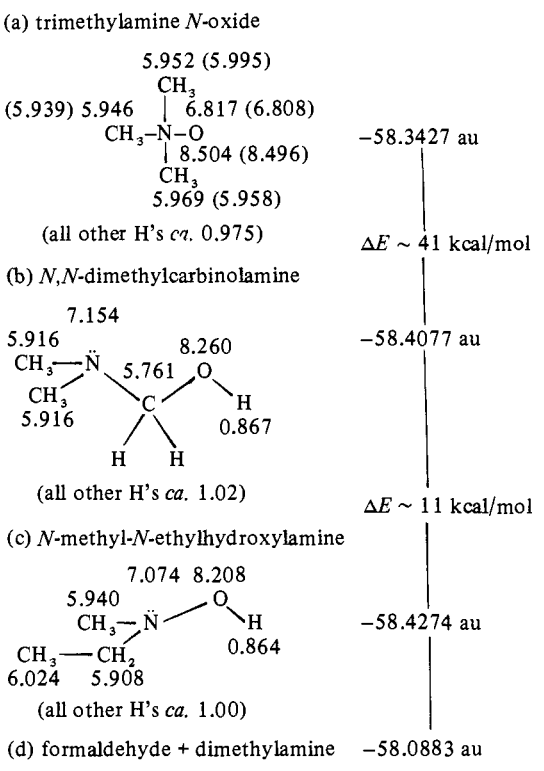


Figure 1. Mulliken atomic populations and energies of C_3H_9NO isomers calculated by the CNDO/2 method. Bond lengths and angles for trimethylamine *N*-oxide from ref 12. All C–H distances 1.083 Å with other bond lengths and angles taken from "Handbook of Chemistry and Physics," 51st ed, R. C. Weast, Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1970, pp F-154 ff. Numbers in parentheses for the N-oxide are charge densities with the molecule solvated.

which is observed experimentally. Although fragmentation to formaldehyde plus dimethylamine appears to be an energy-requiring process from the calculations, the results in Figure 1 do not accurately describe the thermodynamics of the reaction since no term corresponding to the energetically favorable entropy change is included in the calculations.[§]

These electronic structure calculations were carried out on the gas-phase molecules and one needs some estimate of the effects of solvation on both the relative energies and the charge distribution of these species. In aqueous solution the very large negative charge of the *N*-oxide oxygen should allow considerable solvation energy due to the protons of nearby water molecules. We estimate a solvation energy of approximately 40 kcal/mol from this interaction.[#] Moreover, the carbinolamine and hydroxylamine should form four H bonds of a total strength of approximately 15–20 kcal/mol.[**] CNDO/2 calculations on the solvated trimethylamine *N*-oxide [with three waters approaching the oxygen (with R(O–O) = 2.5 Å tetrahedral N–O···O angles) and external water hydrogens trans to the N–O bond] indicate *little change* in the charge distribution of the *N*-oxide (charge densities in parentheses in Figure 1) due to solvation. At neutral pH, the carbinolamine will be largely protonated, increasing its solvation energy.

From the above analysis, it appears unlikely that solvation will change the relative energetics of the carbinolamine and *N*-oxide, although the energy gap may be smaller. It is of interest that an empirical estimate of the energy difference between the carbinolamine and the *N*-oxide predicts the carbinolamine to be more stable by 51 kcal/mol,[17,††] and it is in accord with the relative stabilities predicted by this theoretical treatment.

The electronic structure of the protonated carbinolamine is similar to the neutral species, with most of the positive charge shared by the nitrogen and the methyl and methylene hydrogens. Surprisingly, the Mulliken population on the carbinol carbon increases slightly from 5.761 to 5.785 electrons upon protonation. A referee suggested to us that an alternative rationalization of the carbinolamines' susceptibility to fragmentation might be provided by the relative bond orders of the *N*-oxide, hydroxylamine, and neutral and protonated carbinolamine. The *ab initio* C–N bond orders for the CH_5NO isomers are 0.610, 0.645, and 0.660 for the *N*-oxide, hydroxylamine, and carbinolamine and the corresponding CNDO/2[‡‡] results for the C_3H_9NO isomers are 0.625, 0.636, and 0.647. Protonation of the C_3H_9NO carbinolamine lowers its CNDO/2 bond order to 0.616. This change in bond order is small so not much can be made of this difference; however, good empirical evidence that protonation is an important step in carbinolamine breakdown comes from the stability of amide[18] and carbazole[19] carbinolamines, both of which have much less basic nitrogens.

Thus, the evidence appears strong in support of the thermodynamic instability, but kinetic stability (observed empirically), for the *N*-oxide. These electronic structure calculations provide some rationalization why the *N*-oxide is relatively kinetically stable and the carbinolamine very difficult to isolate. The calculations cannot, however, answer the question of whether the *N*-oxide is an intermediate *in vivo*. Even if one could carry out a precise potential surface study for O transfer in the isolated molecule, this question could not be answered with any confidence, because we know neither the mechanism for oxygen transfer nor the solvation effects on the transfer.

We have attempted to get a more precise estimate for the energy differences by carrying out *ab initio* calculations using an STO-3G basis set[20] as well as CNDO/2 calculations on the isomers of formulas CH_5NO. The charge distribution and relative energies for these species are presented in Figure 2. The charge distributions predicted by the *ab initio* and CNDO/2 methods are very similar, but the *ab initio* energy difference between the *N*-oxide and carbinolamine is almost

§ The difference in energy between the carbinolamine and the aldehyde plus amine is greatly exaggerated in the CNDO/2 studies; the *ab initio* studies of the CH_5NO isomers more accurately reflect these energy differences. Radom, *et al.*,[13] find the CH_5NO carbinolamine to be 14 kcal/mol more stable than formaldehyde and ammonia.

This is slightly more than half the solvation energy of OH^- in the gas phase due to three water molecules.[16] Since the oxygen in the *N*-oxide is considerably less negative than that in OH^-, one would expect weaker hydrogen bonds with water.

** A reasonable average H-bond strength for the four hydrogen bonds is 5 kcal/mol, the N···HOH being stronger and the OH···OH_2 weaker.

†† The N–O bond energy was determined using eq 3.11 of ref 17, p 91.

‡‡ The bond order was calculated using the definition

$$(BO)_{A,B} = \sum_{r}^{A} \sum_{s}^{B} \sum_{i}^{nocc} S_{rs} C_{ri} C_{si}$$

where $(BO)_{A,B}$ is the bond order between atoms A and B, S_{rs} is the overlap matrix element between atomic orbitals r and s, and C_{ri} is the molecular orbital coefficient of atomic orbital r in molecular orbital i. The sums over r and s go over all the AO's on atoms A and B, respectively, and the sum over i goes over all the occupied MO's. This definition of bond order is not rigorous for CNDO/2, because overlap is neglected in determining the coefficients, but the fact that the bond orders are similar in magnitude and give the same trend for the C–N bonds in the three CH_5NO isomers calculated *ab initio* gives us some confidence in this quantity as a reasonable representation of CNDO/2 bond order.

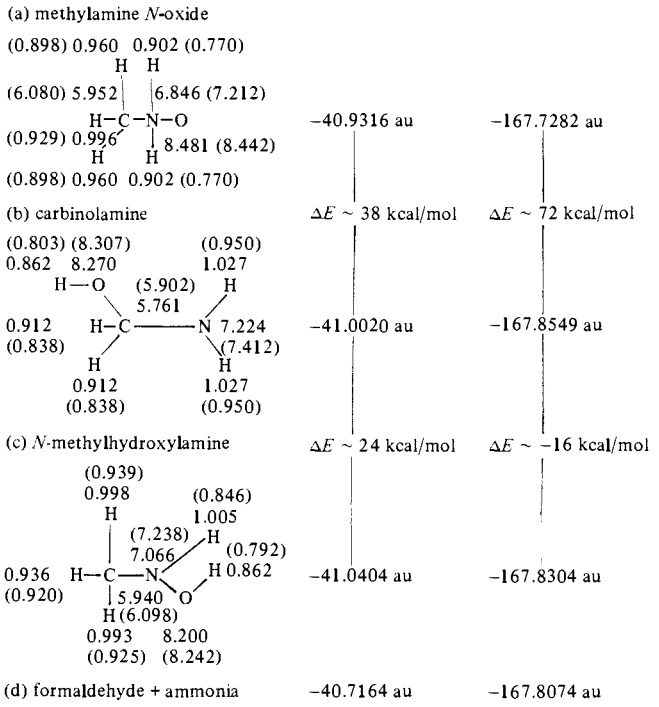


Figure 2. Mulliken atomic populations and energies of CH_5NO isomers found in the CNDO/2 and *ab initio* calculations. Molecular conformations and bond lengths and angles are the same as those of the corresponding C_3H_9NO isomers. The numbers in parentheses are the Mulliken populations from the *ab initio* calculations.

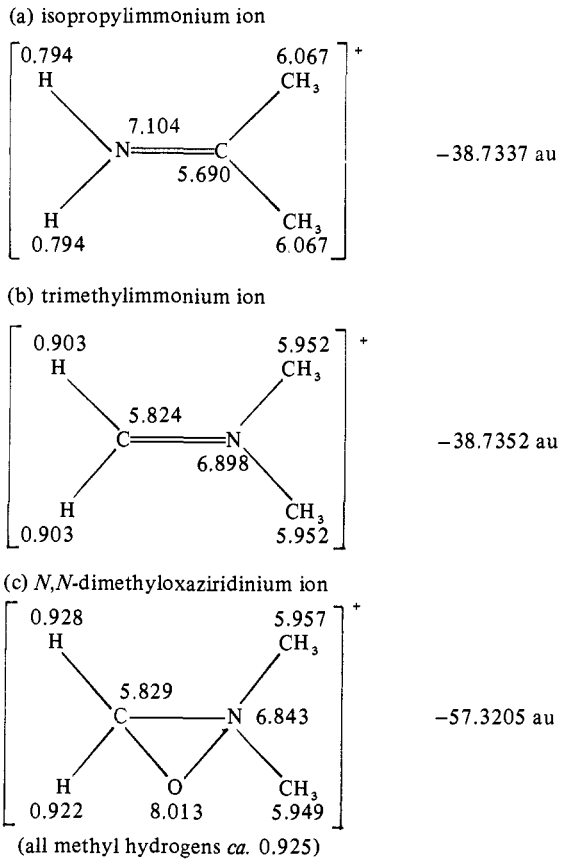


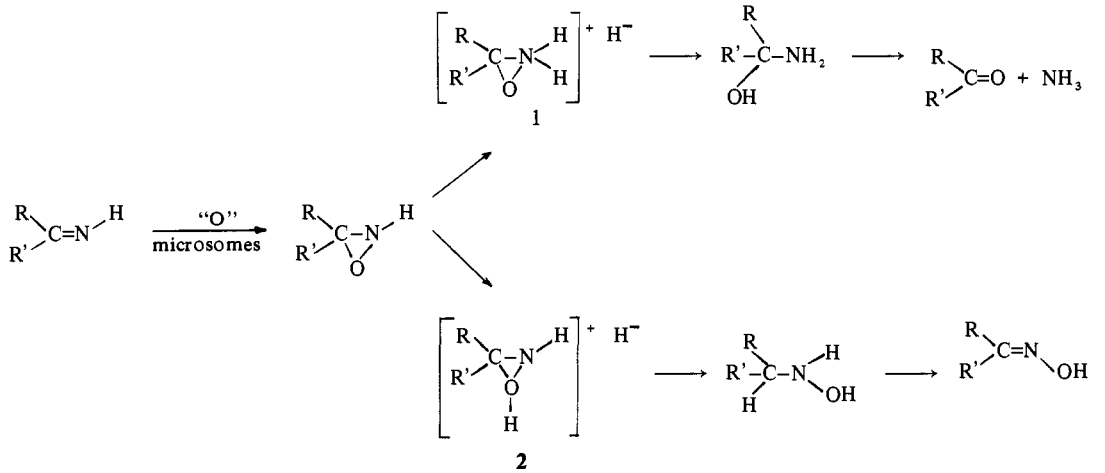
Figure 3. Mulliken atomic populations and energies of immonium ions and an oxaziridinium ion calculated by the CNDO/2 method. See ref 27 for further information on immonium ions.

twice as large as predicted by CNDO/2, supporting our previous conclusions on the relative energies of the two types of species. The relative energies of hydroxylamine and carbinolamine are reversed. More precise *ab initio* calculations support the greater stability of the carbinolamine than the hydroxylamine; Radom, *et al.*,[13] find an energy difference of 38 kcal/mol between the two species. The CNDO/2 charge distributions, however, show why the carbinolamine is kinetically unstable. The *ab initio* atomic populations (Figure 2, in parentheses) show the same trends as the CNDO/2, but the hydrogens generally have less charge and the "heavy" atoms, C, N, and O, more charge in the *ab initio* calculations.

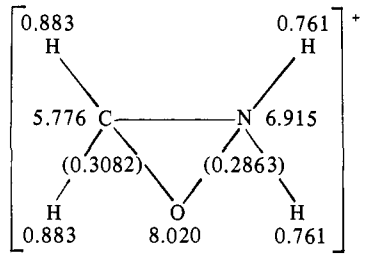
We have also carried out a CNDO/2 calculation on the trimethyl *N*-oxide cation radical and the majority of the charge loss was on the oxygen [ζ(O) = 7.99, ζ(N) = 6.79, ζ(C) = 5.96], but the unpaired electron spent considerable time on the methyl carbons. Thus, one cannot rule out a possible radical mechanism for the *N*-oxide to carbinolamine reaction. This seems more likely than heterolytic cleavage and recombination although one cannot make a definitive statement on this with these calculations. It is, however, noteworthy that free radicals have been observed using CIDNP in thermal rearrangements of *N*-oxide, such as the Meisenheimer rearrangement.[21]

Recent work on oxygenation mechanisms of xenobiotics by microsomal mixed function oxygenases has indicated that epoxides are intermediates in certain aromatic hydroxylations.[22] Although no such intermediates have been detected in amine oxidations, one might speculate that immonium ions or ion radicals may be generated in microsomal systems by some form of hydrogen abstraction with subsequent oxygenation to reactive oxaziridium ions or ion radicals. Results of CNDO/2 calculations on *N,N*-dimethylimmonium

Scheme II

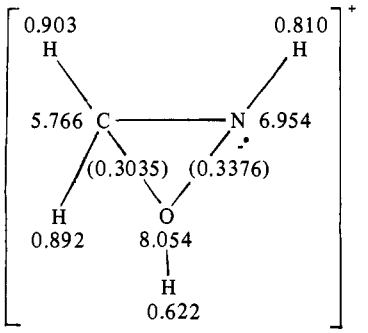


(a) oxaziridinium ion (N-protonated)



−39.9237 au

(b) oxaziridinium ion (O-protonated)



−39.8331 au

Figure 4. Mulliken atomic populations and energies and the C–O, N–O bond orders (in parentheses) of N- and O-protonated oxaziridinium ion. The oxaziridine geometry used in the calculations was taken from J. M. Lehn, B. Munsch, Ph. Millie, and A. Veillard, *Theor. Chim. Acta*, 13, 313 (1969).

ion, isopropylimmonium ion, and *N,N*-dimethyloxaziridinium ion are shown in Figure 3.

The postulate of an intermediate oxaziridinium ion is not completely unfounded considering the work of Parli, *et al.*,[23] who alternatively suggested a direct cytochrome P-450 mediated oxidation of an imine to an oxime. Although none of the recovered products of the *in vitro* oxidation was an oxaziridine, one might speculate that such a species may be an intermediate as outlined in Scheme II.

As shown, the mechanism might involve oxidation to the oxaziridine followed by "protonation" at either the oxygen or nitrogen, possibly by an enzyme as suggested by Watabe and Suzuki[24] for the hydrolysis of aziridines.

Another possibility for the breakdown of the oxaziridine not shown in Scheme II, but suggested by Watabe and Suzuki's[24] work on aziridines, is attack by other nucleophiles such as a hydroxyl group of water to give directly a carbinol hydroxylamine which could then dehydrate to give oxime or ketone. The CNDO/2 calculations (Figure 4) of the two protonated species **1** and **2** indicate favored N-protonation and differences in the C–O *vs.* N–O bond orders (numbers in parentheses) which might lead to preferential opening of the protonated oxaziridine as shown in Scheme II to ultimately produce the observed ketone and oxime.[23,25,26]

The substituent effects on immonium ions are very interesting. Substituting methyl groups on the positive carbon (isopropylimmonium ion) makes the carbon *more* positive [relative to the unsubstituted immonium ion, where $\zeta(C) = 5.75$ and $\zeta(N) = 7.01$ were found in CNDO/2 calculations][27] and the nitrogen more negative. Substitution of methyl groups on nitrogen makes the nitrogen more positive and the carbon more electron rich. These results show that the simple electron-donating inductive model to describe the substituent effect of methyl groups does not work for immonium ions and probably should be applied with caution to any system involving heteroatoms.

In conclusion, our electronic structure calculations on some postulated intermediates in amine metabolism have allowed us to say the following. (1) If the *N*-oxide is an intermediate in oxidative dealkylation, it is probably subjected to electron loss prior to forming the carbinolamine, since the positive character of the adjacent carbon atoms is small in the *N*-oxide. (2) An N-protonated oxaziridinium ion would be energetically favored over an O-protonated form. (3) Methyl groups appear to be electron withdrawing on the positive carbonium immonium ions ($R_2N\text{-}^{+}CR_2'$) when directly bonded to it (R_2') but electron donating to the carbon when bonded to the nitrogen (R_2).

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Effects of Small Changes in Chemical Structure on Stereospecificity

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In further investigation of the effects of changes in chemical structure on stereospecificity it has recently been possible to examine the effects of methylation on the affin-