

Toward the Generation of NO in Biological Systems. Theoretical Studies of the N₂O₂ Grouping

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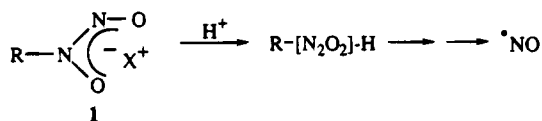
Received September 20, 1994[⊙]

Substituent effects for R[N₂O₂]H (R = NH₂, O⁻, CH₃, H, CF₃, and CN) have been studied by *ab initio* methods and were found to have considerable effect in determining whether or not these derivatives exist as the *N*-nitroso,*N*-hydroxylamine **3** or the tautomeric hydroxydiazonium *N*-oxide **4**. When the substituents (R) were NH₂, O⁻, CH₃, and H, a clear preference for **4** over **3** at the MP2 level was found, attributable to intramolecular hydrogen bonding and favorable substituent effects. The hydroxydiazonium *N*-oxide structures **4** were all planar while the *N*-nitroso,*N*-hydroxylamine structures **3** were tetrahedral, with the exception of R = O⁻. For R = H and NH₂ it is estimated that hydrogen bonding stabilizes **4** by approximately 4.5 kcal mol⁻¹. Although hydrogen bonding was also present to the same extent when the substituents were CF₃ and CN, little preference for either **3** or **4** was found and for these two substituents it is expected that both tautomers are likely to coexist in protic media. Similar charges for both the interior oxygen (O1) and exterior oxygen (O2) in the corresponding anions, suggest that protonation will occur without preference at either O1 or O2 for all substituents, however, when R = NH₂, O⁻, CH₃, and H rapid tautomerism to the thermodynamically more stable **4** is expected. The difference in stabilities between **3** and **4** when the substituent is a nucleophilic residue (for example, NH₂ or O⁻) allows a clear mechanism for the release of NO to be postulated.

Introduction

The development of prodrug analogs which release nitric oxide (NO) is of current importance because of NO's ability to regulate a host of physiological processes. NO has recently been shown to originate from the urea cycle¹ and plays an important role in the control of blood pressure, neurotransmission, blood clotting, immune-system control, destruction of microorganisms and melanoma cells, and learning and memory.²

Salts containing the [N₂O₂]⁻ functionality **1** are ideal candidates for the controlled and gradual release of NO.^{3,4} They are stable in the solid form but are capable of releasing all of the NO contained in the [N₂O₂] moiety (2 equiv) upon dissolution in acidic solution.^{3,4} The rate of NO production from **1** increases with increasing acidity suggesting that protonation to form the corresponding R[N₂O₂]H derivatives is the key step in the route to NO release.



R = NEt₂, NMe₂, polyamine, O⁻

X = Na, K, NH₄, R₃NH.

Structure **2** was first proposed for the [N₂O₂]H grouping in 1895.⁵ It was later found that either the *N*-nitroso,*N*-hydroxylamine structure **3** or the hydroxydiazonium *N*-oxide structure **4** were more plausible, based on the X-ray structure of the dipotassium salt of methylene-*bis*-nitrosohydroxylamine.⁶ More recently, the X-ray crystal structure of several R[N₂O₂]H derivatives (R = phenyl and *cis*-4-methylcyclohexyl), showed unequivocally that they have structure **4** in the solid state.⁷ Although it can be misleading to predict the structure present in solution based upon that observed in the solid state, it was also demonstrated by UV analysis that solutions containing these compounds correlate best with structure **4**.⁷ Compounds of type **4** characteristically display a strong π → π* transition between 225–245 nm (ε = (6.0–9.0) × 10³ M⁻¹ cm⁻¹) while structures **3** are “expected” to exhibit this strong transition along with a weaker n → π* transition between 340–380 nm for the NO chromophore. This latter argument is based primarily on observations that related O-alkylated derivatives of the type **5** display this weak absorption.^{7,8}

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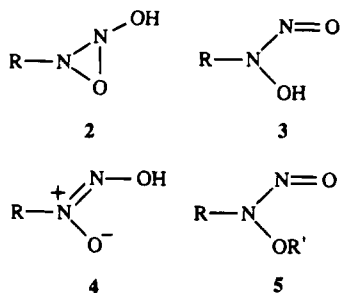
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It is important to emphasize that there has been a general misconception in the earlier literature that the $[\text{N}_2\text{O}_2]\text{H}$ group existed as **3**, presumably because this structure does not involve a separation of charge. This led to conclusions that solutions containing **3** (which must now be regarded as **4** at least for the derivatives known, e.g., R = phenyl and alkyl) did not show the "expected" transition for the N=O chromophore in the electronic spectra due to intermolecular and/or intramolecular hydrogen bonding.⁹ To the best of our knowledge the *N*-nitroso,*N*-hydroxylamine structure **3** has not been observed in the solid state¹⁰ nor has its existence in solution proven conclusively for the derivatives which are currently known.

We felt, however, that the substituent (R) may play an important role in influencing whether or not the $[\text{N}_2\text{O}_2]\text{H}$ grouping exists as either **3**, **4**, or both. For example, if R was a strong electron-withdrawing group then this could be expected to destabilize **4** since the substituent would place a greater positive charge on an already positively charged nitrogen atom, thus favoring tautomer **3**. This type of tautomeric equilibrium would depend on the relative stabilities of **3** and **4**, and in protic media a maximum would be expected where rapid proton exchange from one oxygen atom to the other could occur. Factors of this kind may influence the rate of NO release from these types of systems and consequently should be considered in any design of NO releasing drugs.

Considering the attention that salts of the type **1** are now receiving as possible candidates for the release of NO,^{3,4} coupled with the fact that the as yet unproven structure **3** may exist given the right choice of substituent, we felt that a theoretical study of how substituents are likely to influence whether protonation of salts **1** leads to structures **3** or **4** or both was warranted. This type of study should provide a rationale for why the hydroxydiazonium *N*-oxide structures **4** (R = phenyl or alkyl), which contain a separation of charge, are preferred over the neutral *N*-nitroso,*N*-hydroxylamine structures **3** as well as providing evidence for the mechanism of NO release from derivatives which contain the $[\text{N}_2\text{O}_2]\text{H}$ grouping bonded to a nucleophilic residue (e.g. $\text{N}(\text{Et})_2$, $\text{N}(\text{Me})_2$, polyamine, O^-). The substituents chosen for this study were R = NH_2 , O^- , H, CH_3 , CF_3 , and CN which range from mesomerically donating to strong inductively electron withdrawing.

Computational Details

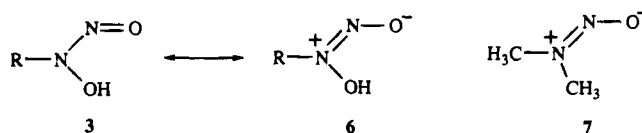
The calculations described here were performed using the GAMESS-UK software package,¹¹ implemented on both a Silicon Graphics Iris Indigo workstation and a CRAY SMP mainframe computer. Full geometry opti-

mizations were performed at the restricted Hartree-Fock (HF) level using 6-31G* basis sets for all atoms. Electron correlation was accounted for by performing calculations which employed second order Møller-Plesset (MP2) perturbation theory at the HF optimized geometry. In order to check the accuracy of these geometries and energies, we recomputed (GAUSSIAN92) the geometries at the MP2 level and redetermined the energies at the MP4 level (SDTQ) for the smallest molecule, $\text{H}[\text{N}_2\text{O}_2]\text{H}$. The effect of diffuse functions on the geometries (HF) and on the energies (MP2) was also examined. The HF wave functions were subsequently analyzed using the MOPLOT¹² and AIMPAC¹³ suites of programs. Details of these analyses are given in the text where appropriate.

Results and Discussion

Geometries of 3 and 4. Optimized geometries of isomers **3** and **4** when R = NH_2 are given in Figure 1 showing the labeling scheme used throughout this work. Geometrical data for all isomers **3** and **4** (R = NH_2 , O^- , CH_3 , H, CF_3 and CN) are given in Table 1. The most significant differences between the two structural types are the geometry at the central nitrogen of the $[\text{N}_2\text{O}_2]$ moiety and the orientation of the hydroxyl group with respect to the $[\text{N}_2\text{O}_2]$ plane.

With the exception of the strong mesomerically donating substituent R = O^- , the structures of **3** are all approximately tetrahedral at the central N1 atom (Figure 1a) with the hydrogen atom of the hydroxyl group out of the N1, N2, and O1 plane. The N2-N1-O1 bond angles were all approximately 115° , slightly larger than the typical tetrahedral value. A shortening of the N1-N2 bond distance was also found; calculated values range from 1.32 to 1.38 Å, compared to expected single bond values of ca 1.45 Å,¹⁴ indicative of double bond character between N1 and N2, although the calculated bond lengths are still longer than typical¹⁴ N-N double bonds (ca 1.22–1.24 Å). This indicates that the resonance contributor **6** makes an important contribution to the overall description of **3**.



When R = O^- the importance of **6** is most noticeable. Comparison of the calculated N1-N2 bond distances for all substituents of structural type **3** (Table 1) shows that when R = O^- this distance is shorter by about 0.1 Å, while the N2-O2 bond distance is longer by approximately 0.1 Å. In fact, when R = O^- the calculated geometry is planar, which implies that **6** is the best description. The importance of resonance contributors

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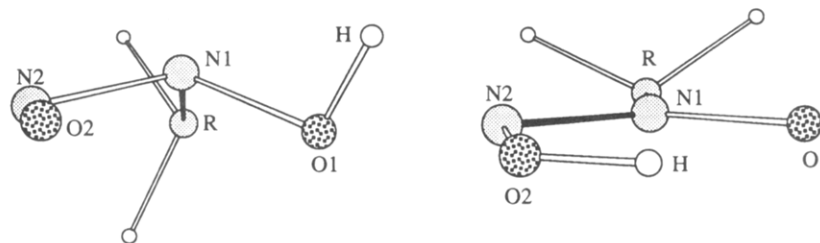


Figure 1. (a) Optimized geometry of **3** (R = NH₂). (b) Optimized geometry of **4** (R = NH₂).

Table 1. Optimized Geometrical Parameters^a for Structures **3** and **4**. Atom Labels are Shown in Figure 1

	isomer	Substituent (R)					
		NH ₂	O ⁻	CH ₃	H	CF ₃	CN
d(N1-N2), Å	3	1.366	1.231	1.323	1.346	1.361	1.376
	4	1.223	1.259	1.225	1.222	1.225	1.232
d(N1-O1), Å	3	1.365	1.376	1.370	1.366	1.360	1.359
	4	1.243	1.258	1.257	1.251	1.310	1.262
d(N2-O2), Å	3	1.169	1.258	1.183	1.173	1.164	1.161
	4	1.337	1.390	1.326	1.324	1.258	1.303
d(N1-R), Å	3	1.384	1.300	1.446	0.998	1.425	1.351
	4	1.374	1.241	1.453	1.007	1.457	1.380
d(O-H), Å	3	0.949	0.950	0.951	0.949	0.950	0.952
	4	0.960	0.957	0.963	0.962	0.965	0.966
∠N1-N2-O2, deg	3	115.1	117.7	115.2	115.2	114.0	113.6
	4	112.3	110.7	112.3	112.2	111.9	111.9
∠N2-N1-O1, deg	3	113.4	119.3	116.6	116.4	115.6	117.7
	4	126.2	121.4	124.3	126.6	125.6	125.3
∠O1-H-O2, deg	4	114.0	119.6	115.5	114.5	114.8	114.9
φ(N2-N1-O1-H), deg	3	-106.5	0.0 ^b	-63.2	93.9	109.9	-97.0
∠O1-N1-R, deg	3	110.9	116.0	111.0	109.6	110.4	114.8
φ(N2-N1-O1-R), deg	3	129.1	176.8 ^b	136.7	126.2	130.2	190.9

^a d, ∠, φ refer to bond distance, bond angle and dihedral angle, respectively. ^b Isomer **3** is planar when the substituent (R) is O⁻.

Table 2. Calculated Energies of the HF Optimized Structures **3** and **4** for the Different Substituents (R)

	calcn type	Substituent (R)					
		NH ₂	O ⁻	CH ₃	H	CF ₃	CN
3	HF ^a	-314.61366	-333.86030	-298.65572	-259.62042	-595.23212	-351.32158
4	HF	-314.61712	-333.88356	-298.65440	-259.61100	-595.21242	-351.29529
Δ(HF) ^b		2.17	14.60	-0.83	-5.91	-12.36	-16.50
3	MP2 ^a	-315.47040	-334.76099	-299.48543	-260.31084	-596.56886	-352.29517
4	MP2	-315.49324	-334.77962	-299.50096	-260.32253	-596.57364	-352.29344
Δ(MP2) ^b		14.33	11.69	9.74	7.34	3.00	-1.09

^a The HF and MP2 energies are given in atomic units, 1 au = 627.5 kcal mol⁻¹. ^b Δ is the difference in energy between isomers **3** and **4**, in kcal mol⁻¹, for either the HF or MP2 calculations. A positive value indicates the amount by which the hydroxydiazene *N*-oxide structure **4** is more stable than the *N*-nitroso,*N*-hydroxylamine structure **3**.

of the type **6** in describing the overall geometries of nitrosamines was also observed in the gas phase structure of *N*-nitrosodimethylamine, for which considerable shortening of the N1-N2 bond length was found.¹⁵ Interestingly, *N*-nitrosodimethylamine exists entirely as **7** in the solid state, again reflecting the importance of resonance delocalization in nitrosamines.¹⁵

As expected, the hydroxydiazene *N*-oxide structures **4** are all planar, existing in the (*Z*)-configuration¹⁶ (Figure 1b) with the hydrogen atom in the [N₂O₂] plane and participating in hydrogen bonding to O1. We will discuss the importance of hydrogen bonding to the stability of **3** and **4** later. The N1-N2 bond lengths are approximately 1.22 Å for all substituents (a slight increase is observed for R = O⁻) and compare favorably with typical N=N bond lengths of 1.22–1.24 Å.¹⁴ This and the other bond distances, bond angles, and dihedral angles in **4** are also in agreement with the experimentally determined geometries for **4** when R = phenyl or *cis*-4-methylcyclohexyl.⁷ The N2-N1-O1 bond angles are slightly larger than the

ideal value of 120° for all derivatives of **4** indicating that some steric repulsion occurs between O1 and the OH moiety. Besides the obvious change in the N1-R bond distance the various substituents had little effect on the overall geometry of the [N₂O₂]H grouping in **4**.

Stabilities of **3 and **4**.** The energies for the optimized geometries of **3** and **4** for the various substituents are collected in Table 2. Calculations at the HF level suggest that the *N*-nitroso,*N*-hydroxylamine structure **3** is of greater stability for all substituents except when R = NH₂ and O⁻. In both of these cases the tautomeric hydroxydiazene *N*-oxide structure **4** is preferred. The preference for **3** over **4** (particularly for R = CH₃) at the HF level is not in agreement with the aforementioned experimental observations, which showed clearly that when R is an alkyl or phenyl substituent the preferred structure of the [N₂O₂]H grouping is **4** in both the solid state, as determined from X-ray analysis, and in solution as determined from UV studies.⁷ This theoretical result is not surprising since methods which include electron correlation may be needed to calculate the relative energies between isomers correctly.¹⁷

The energies of structures **3** and **4** for all substituents

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(16) The (*E*) and (*Z*) configurations are used to denote the arrangement of the O1 and O2 atoms with respect to the N1-N2 double bond.

Table 3. Values^a of r , ρ and $\nabla^2\rho$ for the Normal and Hydrogen-Bonded OH group in Structures 3 and 4 for each Substituent (R)

	substituent (R)							creatine ^c	sarcosine ^c
	NH ₂	O ⁻ (3) ^b	O ⁻ (4)	CH ₃	H	CF ₃	CN		
$r(3)_{\text{O1H}}$	0.1799	0.1716	—	0.1780	0.1794	0.1800	0.1779	—	—
$\rho(3)_{\text{O1H}}$	0.3688	0.3315	—	0.3643	0.3682	0.3593	0.3647	—	—
$\nabla^2\rho(3)_{\text{O1H}}$	-2.1922	-2.0085	—	-2.1891	-2.1965	-2.1175	-2.2028	—	—
$r(4)_{\text{O2H}}$	0.1745	0.5677	0.1758	0.1731	0.1736	0.1722	0.1715	—	—
$\rho(4)_{\text{O2H}}$	0.3517	0.05425	0.3561	0.3475	0.3484	0.3440	0.3420	—	—
$\nabla^2\rho(4)_{\text{O2H}}$	-2.1467	0.2010	-2.1304	-2.1380	-2.1408	-2.1137	-2.1333	—	—
$r(4)_{\text{O1H}}$	0.7346	—	0.6472	0.6967	0.7265	0.7075	0.7067	0.6624	0.6842
$\rho(4)_{\text{O1H}}$	0.03180	—	0.04214	0.03542	0.03204	0.03422	0.03422	0.0350	0.0326
$\nabla^2\rho(4)_{\text{O1H}}$	0.1402	—	0.1708	0.1502	0.1391	0.1478	0.1462	0.1390	0.1301

^a r is in Å, ρ is in e Å⁻³, $\nabla^2\rho$ is in e Å⁻⁵. $r(3)_{\text{O1H}}$ is the distance from the hydrogen nucleus to the OH bond critical point in 3. $\rho(3)_{\text{O1H}}$ is the value of the charge density at the OH bond critical point in 3. $\nabla^2\rho(3)_{\text{O1H}}$ is the value of the Laplacian of the charge density at the OH bond critical point in 3. $r(4)_{\text{O2H}}$ is the distance the hydrogen nucleus to the OH bond critical point in 4. $\rho(4)_{\text{O2H}}$ is the value of the charge density at the OH bond critical point in 4. $\nabla^2\rho(4)_{\text{O2H}}$ is the value of the Laplacian of the charge density at the OH bond critical point in 4. $r(4)_{\text{O1H}}$ is the distance from the hydrogen nucleus to the OH hydrogen-bond critical point in 4. $\rho(4)_{\text{O1H}}$ is the value of the charge density at the OH hydrogen-bond critical point in 4. $\nabla^2\rho(4)_{\text{O1H}}$ is the value of the Laplacian of the charge density at the OH hydrogen bond critical point in 4. ^b This column refers to the planar 3 found when R = O⁻. The values for $r(4)_{\text{O2H}}$, $\rho(4)_{\text{O2H}}$, and $\nabla^2\rho(4)_{\text{O2H}}$, are for O2H hydrogen bond in isomer 3. ^c Reference 23.

(R) were reevaluated incorporating electron correlation at the MP2 level of theory for the minimum energy geometries and these results are also displayed in Table 2. The MP2 energies of tautomers 3 and 4 for R = H and CH₃ are in favor of the hydroxydiazonium *N*-oxide structure 4 by 7.34 and 9.74 kcal mol⁻¹, respectively. Although these *ab initio* results do not incorporate solvent effects, the magnitudes of these energy differences are substantial and suggest that in solution, 4 will exist as the major tautomer (if not exclusively). This argument is supported by the fact that when the substituent is cyclohexyl (or substituted cyclohexyl), which has an almost identical inductive effect as the methyl substituent,¹⁸ only tautomer 4 is observed experimentally.⁷ The energy differences between tautomers 3 and 4 when R is a mesomerically donating substituent (R = NH₂ and O⁻) are even more substantial. At the MP2 level 4 was favored by 14.33 (R = NH₂) and 11.67 (R = O⁻) kcal mol⁻¹. This increase in stability of tautomer 4 over 3 for substituents of this type can clearly be attributed to the mesomeric capabilities of these substituents, stabilizing the positive charge on the central nitrogen atom in 4.

The difference in stabilities between the two tautomeric structures 3 and 4 was less definite for the strong electron-withdrawing groups (R = CF₃ and CN). MP2 energies show that tautomer 4 is favored by 3.00 kcal mol⁻¹ for the CF₃ substituent, while for R = CN tautomer 3 was found to be favored by only 1.09 kcal mol⁻¹. These differences in energies between the two possible structures are small and suggest that in protic media, both tautomers would exist in equilibrium with rapid proton exchange. This is in accordance with the fact that strong electron-withdrawing groups will place a greater positive charge on the already electron deficient nitrogen atom N1 in 4, thus favoring tautomer 3.

Several additional calculations were also performed on H[N₂O₂]H to check the absolute accuracy of our results. Reoptimizing the geometries of 3 and 4 for H[N₂O₂]H at the MP2 level gave an MP2 energy difference of 6.20 kcal mol⁻¹, and the MP4 energy difference of these geometries was 8.10 kcal mol⁻¹. The effect of diffuse functions was also small with the MP2 energy difference being 7.55 kcal mol⁻¹ after reoptimizing the geometry (HF) with diffuse

functions added to the basis set. Thus, the energy difference (HF/MP2) are relatively stable with respect to basis set, geometry, and electron correlation. Finally, it is of interest to mention that structure 2 (R = H), which is another possible isomer for the R[N₂O₂]H grouping, was calculated to be 40.3 kcal mol⁻¹ higher in energy than the corresponding 4 (R = H) at the HF level and was not considered further.

Hydrogen Bonding in 3 and 4. The optimized geometries of 3 are all tetrahedral at N1 (except R = O⁻) with the H atom out of the [N₂O₂] plane (see Figure 1a), and intramolecular hydrogen bonding in these structures was not envisaged. The optimized geometries of 4 are, however, all planar at the central N1 atom (see Figure 1b) with the hydroxyl hydrogen atom (*i.e.*, attached to O2 in 4) also in this same plane. The close proximity of this in-plane hydrogen to the O1 atom prompted us to investigate the possibility of intramolecular hydrogen bonding in these molecules.

The existence of bonds between atoms is readily probed by examining the topology of the total charge density (ρ). Each atomic position represents a local maximum in ρ which may be linked by unique lines along which the electron density is a maximum with respect to any neighboring line. These are termed atomic interaction lines.¹⁹ If the system is at equilibrium, then nuclear-nuclear forces are balanced by the accumulation of charge between the nuclei, and so the atomic interaction lines joining the nuclei are called bond paths, *i.e.*, the atoms are now bonded. Along each bond path there is a bond critical point, where the bond path intersects the interatomic boundary, and the properties of the charge density at this point may be used to characterize the bond.¹⁹ The necessary and sufficient conditions for bonding are thus defined and allow the hydrogen bond to be studied in the same way as other, and perhaps more familiar, types of bonding.

Data for the bonding of H to both O1 and O2 in 3 and 4 are given in Table 3. When the O and H participate in a normal OH bond the value of the charge density at the bond critical point is approximately 0.35 au for both 3 and 4 (see $\rho(3)_{\text{O1H}}$ and $\rho(4)_{\text{O2H}}$). These critical points occur at a distance of about 0.18 Å from H (see $r(3)_{\text{O1H}}$ and $r(4)_{\text{O2H}}$). For hydrogen bonds, which were located between O1 and H in all of the various 4 studied, the

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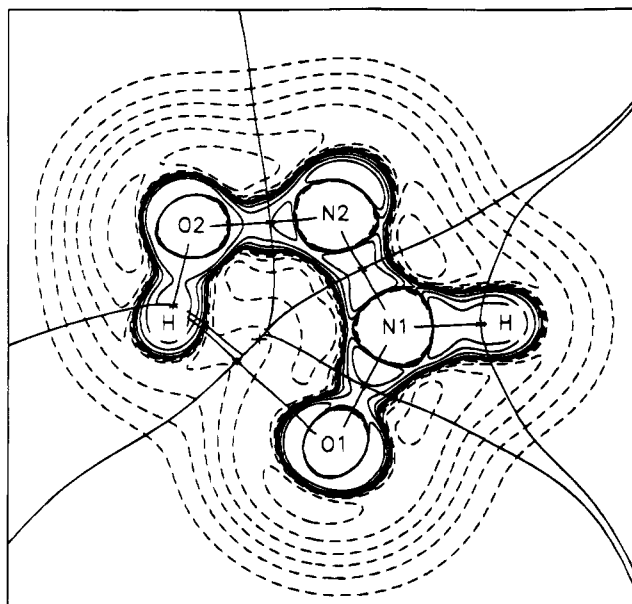


Figure 2. Plots of $\nabla^2\rho$ for the (Z) isomer of **4** when R = H. Bond paths and interatomic boundaries are overlaid and critical points in ρ are also marked (* and + denote bond and ring critical points, respectively). Full contours denote regions where charge is concentrated ($\nabla^2\rho < 0$) and broken contours denote regions where charge is depleted ($\nabla^2\rho > 0$). Note the region of charge concentration in the O2H bonding region compared to the charge depletion in the O1H bonding region.

value of the charge density at the bond critical point (see $\rho(4)_{\text{O1H}}$) is 1 order of magnitude smaller and occurs at a distance of approximately 0.75 Å from H (see $r(4)_{\text{O1H}}$). This large difference in values of ρ for the different types of OH bond are consistent with the fact that hydrogen bonds are much weaker than normal OH bonds.^{20,21}

Bond paths and interatomic boundaries for **4** (R = H) in the $[\text{N}_2\text{O}_2]$ plane are shown in Figure 2. The bond path connecting O1 and H in the (Z) isomer of **4** (Figure 2) is the hydrogen bond, which is obviously much longer than the normal O2H bond. The intramolecular hydrogen bond distance in **4** for all substituents was approximately 1.9 Å which, as might be expected, is considerably shorter than those observed for strong intermolecular hydrogen bonds (2.4 to 2.8 Å).²² The O2H bond length in **4** for all substituents was approximately 0.01 Å longer than the O1H bond length in **3** (see Table 2), reflecting a stretching of this bond due to the participation of the H atom in hydrogen bonding. No bond between O2 and H was found in the various **3** as the H is too far from O2 (ca 2.8 Å) to participate in intramolecular hydrogen bonding.

In addition to the large difference in ρ at the bond critical points, the concentration of charge in the bonding regions between O and H also shows how the two types of bond differ. The bond paths shown in Figure 2 are overlaid onto a plot of the Laplacian of the charge density ($\nabla^2\rho$) which shows where charge density is concentrated or depleted. Values of $\nabla^2\rho$ at the bond critical points for normal OH bonds (see $\nabla^2\rho(3)_{\text{O1H}}$ and $\nabla^2\rho(4)_{\text{O2H}}$ in Table 3) are all approximately -2.1 au which indicates that charge is concentrated at the bond critical point, typical of covalent or shared interactions.¹⁹ The values of $\nabla^2\rho$ at the bond critical points for the hydrogen bonds (see $\nabla^2\rho(4)_{\text{O1H}}$ in Table 3) are typically 0.15 au, *i.e.*, of different

sign which indicates that charge is depleted in the bonding region, typical of ionic or closed-shell interactions.¹⁹ The differences between the two types of OH bond are also shown in Figure 2 where it can be seen that the charge density in the hydrogen bonding region (*i.e.*, between O1 and H) is depleted while in the normal OH bonding region (*i.e.*, between O2 and H) charge is concentrated.

These findings are consistent with previous studies of intramolecular hydrogen bonding in creatine and carbamoyl sarcosine²³ in which a hydrogen bond was defined as the linkage of a proton to both a base atom (by a closed shell interaction) and an acid atom (by an open shell interaction). The values of ρ and $\nabla^2\rho$ in the examples presented here are compared to values obtained for both creatine and carbamoyl sarcosine in Table 3. There is excellent agreement between these results even though the systems are quite different, indicative of the fact that hydrogen bonds have similar quantifiable properties.

The existence of intramolecular hydrogen bonds in **4** is an interesting finding since its importance has not been considered previously, primarily because the $[\text{N}_2\text{O}_2]\text{H}$ grouping was considered as **3** and not **4** for the derivatives known. One should now ask how important the formation of hydrogen bonds is with respect to the formation of other isomers of **4** and if the stabilization energy provided by hydrogen bond formation is enough to make **4** the preferred structure over **3**.

The stabilizing effect associated with hydrogen bond formation can be estimated by rotating the H atom out of the $[\text{N}_2\text{O}_2]$ plane in **4** and these results are shown in Figure 3. The geometry of **4** (R = H) was optimized at the HF level while the dihedral angle $\varphi(\text{N1N2O2H})$ was fixed. The MP2 energies for each of these optimized geometries are shown in Figure 3. The energy is a maximum when the N2O2H plane is almost perpendicular to the N1N2O2 plane because of stronger repulsive interactions between the N2 and O2 lone pairs. This interpretation is supported by the change in the N2–O2 bond length which is initially 1.223 Å when $\varphi(\text{N1–N2–O2–H})$ is 0° and has a maximum value of 1.234 Å when $\varphi(\text{N1–N2–O2–H})$ is 97°. As the O2–H bond is further rotated to 180° the N2–O2 bond decreases to 1.226 Å, close to its original value. At this value of $\varphi(\text{N1–N2–O2–H})$ the energy is higher than the starting geometry by 4.58 kcal mol⁻¹, a consequence of the lack of intramolecular hydrogen bond formation (See Figure 3). Thus, from these results, it is probably fair to say that the stabilization energy provided by the formation of a hydrogen bond in **4** (R = H) is of the order of 4.5 kcal mol⁻¹. This value is similar to those measured for the hydrogen bond strength between carboxylic acids.²⁴

The values of ρ and $\nabla^2\rho$ for the hydrogen bonds in the various structures of **4** are all similar (Table 3) indicating that any stabilization energy due to the formation of the hydrogen bond will be similar in each, *i.e.*, of the order of 4.5 kcal mol⁻¹. To confirm this the O2H bond was rotated out of the N_2O_2 plane for **4** (R = NH₂) in an identical manner to that performed for **4** (R = H). Even though this substituent showed the greatest difference in energies between **3** and **4**, rotation of the O2H bond through 180° resulted in a geometry higher in energy by 4.54 kcal mol⁻¹ due to loss of intramolecular hydrogen bonding. When R is H or CH₃ a substantial portion of

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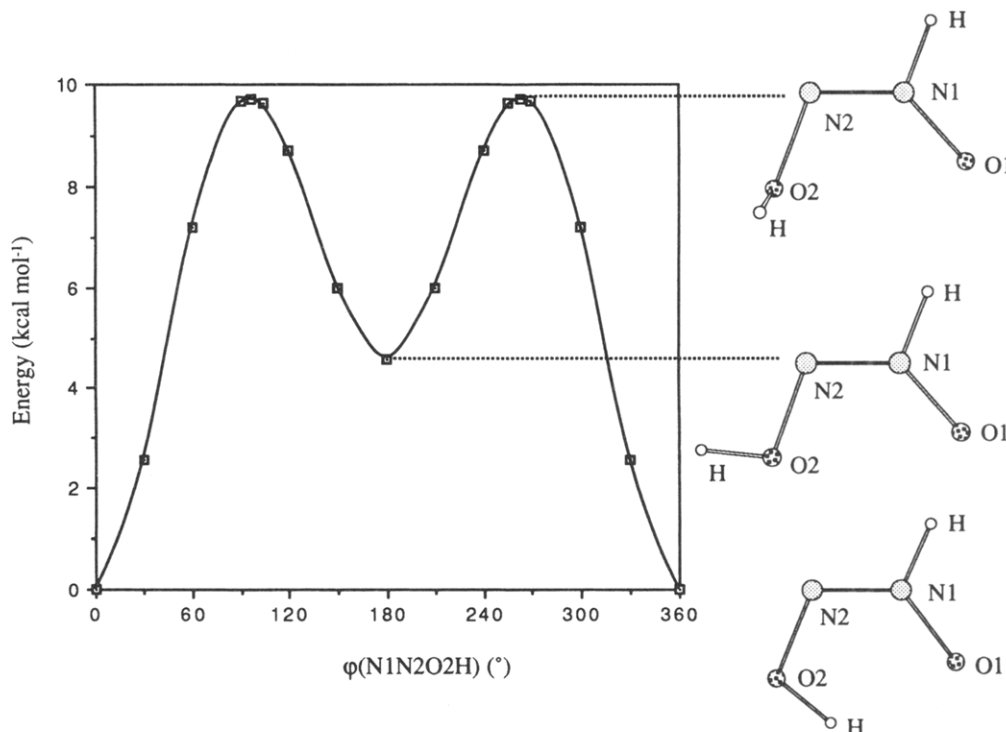


Figure 3. A plot of the change in energy as the O2H bond is rotated out of the $[N_2O_2]$ plane for **4** ($R = H$).

Table 4. Atomic Charges for the (*E*) and (*Z*) Isomers of the Anions **1** for All Substituents (*R*), along with the Differences in Energy between the Optimized (*E*) and (*Z*) Geometries at the HF and MP2 Levels

	substituent										
	NH ₂		O ⁻	CH ₃		H		CF ₃		CN	
	<i>E</i>	<i>Z</i>	<i>E/Z</i> ^a	<i>E</i>	<i>Z</i>	<i>E</i>	<i>Z</i>	<i>E</i>	<i>Z</i>	<i>E</i>	<i>Z</i>
N1	0.186	0.183	0.464	-0.260	-0.273	-0.119	-0.140	-0.213	-0.247	-0.405	-0.447
N2	0.274	0.250	0.084	0.291	0.264	0.284	0.260	0.343 ^c	0.364	0.417	0.383
O1	-0.734	-0.723	-0.846	-0.764	-0.759	-0.755	-0.752	-0.710	-0.712	-0.699	-0.692
O2	-0.802	-0.735	-0.811	-0.790	-0.727	-0.788	-0.734	0.606	-0.659	-0.679	-0.632
R ^b	0.076	0.029	-0.897	0.510	0.499	0.380	0.364	0.186	0.239	0.367	0.388
HF ^c		8.73	—		1.86		4.60		5.59		1.48
MP2 ^d		8.39	—		0.53		2.86		6.78		3.10

^a These isomers are the same. ^b Summation of atomic charges on all atoms in the substituent *R*. ^c The amount by which the optimized (*E*) or (*Z*) geometry is lower in energy than the other, in kcal mol⁻¹, is given in the appropriate column. ^d The amount by which the optimized (*E*) or (*Z*) geometry is lower in energy than the other at the MP2 level, in kcal mol⁻¹.

the energy difference between **3** and **4** can be attributed to intramolecular hydrogen bonding. For the mesomerically donating substituents ($R = NH_2$ and O^-) which are favored over **3** by 14.33 and 11.69 kcal mol⁻¹, respectively, the stabilization energy gained from hydrogen bonding is reinforced by favorable mesomeric donation from the substituents, stabilizing the positive charge on N1 in **4**. It is interesting to note that the *N*-nitroso,*N*-hydroxylamine, **3**, for $R = O^-$ was found to be planar and that the hydrogen atom participated in intramolecular hydrogen bonding with O2, again reflecting the mesomeric capabilities of the O^- substituent.

Although hydrogen bonding exists to the same extent for the two strong electron-withdrawing substituents **4** ($R = CN$ and CF_3) this stabilization is counter-balanced by the destabilizing effects of placing these substituents next to the positively charged N1 atom in **4**. Finally, it is also worth mentioning that hydrogen bond strengths may be related to the pK_a of the free acids and are expected to increase with increasing acidity.²⁴ The pK_a of cupferron (**4**, $R = Ph$) has been measured²⁵ as 4.42 and

is again indicative of strong intramolecular hydrogen bonding in these systems.

Anions of 1. The relative stabilities of **3** and **4** for the two strong electron-withdrawing groups (CF_3 and CN) were approximately the same suggesting that both isomers coexist. Although isomer **4** was found to be significantly more stable than **3** for the other substituents (NH_2 , O^- , CH_3 , H) due to favorable hydrogen bond formation and substituent effects, there still exists the possibility that upon dissolution, salts of type **1** containing these substituents will lead to **3**, **4**, or both. Since **3** was found to be thermodynamically less stable, rapid tautomerism to **4** could occur and with this in mind the anions of **1** were studied in the same way as that described above for the protonated species. In particular, the charges on the interior (O1) and exterior (O2) atoms are of interest as these should reflect any preferences for protonation and hence give some idea about the possibility of such an equilibrium.

Geometries of the anions were optimized at the HF level using 6-31G* basis sets, with MP2 energies calculated for the optimized geometries. Each of the anions were found to have a planar geometry at the N1 atom, consistent with an N1–N2 double bond. Bond lengths

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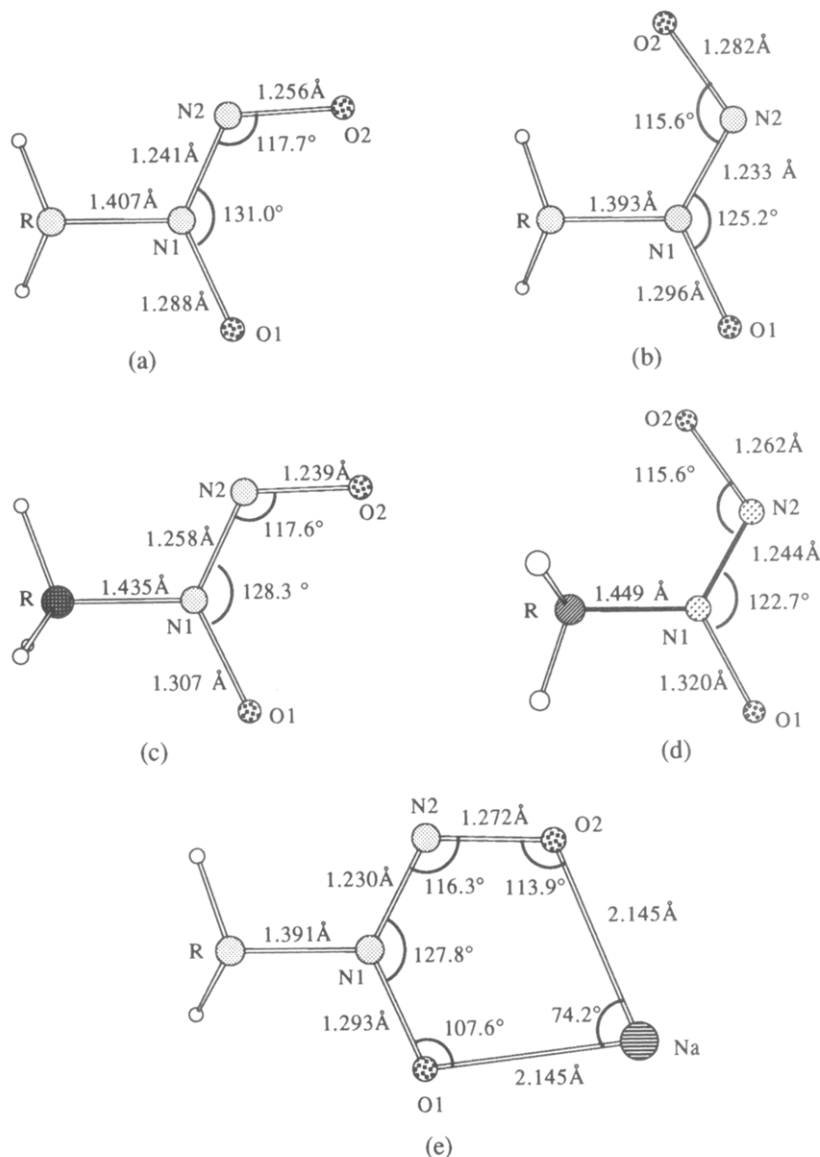
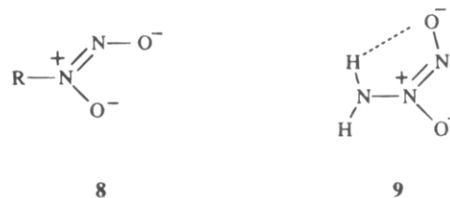


Figure 4. Optimized geometries of $R[N_2O_2]^-$: (a) $R = NH_2$, (*Z*) configuration; (b) $R = NH_2$, (*E*) configuration; (c) $R = CH_3$, (*Z*) configuration; (d) $R = CH_3$, (*E*) configuration; (e) $R = NH_2$, (*Z*) configuration with the counterion Na^+ .

and angles for the $R[N_2O_2]^-$ anions when $R = NH_2$, and CH_3 are shown in Figure 4 for both (*E*) and (*Z*) isomers. The geometries of the anions for the other four substituents are not shown since these are not significantly different to those given in Figure 4.

Atomic charges in each of the anions are given in Table 4. These were calculated by integrating the electron density over the basin of the atom,¹⁹ where the atomic boundaries are analogous to those shown in Figure 2. In each anion, the charge on O1 is approximately the same as that on O2, with the tendency for O2 to have greater charge in the (*E*) isomers while for the (*Z*) isomers the charges on O1 and O2 are quite similar. In fact the largest variation in charge for the different substituents occurs at N1. When the R group is a strong inductive electron-withdrawing group, as in CF_3 and CN, the destabilizing effect of the substituent is counterbalanced to some extent by the transfer of charge from other atoms, as reflected by the negative charge on N1 (Table 4). When the substituent is mesomerically donating, as in NH_2 and O^- , the charge on N1 is slightly positive indicating that charge has been transferred from N1. These results suggest that of the possible resonance descriptions that may be drawn, the most appropriate is

that shown in **8**, where the interior and exterior oxygen atoms bear equal charge.



The relative stabilities of the (*E*) and (*Z*) isomers for the anions are also given in Table 4. When R is either CH_3 , H, or CN there is little preference for the (*E*) or (*Z*) configuration. For the CF_3 substituent the enhanced stability of the (*Z*) isomer can be attributed to reduction of steric repulsions between O2 and the CF_3 moiety. The increased stability of the (*E*) over the (*Z*) isomer for the case when $R = NH_2$ is a consequence of intramolecular hydrogen bond formation between an hydrogen atom of the NH_2 substituent and O2, analogous to that described above (see **9**). The values of ρ and $\nabla^2\rho$ at the O2-H bond critical points are 0.0314 and 0.131, consistent with those shown in Table 3. The contribution of hydrogen bond

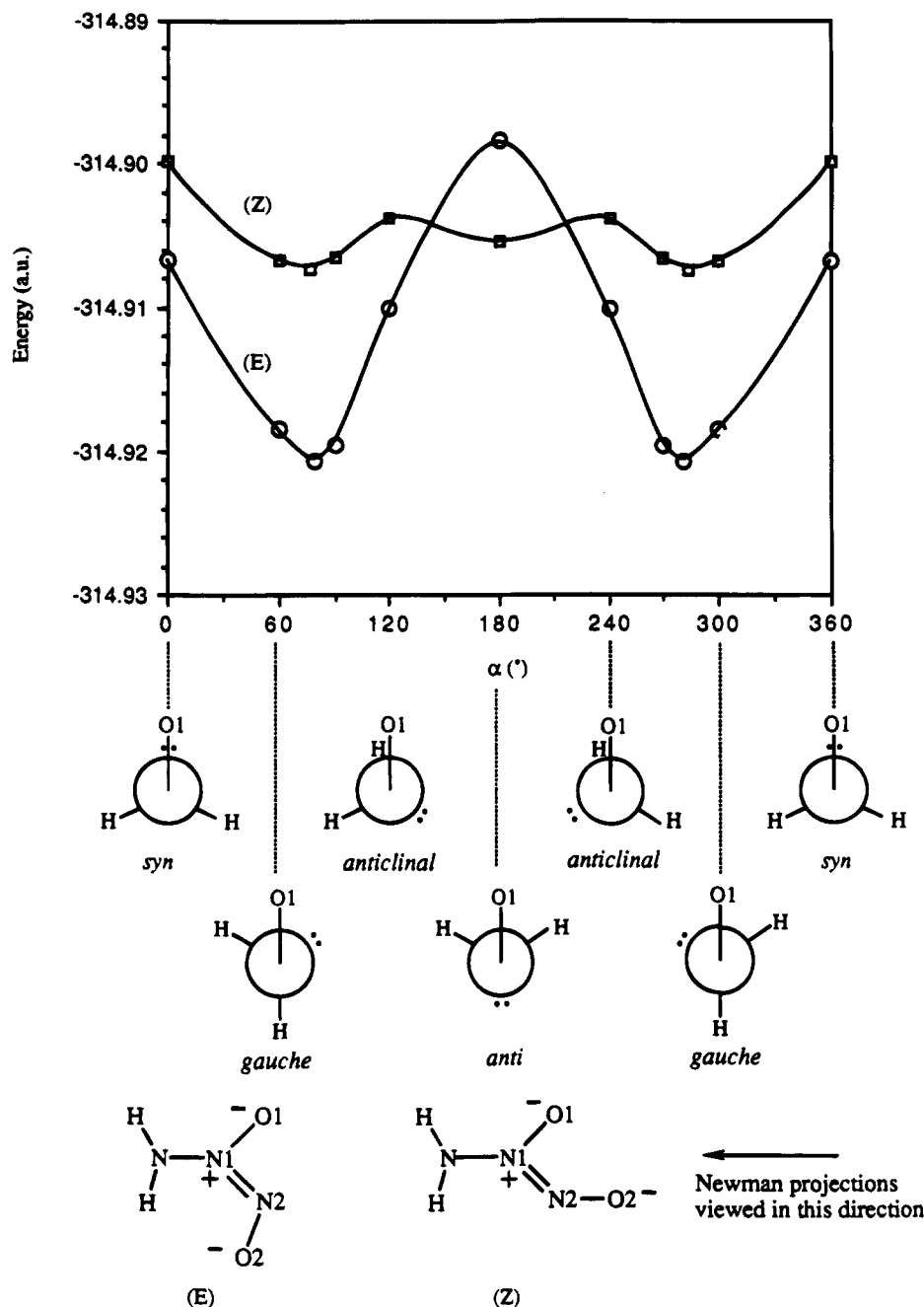


Figure 5. A plot of the variation of the O1-N1-N-lone pair dihedral angle, α . The MP2 energy at the HF optimized geometry (with the various dihedral angles fixed) is shown for the (E) (○) and (Z) (□) isomers.

formation to the stability of the (E) isomer was studied by changing the dihedral angle (α) defined by the angle between the planes defined by O1, N1, N and N1, N, lone pair. At each of the various α , the geometry was optimized at the HF level and the MP2 correction to the energy calculated. For completeness we also performed the same series of calculations for the (Z) isomer. The variation of MP2 energies with α for both the (E) and (Z) isomers is plotted in Figure 5.

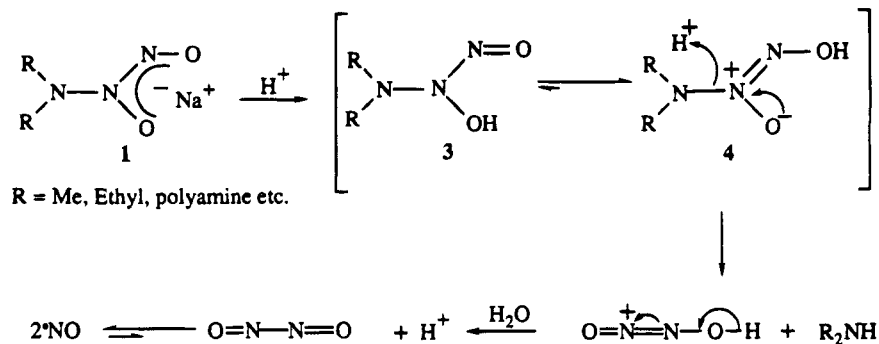
The decrease in energy of the *gauche* ($\alpha = 72^\circ$) with respect to the *syn* conformation for the (E) isomer is due to hydrogen bond formation and the removal of the unfavorable repulsive interaction between O1 and the lone pair of NH_2 . As the NH_2 group is rotated the energy increases because lone pair–O2 repulsive interactions are increased. When the *anti* conformation is reached the energy is a maximum, as expected, because the nega-

tively charged O2 atom is in close proximity to the lone pair, which results in substantial destabilization.

The (Z) isomer is consistently higher in energy than the (E) isomer for $\alpha = 0$ to 120° as no intramolecular hydrogen bond formation is possible and also because of unfavorable interactions between the negatively charged O1 and O2 atoms. The *anti* conformation for the (Z) isomer is not, however, of highest energy as the NH_2 lone pair is further away from O2. The decrease in energy of the *anti* with respect to the *anticlinal* conformations is due to the differences between the O1–lone pair and N2–lone pair interactions.

These results show that intramolecular hydrogen bond formation is viable, and provides theoretical evidence for bonding of this type recently proposed⁴ in anionic species of 1 (R = polyamine). A possible consequence of intramolecular hydrogen bond formation in these anions is that

Scheme 1. Proposed mechanism for NO release from salts of type 1.



they will have longer half-lives in solution which will affect the rate of NO production.

The sodium salt of the R = NH₂ anion was also studied, and while a preference for the (*E*) over the (*Z*) isomer was found for the anion, the (*Z*) isomer was found to be most stable upon complexation to sodium (Figure 4e). In the favored geometry (obtained by optimization at the RHF/6-31G* level of theory) the sodium atom was found to be equidistant between O1 and O2, and bonded to both. In addition, the MP2 energy of this structure was lower than the MP2 energies of isomers formed by complexation of only one of O1 or O2 by 11.7 and 17.5 kcal mol⁻¹, respectively. This result is in agreement with the crystal structure of the dipotassium salt of methylenebisnitrosohydroxylamine⁶ where the potassium atom is between O1 and O2, though not exactly equidistant due to coordination to another [N₂O₂] group in the unit cell.

Although complexation of a counterion (Na⁺) occurs equidistant from the interior and exterior oxygen atoms in anions of type 1 in the thermodynamically desirable (*Z*) configuration, without a counterion the configurations of the anions are quite substituent dependent. Nevertheless, analysis of the atomic charges for both the (*E*) and (*Z*) forms of the anions shows that the charges on the interior and exterior oxygen atoms are virtually the same (see Table 4). This suggests that upon dissolution, either oxygen atom would be susceptible to protonation to give either 3 or 4. Once protonated, however, tautomerism to the more stable isomer 4 occurs if the substituent is either NH₂, O⁻, CH₃, or H.

Mechanism for NO Release. Salts of type 1 formed from the reaction of NO with primary, secondary, and polyamines spontaneously decompose upon dissolution in acidic media with first-order kinetics. Virtually all of the NO in the [N₂O₂] moiety⁸ is released as is the amine portion as the free amine. Although the structure of the free acids of these salts (*i.e.*, R₂N[N₂O₂]H) is unproven, our results clearly suggest that the [N₂O₂]H grouping will be in the form of the more thermodynamically stable hydroxydiazonium *N*-oxide structure 4. Knowing this, a simple mechanism for the release of NO may be postulated (Scheme 1).

Upon dissolution of salts of type 1, protonation may occur on either O1 or O2 to give structures 3 and 4, respectively, as reflected by the charges on these oxygen atoms. Tautomerization of 3 to 4 is expected based on the greater thermodynamic stability of 4 over 3. In acidic media 4 then collapses with release of the amine portion of the derivative. Loss of a proton will afford the nitric oxide dimer N₂O₂ which is known to exist almost exclu-

sively as free nitric oxide at ambient temperature.²⁶ This mechanism is in agreement with recent findings that alkylation on O2 virtually ceases all NO release.⁸ In the case when the substituent is O⁻ a similar mechanism is likely with loss of water.

Little evidence concerning the extent of NO release and the products formed is available for cases in which the substituent is not a nucleophilic residue. For example, cupferron (4, R = phenyl) has been reported to decompose rapidly at ambient temperature (a result confirmed by us²⁷) in the presence of trace amounts of acid to afford many products including NO and nitrosobenzene.²⁸ Clearly the mechanism for NO release is different in this case to that proposed above, primarily because the substituent is not a nucleophilic residue and cannot be resolved fully until detailed product analyses are carried out. Understanding how compounds containing the [N₂O₂] grouping bonded to non-nucleophilic residues can be employed as NO releasing prodrugs will be the subject of future work.

Conclusions

The planar hydroxydiazonium *N*-oxide structure 4 is thermodynamically more stable than the tetrahedral tautomeric *N*-hydroxyl,*N*-nitrosoamine structure 3 at the MP2 level for the substituents NH₂, O⁻, CH₃, and H by 14.33, 11.69, 9.74, and 7.34 kcal mol⁻¹, respectively (see Figure 1 and Tables 1 and 2). The differences in stabilities of 3 and 4 for the two strong electron-withdrawing groups, CF₃ and CN, were less defined, attributable to the destabilizing effect of attaching these strong inductive groups to the positively charged N1 in 4. Analysis of the charge density between the O1 and H atoms showed that intramolecular hydrogen bonding occurs in all of 4 considered (see Figure 2 and Table 3). For the substituent R = H the contribution of intramolecular hydrogen bond formation was examined by rotating the hydroxyl group with respect to the [N₂O₂] plane (see Figure 3). The energies of these variants of 4 when R = H suggests that intramolecular hydrogen bonding in 4 contributes 4.58 kcal mol⁻¹ to the total energy. Similar calculations were performed for 4 with R = NH₂ and a contribution of 4.54 kcal mol⁻¹ was found, leading us to conclude that intramolecular hydrogen bond formation stabilizes these geometries by approximately 4.5 kcal mol⁻¹.

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Anions formed by the loss of a proton from either **3** or **4**, were also studied. For the substituents R = CH₃, H, and CN the (*E*) or (*Z*) configurations have similar stability. When the substituent is the CF₃ group the stability of the (*Z*) isomer is enhanced which can be attributed to reduction of steric repulsions between O2 and the CF₃ moiety. Intramolecular hydrogen bond formation between an hydrogen atom of the NH₂ substituent and O2 results in increased stability of the (*E*) over the (*Z*) isomer when R = NH₂ (see Figure 5). Charges on both the interior (O1) and exterior (O2) oxygen atoms are equivalent in each of the anions, which leads us to infer that there is no preference for protonation at either O1 or O2 (see Table 4). Consequently, rapid formation of either **3** or **4** is expected upon dissolution of the corresponding salts, with tautomerism to give the more thermodynamically stable isomer **4** when R = NH₂, O⁻, CH₃, and H. Complexation of sodium to the anion when R = NH₂ was also studied giving a structure in which the sodium atom was bonded to both O1 and O2 atoms (see Figure 4e), in agreement with the known crystal structures of salts of this type.⁶

Derivatives which contain the [N₂O₂]H grouping bonded to a nucleophilic residue (*e.g.* NEt₂, NMe₂, polyamine, or O⁻) are currently of interest because of their ability to release NO. This study clearly suggests that the [N₂O₂]H

grouping in derivatives containing these types of substituents will be in the form of the hydroxydiazonium *N*-oxide structure **4**. This allows a mechanism for NO formation to be postulated (see Scheme 1) which is in agreement with known experimental facts:⁸ (a) that alkylation on O2 stops the generation of NO, (b) increasing the acidity also increases the half-life for NO production, and (c) when the substituent R is a substituted amine, it is released as the free amine.

Acknowledgment. We are indebted to the National Science Foundation, grant nos. CHE 91-13634 (MBH) and CHE 91-09878 (DHRB), and the Welch foundation, grant nos. A-648 (MBH) and A-1036 (DHRB), for financial support of this work.

Supplementary Material Available: Tables of optimized geometrical data and total energies for the various structures used to construct Figures 3 and 5 as well as the geometrical data and total energies for the anions of **3** and **4**. Also included, geometrical data and total energies of **3** and **4** (R = H) incorporating diffuse functions as well as their MP4 (SDTQ) energies (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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