

MO-Calculations on Nitroxide Radicals

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Molecular orbital (MO) calculations by the INDO method have been performed for some free nitroxide radicals, which might be of importance in cancer therapy.

The calculated order of red/ox potentials was found to be consistent with the order experimentally found.

The spin densities were calculated to be greater on O (70 %) than on N, in contrast to the results deduced from paramagnetic resonance measurements. The INDO calculations also indicate a considerable change in the ratio between total spin density and $2s_N$ spin density on bending the NO-bond. This leads to a possible explanation of the inconsistency between these calculations and the conclusion drawn from paramagnetic resonance measurements.

Nitroxide radicals constitute a group of remarkably stable free radicals. They are hence used for spin labelling of a wide range of compounds.¹ Experiments have demonstrated that this class of free radicals may act as radiosensitizing agents,²⁻⁸ possibly through binding to radiation induced DNA radicals.

The ability of an organic nitroxide radical to react with various compounds will to a large extent depend on the red/ox potentials of the radicals, steric hindrance, and the charge and spin distribution. These properties are also important for the explanation of the reactions between nitroxide radicals and DNA or RNA radicals, as well as for their spin labelling ability.

The present paper gives the results of a semi-empirical MO-calculation for some free nitroxide radicals.

To our knowledge no semi-empirical MO-calculation on nitroxide radicals has previously been published.

METHOD OF CALCULATION

The calculations were performed according to the INDO (Intermediate neglect of differential overlap) method introduced by Pople *et al.*⁹⁻¹³ All parameter values were chosen according to the original scheme. INDO and the closely related CNDO/2 method include explicitly all valence electrons. INDO gives a better description than does

CNDO/2, of spin densities in open shell systems. This is because it retains all one-center two-electron integrals. The calculations were performed on a CDC 3300 computer.

As to the INDO energy difference ΔE used for comparison with experimental red/ox potentials, ΔE has been used as an approximate value for ΔH , assuming small volume changes. It is further assumed that the ratio between the calculated energy differences can approximately be compared to the ratio between the experimentally found red/ox potentials.

RESULTS AND DISCUSSION

The compounds studied were (Fig. 1): Nitric oxide (I), dihydro nitric oxide II (non-existent), di-*t*-butyl nitroxide (III), 2,2,5,5-tetramethyl-3-carboxamidopyrroline-1-oxyl (IV), 2,2,6,6-tetramethyl-4-piperidone-1-oxyl(V),

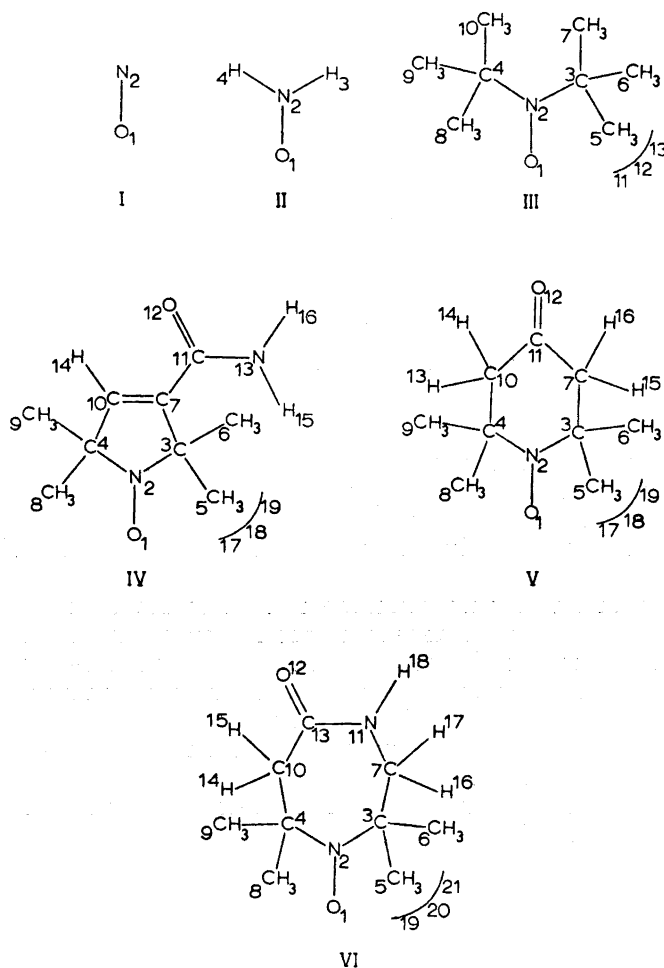


Fig. 1. Numbering of the atoms in the molecules investigated. The numbering refers to the calculations and is not in agreement with standard chemical numbering.

Table 1. Interatomic angles used.

Angles/Molecule (see Fig. 1)	II	III	IV	V	VI
1-2-3	120.0	112.0	123.4	117.0	112.0
1-2-4	120.0	112.0	123.4	117.0	112.0
2-3-5		112.0	112.0	110.0	112.0
2-3-7		112.0	100.0	112.0	112.0
3-5-19		110.0	110.0	110.0	110.0
3-7-11			140.0	112.0	112.0
3-7-10			113.4	—	—
3-7-16			—	110.0	110.0
4-10-7			113.4	—	—
4-10-13			—	110.0	112.0
4-10-14			126.6	110.0	110.0
5-3-6			112.0	110.0	112.0
7-11-10			—	117.0	—
7-11-12			122.0	121.5	—
7-11-13			113.0	—	126.7
10-7-11			100.6	—	—
10-13-11			—	—	120.2
10-13-12			—	—	120.0
11-13-12			—	—	120.0
11-13-16			107.0	—	—

usually called TAN, and 2,2,7,7-tetramethyl-1,4-diazacycloheptan-5-on-1-oxyl (VI).

The angles and distances used are given in Table 1 and 2. Experimental data are not available for all the nitroxide radicals. Standard values are therefore used, constructed from comparison with similar molecules.¹⁵ The radicals

Table 2. Interatomic distances used.

Distances (Å) (see Fig. 1)	I	II	III	IV	V	VI
1-2	1.1500	1.2300	1.2800	1.2800	1.2800	1.2800
2-3		1.0000	1.5120	1.5120	1.5120	1.5120
2-4		1.0000	1.5120	1.5120	1.5120	1.5120
3-5			1.5360	1.5360	1.5360	1.5360
3-7			1.5360	1.5000	1.5360	1.5360
4-8			1.5360	1.5360	1.5360	1.5360
5-19			1.1050	1.1050	1.1050	1.1050
7-10				1.3332	—	—
7-11				1.4800	1.5000	1.4500
7-16				—	1.1050	1.1050
10-14				1.0860	1.1050	1.1050
10-13				—	1.1050	1.5000
11-12				1.2200	1.2200	—
11-13				1.3600	—	1.3213
11-17				—	—	0.8500
12-13				—	—	1.2200
13-16				1.0200	—	—

Table 3. Some quantities comparing the two different configurations of TAN (V), see text.

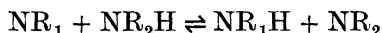
E_{tot} (a.u.)	$E_{\text{TANH}} - E_{\text{TAN}}$ (a.u.)	μ_{tot} (D)	Spin N	Spin O	Charge N	Charge O	a_{N} (gauss)
Standard geometry -120.587480	-0.745675	0.607	0.2961	0.7230	0.0015	-0.2359	6.08
Experimental -120.597374	-0.750373	1.780	0.2564	0.7408	-0.0035	-0.2285	8.91
Difference 0.009894	0.004698	1.173	0.0397	-0.0178	0.0050	-0.0074	-2.83

are also assumed to be co-planar in the NO-part, *i.e.* the atoms 1, 2, 3, and 4 (see Fig. 1) constitute one plane (called the NO-plane).

To get a measure of the error introduced by not having an experimentally or energetically optimized configuration, one alternative calculation is performed for TAN (V). In this calculation preliminary results obtained by electron diffraction are used.¹⁹ Not only distances and angles are here different, but also the configuration; a "twisted boat" form (and not co-planar in the NO-part), whereas all the standard configurations are in "chair" form (and co-planar).

From Table 3 it is seen that the dipole moment and the total energy are significantly different in the two cases, whereas predicted charge, spin density, and the red/ox energy differences are more constant. Therefore, a discussion of these last mentioned quantities should be justified without further knowledge of the molecular geometries.

(a) *Relative red/ox potentials.* Consider the reaction



where NR is an organic nitroxyl radical and NRH is the corresponding hydroxylamine.

The calculated relative red/ox potentials of the nitroxide radicals are given in Table 6, together with experimental values recently obtained (Tomas, Efskind and Nakken; in press).

The sequence of the red/ox potentials is the same for experimental as for calculated values, VI being the most readily reducible. It is further seen from Table 6 that the relative position of V between the two others is about the same for calculated and for experimental potentials.

It is not relevant to draw more quantitative conclusions (see discussion in Method of calculation).

(b) *Spin and charge distribution.* Nitric oxide (I) was found to have a total spin density of about 70 % on N and 30 % on O. All the other nitroxide radicals have the reverse distribution, *i.e.* 30 % on N and 70 % on O (see Table 5, atoms 1 and 2). Nearly all the spin density originates from $2p_{\pi}$ atomic orbitals.

In contrast, Stone *et al.*¹⁶ and McConnell *et al.*¹ deduced from paramagnetic resonance measurements that 70–90 % of the spin of the nitroxide radicals

Table 4. Atomic charges using standard geometry given in Tables 1 and 2.

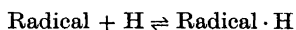
Atom	I	II	III	IV	V	VI
1	-0.0407	-0.2085	-0.2533	-0.2399	-0.2359	-0.2440
2	+0.0407	+0.0401	+0.0025	+0.0033	+0.0015	-0.0041
3		+0.0842	+0.1365	+0.1483	+0.1311	+0.1118
4		+0.0842	+0.1365	+0.1228	+0.1311	+0.1118
5			+0.0548	+0.0393	+0.0415	+0.0425
7			+0.0548	-0.1105	-0.0201	+0.1612
8			+0.0548	+0.0475	+0.0415	+0.0425
10			+0.0548	+0.0367	-0.0201	+0.1612
11			-0.01-02	+0.4418	+0.3013	-0.2298
12				-0.4177	-0.3086	-0.4200
13				-0.3073	+0.0022	+0.4270
14				+0.0190	+0.0022	-0.0005
16				+0.1513	-0.0022	-0.0370
17				-0.01-2	-0.01-2	+0.1368
18						-0.01-2
$-E_{\text{tot}}$ (a.u.)	28.557414	30.209310	97.682814	130.815825	120.587480	132.449727
μ (debyes)	0.167	2.616	2.641	2.174	0.607	4.363

is located in the $2p_{\pi}$ -atomic orbital on N. This will be further discussed in the next section.

Since the radicals are not planar, there is strictly no σ - π -separation of MO's. However, for the purpose of comparison with the above mentioned results of Stone and McConnell, it is of interest to note that the highest filled MO in the INDO calculations has a nearly 100 % π -character in the NO part.

Table 5. Spin densities (total).

Atom	I	II	III	IV	V	VI
1	+0.3038	+0.7252	+0.7272	+0.7097	+0.7230	+0.7353
2	+0.6962	+0.3197	+0.2919	+0.3118	+0.2961	+0.2845
3		-0.0179	-0.0142	-0.0237	-0.0216	-0.0202
4		-0.0179	-0.0142	-0.0234	-0.0216	-0.0201
5			+0.0021	+0.0093	+0.0102	+0.0091
7			+0.0049	-0.0001	+0.0027	+0.0027
8			+0.0021	+0.0091	+0.0102	+0.0089
10			+0.0049	+0.0002	+0.0027	+0.0028
11			-0.002-10	-0.0008	-0.0025	-0.0005
12				-0.0005	+0.0024	+0.0002
13				-0.0001	-0.0008	-0.0005
14				-0.0001	-0.0008	-0.0007
16				-0.0007	-0.0008	-0.0007
17				-0.0005-12	-0.0003-13	0.0000
18						-0.0001-12

Table 6. The red/ox energy ΔE of the reaction:

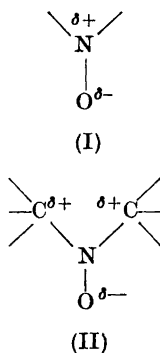
using the INDO hydrogen atom value (0.638730 a.u.). Experimental potentials are taken from Tomas, Efskind and Nakken (in press).

Molecule ΔE (INDO) in a.u.	IV	V	VI
	-0.103128	-0.106945	-0.111013
Experimental red/ox potentials (pH=7); in V	0.004	0.137	0.252

About 70 % of the spin density contribution from this MO is located on N. Hence, the greater total spin on O is caused by contributions from the lower spin-polarized MO's.

According to our calculations there is hardly any smear-out effect of the spin outside the NO-group, the nearest carbon neighbours (atoms 3 and 4, Table 5) having a small negative spin density.

Nitric oxide (I) has a fairly small charge polarization (Table 4), whereas the oxygen in the NO-part of the nitroxyl radicals has a net charge of -0.20 to -0.25 (atom 1, Table 4). the nitrogen retaining its slightly positive charge unchanged (atom 2, Table 4). The charge on oxygen is mostly compensated by positive charges on the nearest carbon neighbours (atoms 3 and 4, Table 4).



Stone *et al.*¹⁶ postulate a polar bond structure (I). The present calculation modified this rather to be II leaving the nitrogen neutral.

The carbonyl group in IV, V, and VI is heavily polarized, the oxygen (atom 12, Table 4) being significantly more negative than in the NO-part.

This may be one of the explanations why these radicals react more readily in the carboxyl part than in the free spin part.

(c) *Hyperfine coupling constants for nitrogen.* Stone¹⁶ and McConnell's¹ conclusion of a high spin density on nitrogen is based on observed hyperfine

Table 7. $2s_N$ spin densities and the corresponding hyperfine coupling constants (a_N). The configurations are coplanar in the NO part. Experimental values for a_N are about 15.5 gauss for the nitroxide radicals and about 19.5 for the NH_3^+ ion. The configuration of NH_3^+ ion is taken planar with an NH bond length of 1.02 Å and an H–N–H angle of 120°.

	$\text{NH}_3(+)$	II	III	IV	V	VI
$\rho_N(2s)$	0.0537	0.0157	0.0164	0.0176	0.0160	0.0160
$\rho_N(2p\pi)$	1.00	0.29	0.27	0.28	0.27	0.26
$\rho_N(\text{total})$	1.13	0.32	0.29	0.31	0.30	0.28
$a_N(\text{gauss})$	20.4	5.9	6.2	6.7	6.1	6.1

coupling constants, assuming a proportionality between the large π -electron spin density and the small σ -electron spin density on nitrogen in planar π -electron radicals.

From Table 7 it is seen that the INDO results on radicals, which are coplanar in the NO part, are consistent with this proportionality rule. The NH_3^+ ion is included for comparison as it is a planar system with unity π -electron spin density on nitrogen. In going from NH_3^+ to the nitroxide radicals, the spin density on nitrogen is reduced (according to INDO) to about 0.3. According to the proportionality rule, this gives a value for a_N of about $20 \times 0.3 = 6$ gauss, in good agreement with the calculated values (Table 7).

The coupling constants in Table 7 are calculated according to Pople *et al.*¹⁴ using the $2s_N$ spin density.

Some experimental evidence indicates, however, that the nitroxide radicals are not coplanar in the NO part, giving an angle of 14–21°^{17–19} between the NO-bond and the plane defined by C(3)–N(2)–C(4). One would expect a larger $2s_N$ spin density for these non-planar radicals where the σ – π separation is further removed. This is found to hold true by calculating the radicals using an out of plane angle of 20° for the NO-bond (Table 8). The coupling constants

Table 8. Some properties of the bent nitroxide radicals. The NO bond is 20° out of the plane defined by atoms 2, 3, and 4 (see Fig. 1). The bond length of NO is 1.26 Å (1.23 Å for II).

	II	III	IV	V	VI
$\rho_{\text{total}}(\text{N})$	0.2909	0.2917	0.2981	0.2886	0.2819
$\rho_{\text{total}}(\text{O})$	0.7238	0.7173	0.7069	0.7175	0.7275
$\rho_{2s}(\text{N})$	0.0252	0.0207	0.0233	0.0215	0.0201
$a_N(\text{gauss})$	9.5	7.9	8.8	8.2	7.6
$-E_{\text{total}}(\text{a.u.})$	30.209744	97.684538	130.819521	120.590555	132.451818

* The equilibrium configuration of H_2NO has a total INDO energy of -30.229851 a.u., an NO bond length of 1.23 Å and an NH bond length of 1.07 Å. The angle H–N–H is 113° and the out of the plane angle for the NO bond is 35°. The other radicals would not give such a high out of the plane angle because of the hindrance of the methyl groups.

rise to about 8 gauss as compared to the coplanar values of about 6 gauss. None of the configurations are, however, equilibrium configurations, where one should expect a still higher value. Indeed the coupling constant for the calculated equilibrium configuration of H_2NO^* is 16.3 gauss, as compared to the experimental value of about 15.5 gauss for the nitroxide radicals.

The improved coupling constants for the bent configurations are consistent with the idea that the nitroxide radicals are not, as assumed by Stone and McConnell, coplanar in the NO-part. But even for the bent configurations as calculated by INDO, the total spin density on nitrogen remains as low as 0.3 (Table 8), *i.e.* about the same as for the coplanar configurations (Table 5).

The conclusion of Stone and McConnell, that the value for a_N indicates a large spin density (0.7–0.9) on nitrogen, might therefore be erroneous. According to the INDO calculations there is a considerable change in the proportionality between total spin density and $2s_N$ spin density if the NO-bond is bent. It is the bent structure that accounts for the large $2s_N$ spin density and hence the large a_N value, and not a high total spin density. The reservation must be made that this conclusion is completely dependent on the ability of the INDO method to predict spin densities.¹³ It may turn out that the small basis set in INDO does not give an adequate description of the spin distribution in this case. *Ab initio* calculations with large basis sets would probably be of importance in deciding this question.

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