

EPR as a Diagnostic Tool to Verify Semiempirical Simulation of the Reactivity of Nitrones as Spin Traps

F. N. Dultsev¹ and G. G. Dultseva²

¹ Institute of Semiconductor Physics, Russian Academy of Sciences,
Novosibirsk, Russian Federation

² Institute of Chemical Kinetics and Combustion, Russian Academy of Sciences,
Novosibirsk, Russian Federation

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Abstract. The application of electron paramagnetic resonance spectroscopy as a diagnostic tool to verify the results of quantum chemical calculations is demonstrated. By using semiempirical MNDO/PM3 procedures and molecular mechanics MM2, it is possible to predict some characteristics of spin trapping with nitrones as spin traps, for example, the position of radical addition to a nitron, probability of the spin adduct to be formed, and the stability of the spin adduct.

1 Introduction

Quantum chemical simulation is becoming an increasingly important method in chemistry to describe the structure and properties of complicated organic molecules, including those possessing unpaired electrons. In addition, much attention is paid at present to the use of organic compounds as receptors to detect reactive species in the gas phase or in liquid. The use of quantum chemical calculation procedures enables one to simplify the search for a required receptor [1]. However, it is still necessary to have experimental methods in hand to confirm the correctness of calculation results. Electron paramagnetic resonance (EPR) provides a good chance to verify reaction routes expected in the systems containing free radicals. Not only advanced EPR procedures like time-resolved ones but also conventional stationary methods can be fruitful for this purpose. For instance, an informative possibility to carry out experimental verification of radical addition to a nitron molecule and of the position at which the addition proceeds is provided by the spin-trapping procedure.

Spin trapping is a chemical tool to detect and identify short-lived free radicals, which are known to play key roles in many chemical and biological proc-

esses [2]. The addition of a short-lived free radical to the double C=N bond of a nitrone molecule – spin trap (ST) results in the formation of a spin adduct (SA) which is a stable nitroxide radical. Its structure can be determined on the basis of the resulting EPR spectrum with the hyperfine splitting at the nitrogen atom of the nitrone group (and additional splitting at H atoms, if there are any, in the β -position to the radical center formed).

Nitroxide radicals are widely used in experimental chemistry as spin labels and paramagnetic probes to investigate molecular dynamics and structure of liquids, polymers and biological systems. In addition, nitroxide radicals are suitable species to test the applicability of various approximations in quantum chemical simulations of the potential reactivity. The reactivity of nitrones as STs depends on substituents in a nitrone molecule. The available experimental data [3] suggest that the selectivity of STs to various kinds of free radicals may be achieved by choosing nitrones with different substituents. So, with the help of EPR, we may carry out reliable experimental verification of the results of quantum chemical calculations.

2 Materials and Methods

We propose to simulate the radical addition reaction through the formation of a hypothetical adsorption complex (AC). This method allows us not only to predict the position of radical addition but also, which is more important, to estimate the probability of the reaction to proceed. The structure of SA and AC was calculated using semiempirical (MNDO/PM3) procedures and molecular mechanics (MM2). Simulation was carried out with the help of CS Chem 3D Pro, Version 5.0 (Cambridge Soft Corporation, USA). Simulation results were compared with the experimental data on the reactivity of substituted nitrones with respect to short-lived free radicals [4–6]. In order to verify some predictions based on simulation results, additional experiments were carried out. Simulation of nitrone molecules as STs for the hydroxyl radical OH was carried out.

Generation of free radicals. Short-lived free radicals were generated by ultraviolet irradiation of aqueous solutions of H_2O_2 (to produce $\cdot\text{OH}$), with methanol or ethanol added (to produce $\cdot\text{CH}_2\text{OH}$ or $\cdot\text{CH}_2\text{CH}_2\text{OH}$, respectively), using the same generation procedure as that described in ref. 4. For nitrones poorly soluble in water, a mixture of water with acetonitrile (1:3 by volume) was used as a solvent to improve the solubility.

EPR measurements. EPR spectra of SAs were recorded on a Bruker EMX spectrometer in a standard quartz cell. The stability of magnetic field was 2 mT/h. Microwave power was not higher than 10 mW, modulation amplitude was 0.03–0.10 mT. Modulation frequency was 100 kHz, receiver gain was $1 \cdot 10^4$ – $6.32 \cdot 10^5$. Time constant was 0.2 s, scan rate was 10 mT/200 s. Microwave frequency was 9.6 GHz (X-band). Instrumental error of measuring the constants of hyperfine splitting was 0.004 mT.

2.1 Modeling and Verification

2.1.1 Adsorption Complex

We use the term “adsorption complex” to indicate a hypothetical state through which the formation of a SA occurs. Simulation involving an AC allows us not only to estimate the probability of SA formation but also to predict the position in a nitron molecule at which the radical gets added. An AC designates physical adsorption of a short-lived free radical ($\cdot\text{OH}$) on a nitron molecule; AC involves weak interactions caused by some changes in electron motion in the adsorbate and adsorbent molecules. The adsorbed particles conserve their chemical nature. We calculate small changes in energy levels of the nitron molecule induced by the approaching short-lived free radical and then compare the energies of the initial unperturbed nitron molecule, of the AC, and of SA – the product of radical addition. The probability of addition to proceed may be evaluated from relative positions of these three energy levels.

2.1.2 Calculation Procedures

The semiempirical methods can be optimized for different purposes. For instance, MNDO, AM1 and PM3 methods were designed to reproduce heats of formation and structures of a large number of organic molecules. PM3 is much better than AM1 in calculating heats of formation for nitrogen-, sulfur- or phosphorus-containing organics (the accuracy is 4–5 times better in comparison with AM1). PM3 is used to calculate spin densities (for radical structures).

In order to achieve this goal, we calculated the interaction of OH with nitrones using quantum-chemical semiempirical methods (MNDO, AM1, PM3) and molecular mechanics (MM2).

We searched for the minimum of the total energy as a function of atomic coordinates. The procedure in general allows obtaining the data on molecular geometry, dipole moment, heat of formation [7, 8].

2.1.3 Calculation Algorithm

MNDO, AM1, PM3 procedures were applied to optimize the structures of nitron molecules and the corresponding ACs. MNDO- and AM1-optimized structures were further optimized using MM2, to calculate the structure of ACs. The following steps of simulation were realized: (i) MNDO/PM3: determination of a site at which a radical gets adsorbed. The criterion of adsorption to occur is reasonably short distance between the radical and the nitron, corresponding to the minimal energy (the formation of AC); (ii) PM3 + MM2: determination of the structure of this AC (bond lengths, valence angles); (iii) MM2: determination of the structure of SA.

3 Results and Discussion

The compounds containing two nitron groups were considered. Their structures are shown in Fig. 1. For convenience of consideration, some atoms are numbered.

3-Hydroxy-2,2,5-trimethyl-2,3-dihydropyrazine-1,4-dioxide (HDPDO) [5, 6] (Fig. 1a) has two conjugated nitron groups. These groups differ in the number of H atoms in β -position (with respect to the radical center formed when OH is trapped). So, the EPR spectra of OH SAs with this nitron formed at the two possible positions must differ from each other. The position of radical addition to this nitron can be determined on the basis of the number of lines in the EPR spectrum of the SA. Thus, simulation results can be easily verified on the basis of the experimental EPR spectra.

Possible positions for OH addition to HDPDO are 5 and 6. Simulation of the structures of the corresponding two kinds of SAs shows that the addition of OH at position 5 (CH_3) results in two possible SA configurations differing from each other in energy by 17 kcal/mol; if the energy level of the most profitable configuration of SA formed at position 5 is taken as zero, the addition at position 6 corresponds to the energy of 13 kcal/mol. Therefore, OH addition can proceed only at position 5. Consideration of the AC formation shows that position 5 is the most favorable one for $\cdot\text{OH}$ addition.

SAs with only one radical center were observed experimentally for dinitron HDPDO; no biradicals were detected. The experimental EPR spectrum of the OH-HDPDO SA is a triplet of doublets containing six lines [6]. Therefore, OH adds to position 5 only. So, theoretical results are in good agreement with the experimental observation of the position of OH addition to HDPDO.

Simulation was performed for 2,2-dimethyl-3-phenyl-2,3-dihydropyrazine-1,4-dioxide (DPDPO) shown in Fig. 1b and showed that DPDPO forms an adduct with $\cdot\text{OH}$. Position 5 is preferable for the formation of an AC and for the formation of the final product of radical addition. Addition to a different position is by 14 kcal/mol less profitable. To carry out experimental tests of the reactivity of DPDPO, we performed spin-trapping experiment with this compound and discovered that it traps OH with the formation of a SA. The experimental EPR spectrum of the adduct contains 12 lines ($a_{\text{N}} = 1.24$ mT, $a_{\text{H}}(1) = 0.65$ mT, $a_{\text{H}}(2) =$

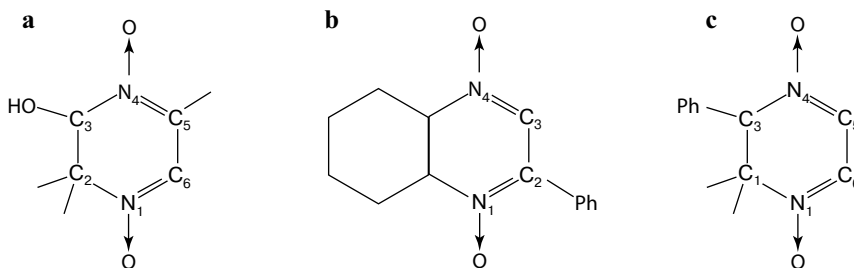


Fig. 1. Structures of nitrones involved in the present study.

0.21 mT, indicating the interaction with one N and two nonequivalent H atoms). The number and shapes of lines suggest that OH adds at position 5, so we observe the interaction with N_4 , one H atom bound with C_5 and another H atom at C_3 (otherwise, if the radical adds at position 6, the spectrum would contain only a triplet of doublets, 6 lines, because there is only one β -hydrogen atom at C_6) (see Fig. 1b). So, theoretical calculations suggest that radical addition proceeds at position 5, in agreement with the experimental observation.

Thus, the number of components in the EPR spectrum of SA can be predicted rather reliably on the basis of simulation results.

The next example explains why no adducts of OH are formed with hexahydroquinoxaline-1,4-dioxide having a phenyl substituent in position 2 (HDPOPh (Fig. 1c)). This compound is another conjugated dinitrone differing from HDPDO by the presence of phenyl group (in position 2) and a cycloalkyl fragment. These differences in structure bring about sharp differences in the reactivity: compound HDPOPh does not form any adducts with $\cdot\text{OH}$; however, it was observed to trap hydroxyethyl radical $\cdot\text{C}_2\text{H}_4\text{OH}$ [6]. Let us consider the addition of short-lived free radicals through the formation of an AC. Simulation shows that compound HDPOPh forms an AC with radicals at position 3. The structure of AC is shown in Fig. 2. The calculated energy diagrams of the reactions for $\cdot\text{OH}$, $\cdot\text{CH}_2\text{OH}$, $\cdot\text{C}_2\text{H}_4\text{OH}$ are shown in Fig. 3. Though the formation of an AC with OH is profitable from the viewpoint of energy (see Fig. 3), about 30 kcal/mol is necessary for the final product to be formed (by $\cdot\text{OH}$ addition to position 3); this means that the addition of OH does not give any energy gain; the probability of this reaction is very low. So, adduct with OH at position 3 is not formed.

In the case of hydroxymethyl radical (see Fig. 3), energy is released with the formation of AC (~ 12 kcal/mol); further, with the addition of the radical, about 2 kcal/mol is released. A similar situation is observed for hydroxyethyl radical (see Fig. 3), but energy gain in the formation of the SA is 9 kcal/mol. So, it may be stated on the basis of calculations that addition at position 3 is most profitable for the hydroxyethyl radical. The addition of hydroxymethyl radical is also possible at this position, but the concentration of the formed SA should

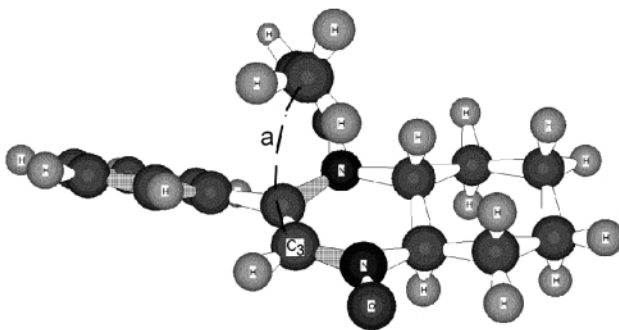


Fig. 2. Structure of AC for dinitrone (HDPOPh), a – minimal distance between the nitrone molecule and $\cdot\text{CH}_2\text{CH}_2\text{OH}$.

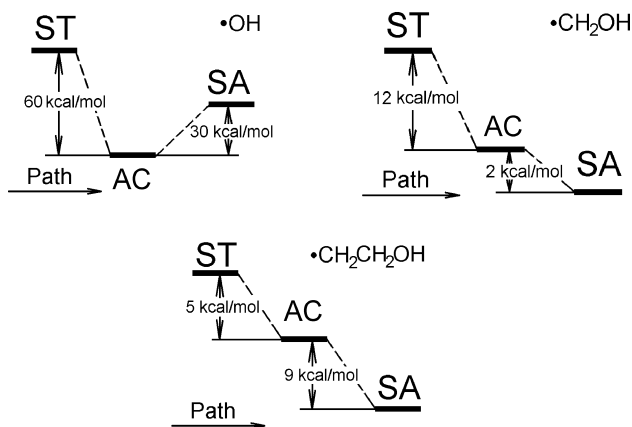


Fig. 3. Energy diagrams of the SA formation through AC for $\cdot\text{OH}$, $\cdot\text{CH}_2\text{OH}$, $\cdot\text{CH}_2\text{CH}_2\text{OH}$. ST: dinitrone (HDPOPh).

be lower. No experimental data were available for this nitrone as a trap for $\cdot\text{CH}_2\text{OH}$, so we performed the necessary experiments involving trapping of $\cdot\text{OH}$, $\cdot\text{CH}_2\text{OH}$ and $\cdot\text{C}_2\text{H}_4\text{OH}$ radicals with compound HDPOPh in order to compare its reactivity toward these radicals under identical experimental conditions. Experimental results confirmed the calculated data. The EPR spectra of SAs recorded in the experiment are shown in Fig. 4 (SA with $\text{C}_2\text{H}_4\text{OH}$: $a_{\text{N}} = 1.14$ mT, $a_{\text{H}} = 0.81$ mT; with CH_2OH : $a_{\text{N}} = 1.15$ mT, $a_{\text{H}} = 0.93$ mT). The number of lines in these EPR spectra is 6 (a triplet of doublets), suggests that the radicals add at position 3 of nitrone HDPOPh. Quantitative estimation of the number of SAs formed in a sample suggests that the adduct of hydroxyethyl radical is formed at higher

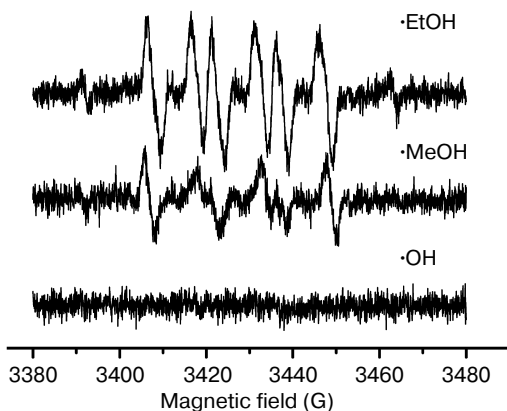


Fig. 4. Examples of EPR spectra of SAs of dinitrone (HDPOPh) with different radicals.

concentration than that of hydroxymethyl. This confirms our theoretical result that the concentration of CH_2OH adduct should be lower. No adduct is observed with OH, exactly as predicted by simulation. In this case we also observe good agreement between theory and experiment, which confirms our model of SA formation through AC.

4 Conclusions

Thus, on the basis of results obtained in semiempirical simulation of the ACs and the resulting SAs, one may predict the structure of SA, and the reactivity of nitrones as STs. If one knows at which position a radical adds to a nitron, the appearance of EPR spectrum of the resulting SA can be easily predicted. The results of simulation obtained in the present work exhibit a good agreement with the experimental results. It is shown that the energy diagrams depicting the formation of AC between a short-lived free radical and a nitron molecule help predicting the stability of SAs. So, semiempirical calculations can be used to predict the reactivity of nitrones as STs thus saving synthetic effort and simplifying experimental search for compounds to trap, and identify short-lived free radicals of different kinds.

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Authors' address: Fedor N. Dultsev, Institute of Semiconductor Physics, Russian Academy of Sciences, Prospekt Lavrentyeva 13, Novosibirsk 630090, Russian Federation
E-mail: fdultsev@thermo.isp.nsc.ru