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Superoxide Radical Anion Adduct of 5,5-Dimethyl-1-pyrroline *N*-oxide. 6. Redox Properties

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Abstract

Cyclic nitrones have been employed for decades as spin trapping reagents for the detection and identification of transient radicals, and have been employed as pharmacological agent against ROS-mediated toxicity. The short half-life of the nitron-superoxide adducts has limit the application of nitrones in biological milieu, and therefore investigation of the redox properties of the superoxide adducts is important. Moreover, computational investigation of the redox properties of the nitrones and their corresponding spin adducts may provide new insights into the nature of their pharmacological activity against ROS-induced toxicity. In general, electron-withdrawing group substitution at the C-5 position results in higher EAs and IPs making these substituted nitrones more susceptible to reduction but more difficult to oxidize compared to DMPO. One-electron reduction and oxidation of nitrones both resulted in elongated N-C₂ bonds indicating the tendency of radical anion and cation forms of nitron to undergo ring opening. The EAs and IPs of various O₂^{•-} adducts indicate that DEPMPO-O₂H is the most difficult to reduce and oxidize compared to the O₂^{•-} adducts of DMPO, EMPO and AMPO. In general, nitroxides gave higher EAs compared to nitrones making them more susceptible to reduction. One-electron oxidation of nitroxides leads to elongation of the N-C₂ bond but not for their reduction. The energetics of redox reaction of O₂^{•-} adducts was also explored and results indicate that the reduction of O₂^{•-} adducts with O₂^{•-} is preferred followed by their oxidation by O₂ and then by O₂^{•-} but the maximum difference between these free energies of redox reactions in aqueous solution is only 0.21 kcal/mol. The preferred decomposition pathways for the one-electron oxidation and reduction of nitroxides was also explored and formation of potentially biologically active products such as NO, H₂O₂ and hydroxamic acid were predicted.

1. Introduction

Almost four decades after the use of the cyclic nitron, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), was first demonstrated^{1,2} for radical detection using electron paramagnetic resonance (EPR) spectroscopy, DMPO has been the reagent of choice for most spin trapping applications.^{3,4} The popularity of DMPO for the identification of superoxide radical anion (O₂^{•-}) by spin trapping can be attributed to the distinctive EPR signal that the DMPO-O₂^{•-} adduct (from hereon will be referred to its final form in solution as DMPO-O₂H) exhibits in spite of its short half-life. The fact that one can discern the various radical adducts generated by DMPO from DMPO-O₂H based on the EPR spectral profile they exhibit makes DMPO a more preferred spin trap for the detection of O₂^{•-} compared to other nitrones such as α -phenyl-*N*-tert-butyl nitron (PBN).

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Supporting Information Available. Bottom-of-the-well energies, free energies and enthalpies for all the compounds are available as supporting information. This information is available free of charge at <http://pubs.acs.org>.

Superoxide is produced from one-electron reduction of molecular oxygen via enzymatic,⁵ chemical⁶ and photochemical⁷ means, and is the main precursor of highly reactive species such as $\text{HO}^\bullet$, $\text{CO}_3^{\bullet-}$, $\text{ONOO}^\bullet$, HOCl and $\text{GSSG}^{\bullet-}$ which are known to exist in biological systems.⁸ Therefore, detection of $\text{O}_2^{\bullet-}$ in cellular system is important as it signals the first sign of oxidative burst which, in unregulated concentrations, can cause oxidative damage to key biomolecules, and subsequently leading to cell injury or death.⁸ Aside from the popularity of spin trapping using DMPO for $\text{O}_2^{\bullet-}$ detection in biological systems, there has also been a growing interest for this application in the fields of fuel cell research,⁹ drug metabolism,¹⁰ photodynamic therapy,¹¹ environmental remediation,¹² tobacco smoking,¹³ cosmetology,¹⁴ sonolysis¹⁵ and beverage research.¹⁶ Indeed, radical detection using spin trapping has contributed significantly toward the understanding of some of the fundamental free radical-mediated processes in chemical and biological systems.^{17,18}

Relative to the hydroxyl and alkyl radical adducts of cyclic nitrones that persist for hours, the half-lives of the $\text{O}_2^{\bullet-}$ adducts of cyclic nitrones such as DMPO, AMPO, DEPMPO, and EMPO (see Figure 1) are generally short (< 1 min to ~ 14 min).^{4,19–23} Several approaches have been employed to prolong the half-lives of the DMPO $\text{O}_2^{\bullet-}$ adduct such as by freezing,^{24,25} conjugation²⁶ to β -cyclodextrin or adduct generation in the presence of β -cyclodextrin.²⁷ Although these half-lives reflect unimolecular processes,^{4,21,23,28,29} but in a more complex matrix such as in vitro or in vivo systems this mechanism seems irrelevant due to the presence of oxidoreductants as spin adducts, like all nitroxides, are also very susceptible to redox reactions.^{30,25} As demonstrated by Khan, et al.,²⁵ the hydroxyl adduct half-lives of cyclic nitrones are greatly reduced ($t_{1/2}$ = 0 to 5 min) in the presence of cell suspension using the spin traps DMPO, DEPMPO, and EMPO while the first-order half lives for the hydroxyl adduct of cyclic nitrones were found to be generally longer (40–160 min)³¹ in phosphate buffer at neutral pH.

The reactivity of stable nitroxides such as the tetra-alkyl β -substituted pyrrolidine- and piperidine-based aminoxyl radicals, PROXYL and TEMPO, to Fe(II) ³² and $\text{O}_2^{\bullet-}$ ³³ have been investigated, and their oxidation and reduction to form oxoammonium cation and hydroxylamine, respectively, are mostly reversible in simple solvent systems (Scheme 1).^{34,35} In contrast, the redox properties of nitroxides bearing a β -H such as in the case of the spin adduct of cyclic nitrones have not been explored. Samuni et al.³⁶ have shown that $\text{O}_2^{\bullet-}$ depletes hydroxyl and methyl adducts of DMPO but their fate was not well understood. The role of β -H in the instability of the spin adducts was further supported by the increased stability of the spin adduct upon substitution of the β -H by a methyl group.³⁷ The intrinsic half-lives of the $\text{O}_2^{\bullet-}$ adducts in aqueous solution are short relative to the more stable TEMPO and PROXYL nitroxides, and the presence of oxidoreductants in biological milieu exacerbates this problem thereby making them even more difficult to detect by EPR. In most cases, $\text{O}_2^{\bullet-}$ adduct formation in biological systems can only be inferred from inhibition of hydroxyl radical adduct formation using superoxide dismutase.^{38,39}

There are several reasons why investigation of the redox properties of $\text{O}_2^{\bullet-}$ adducts of cyclic nitrones is relevant. First, oxidoreductants such as ascorbic acid and metal ions are ubiquitous in biological systems and can react with the redox active nitroxide-based spin adducts. Also, the $\text{O}_2^{\bullet-}$ adduct is concurrently generated with $\text{O}_2^{\bullet-}$ production in solution and reaction of $\text{O}_2^{\bullet-}$ with $\text{O}_2^{\bullet-}$ adducts may lead to shorter adduct half-life. Moreover, cyclic nitrones impart pharmacological activity against ischemia and reperfusion induced injuries^{40,41} but it is not clear if the cardioprotection offered by nitrones is due to the nitrone itself, the aminoxyl adduct or its metabolic products. Evidences show that nitroxides or hydroxylamines, which are potential products of spin trapping, are cytoprotective⁴² and that the NO decomposition product of $\text{O}_2^{\bullet-}$ adducts⁴³ can potential offer bioactivity. Due to the growing evidence that shows $\text{O}_2^{\bullet-}$ generation during ischemia and reperfusion,^{44,45} and the that cyclic spin traps offer

cardioprotective properties, studies of the redox properties of nitrones and their respective $\text{O}_2^{\bullet-}$ adducts, are therefore relevant and may yield interesting chemistries that may help explain the molecular basis of their pharmacological activity.

2. Computational Methods

All calculations were performed using Gaussian 0346 at the Ohio Supercomputer Center. Density functional theory (DFT)⁴⁷ at the B3LYP/6-31G(d) level of theory was employed in this study to determine the optimized geometry of the nitrones, $\text{O}_2^{\bullet-}$ adducts, as well as their respective one-electron oxidized and reduced forms. All the structures yielded no imaginary vibrational frequency. Negligible spin contamination of $0.75 < \langle S^2 \rangle < 0.76$ were obtained for the aminoxyl compounds. Scaling factors of 0.980648 was used for the zero-point vibrational energy (ZPE) corrections for all the B3LYP/6-31G(d) geometries. Free energies were obtained from the calculated thermal and entropic corrections at 298 K using the unscaled vibrational frequencies. In this study, the BHandHLYP/6-311G(d,p)//B3LYP/6-31G(d) level of theory was chosen since it gave bottom-of-the-well energy barrier estimates that are close to the more accurate QCISD/cc-PVDZ//B3LYP/6-31G(d) based on our previous studies.⁴⁹ The effect of solvation was also investigated using the PCM,⁵⁰⁻⁵¹ while the spin and charge densities were obtained from natural population analysis (NPA) approach⁵² at the PCM(water)/BHandHLYP/6-311G(d,p). Procedure for the calculation of $\text{p}K_a$'s is described in our previous works.⁵³⁻⁵⁵

3. Results and Discussion

3.1. Redox Properties of Nitrones

Table 1 shows the electron affinities (EA) and ionization potentials (IP) of the various nitrones at the PCM/BHandHLYP/6-311G(d,p)//B3LYP/6-31G(d) level. Compared to all the nitrones, DMPO gave the lowest EA (along with EMPO) and IP, while AMPO gave the highest EA and IP compared to other nitrones. The same trend was observed for AMPO and only DMPO gave the lowest EA and IP at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level of theory (see Table S1). In general, electron-withdrawing group substituted nitrones at the C-5 position results in higher calculated EAs and IPs making them more susceptible to reduction but are more difficult to oxidize. Examination of the NPA charge densities of the nitrones show that AMPO has the most positive charge density on the nitronyl-C, least positive charge on the nitronyl-N and has the most negative charge on the nitronyl-O (Table 2) which may account for its relative ease of reduction and difficult oxidation compared to other nitrones.

Table 2 shows the bond lengths and charge densities of the nitronyl group for the nitron and its reduced and oxidized forms (as radical anion and radical cation, respectively). Examination of the various bond lengths shows that the N-C₂ and N-O are, in general, longest for the radical anions compared to the neutral nitrones and the radical cations. The C₅-N bonds, with the exception of EMPO, are lowest for the radical anions. Only slight increase (0.02–0.04 Å) in the N-C₂ bond lengths was observed for the radical cations. These results indicate that the radical anions may have more propensity to undergo ring opening than the radical cations. There are also significant changes in the charge densities that can be observed during the one-electron reduction and oxidation of nitrones. For example, the charge densities on the nitronyl-N, -O and -C₂ are most negative for the radical anions compared to the neutral forms and radical cations, while higher positive charges on the nitronyl-C₂ can be observed for the radical cations compared to the neutral and radical anions. The spin densities are highest on the N (0.12–0.43 e) and C₂ (0.42–0.81) for the radical anions, while for the radical cations, the spin densities are mostly localized on the O (0.73–0.74) and C₂ (0.27–0.32). These spin density distributions are represented in Figure 2 showing the possible resonance structures for the radical anion and cation. No significant changes in the electronic properties of C₅ and X₆, either

from the radical anions and cations, indicating that the nitronyl moiety is mostly susceptible to redox reaction and not the substituents.

3.2. Redox Properties of $O_2^{\bullet-}$ Adducts

Comparison of the calculated EA's (Table 1) for the more stable nitroxides shows that PROXYL is harder to reduced compared to TEMPO further supporting the previous findings showing a more facile reduction of six-membered ring nitroxides compared to the five-membered ring ones due to the conformational flexibility of the aminoxyl moiety in the former.⁵⁶ However, no significant difference in the IP's were predicted for PROXYL and TEMPO. For nitroxides bearing a β -H, lower EA values for the DMPO-CH₃ and DMPO-OH adducts were predicted compared to the EA's of all the $O_2^{\bullet-}$ adducts suggesting that the $\bullet$ CH₃ and $\bullet$ OH adducts are harder to reduce compared to the $O_2^{\bullet-}$ adducts. This observation follows the same qualitative trend in half-lives of $\bullet$ CH₃ and $\bullet$ OH adducts in aqueous solution compared to $O_2^{\bullet-}$ adducts where the $\bullet$ CH₃ and $\bullet$ OH adducts³¹ are generally more persistent than the $O_2^{\bullet-}$ adducts.

The calculated EAs and IPs for the various $O_2^{\bullet-}$ adducts are shown in Table 1. Results indicate that in general, DEPMPO-O₂H gave the lowest EA and high IP values compared to other adducts suggesting that DEPMPO-O₂H is relatively harder to reduce and oxidize than the other adducts. DMPO-O₂H gave the lowest IP while AMPO-O₂H gave the highest EA, and therefore, these adduct may be more susceptible to oxidation and reduction, respectively. The experimental first-order half-life of DMPO-O₂H^{19–21} is only ~ 1 min, however, AMPO-O₂H,⁴ and EMPO-O₂H²² gave half-lives of ~ 8–10 min while DEPMPO-O₂H²³ has the longest with $t_{1/2}$ ~ 14 min. First-order decomposition of adducts have been shown to be due to the aminoxyl C₂-N bond cleavage with concomitant release of NO.^{41,43} Our previous study⁴¹ on the kinetics and thermodynamics of the unimolecular decomposition of the $O_2^{\bullet-}$ adducts via C₂-N bond cleavage shows preference for DMPO-O₂H, but no significant difference in the energetics of decomposition was predicted among AMPO-O₂H, EMPO-O₂H and DEPMPO-O₂H and their respective isomers. This observation leads us to suggest that other modes of decomposition maybe operative. Since DEPMPO-O₂H has the lowest EA and high IP (as shown in Table 1) compared to other $O_2^{\bullet-}$ adducts could indicate that DEPMPO-O₂H is less susceptible to reduction and oxidation, consistent with its long half-life in solution. Therefore, redox reaction may play a more important role in adduct decay in solution possibly via further reaction with $O_2^{\bullet-}$, disproportionation reaction, or reaction with molecular oxygen which will be discussed in details in the succeeding sections.

Table 3 shows the pertinent bond lengths of the various adducts, and of their reduced and oxidized forms. The aminoxyl compounds show an N-O bond length of 1.27–1.29 Å, similar to that observed experimentally of 1.27 Å.⁵⁷ The reduction of the aminoxyl compounds shows a more elongated N-O bond (1.39–1.43 Å) indicative of more resonance contribution from the singly bonded N-O consistent with the formation of *N*-olate anion, while oxidation of the aminoxyl compounds gave shorter N-O bond length (1.19–1.20 Å) confirming the formation of oxoammonium cation with doubly bonded N=O. Data also indicate elongation of the N-C₂ bond for oxidized aminoxyl compounds which could imply potential for ring opening decomposition pathway during oxidation of the $O_2^{\bullet-}$ adducts. Table 4 shows the NPA charges of the relevant atoms and results show that compared to the aminoxyl compounds, the reduced forms gave more negative charge density on the aminoxyl-O, -N and β -O, while for the oxidized forms, less negative charges were observed for the aminoxyl-O and β -O but positive charge on the N.

3.3. Reactivity of Aminoxy Radicals with $O_2^{\bullet-}$

The more stable nitroxides such as TEMPO and PROXYL as well as the adducts, DMPO-OH and DMPO-CH₃, show lower EAs that ranges from 1.94 eV to 2.40 eV (see Table 1) and a lower IP range of 5.35–5.52 eV compared to the $O_2^{\bullet-}$ adducts which have EAs and IPs ranging from 2.91–3.39 eV and 5.56–6.06 eV, respectively. The higher EAs and IPs for $O_2^{\bullet-}$ adducts indicate that they are easier to reduce and harder to oxidize than the more stable nitroxides. The adduct DEPMPO-O₂H has been experimentally observed to have the longest first-order half-life in aqueous solution compared to other $O_2^{\bullet-}$ adducts, with its lower EA being consistent with those of the more stable nitroxides but not the high IP predicted for DEPMPO-O₂H. Since thermodynamically, reduction of DEPMPO-O₂H by O_2 is improbable, and therefore, the steady flux of $O_2^{\bullet-}$ or oxidation by oxygen could be a more important factor affecting $O_2^{\bullet-}$ adduct half-life. By virtue of $O_2^{\bullet-}$ oxidation state, it can serve as both a reducing and oxidizing agent leading to the formation of oxygen (O_2) and peroxide (O_2^{2-}), respectively.

Table 5 shows the thermodynamic parameters for the reduction and oxidation of various $O_2^{\bullet-}$ adducts and those of the more stable nitroxides (i.e., TEMPO, PROXYL, DMPO-OH and DMPO-CH₃) to *N*-olate anion and oxoammonium cation forms, respectively. Results show that in general, $O_2^{\bullet-}$ adducts reduction gave $\Delta G_{rxn,aq}$ range of –0.01–0.01 kcal/mol while reduction of the more stable adducts is only slightly more endoergic $-G_{rxn,aq}$ 0.02–0.04 kcal/mol indicating that $O_2^{\bullet-}$ adducts are relatively susceptible to both reduction and oxidation in solution. However, no significant trend in the energetics of oxidation between $O_2^{\bullet-}$ adducts and the more stable adducts can be observed. Comparison of the overall energetics between reduction and oxidation of aminoxy by $O_2^{\bullet-}$ shows that the former is slightly more favored by $\sim 0.18 \pm 0.02$ kcal/mol. However, comparison of the energetics of oxidation of aminoxy to oxoammonium cation shows that oxidation by O_2 is more favorable than oxidation by $O_2^{\bullet-}$ (see Table 5) giving $\Delta G_{rxn,aq}$'s ranging from 0.09–0.11 kcal/mol for the former. Therefore, in aqueous solution and in the presence of O_2 with steady flux of $O_2^{\bullet-}$, the order of preference for nitroxide decomposition is via reduction by $O_2^{\bullet-}$ followed by oxidation by O_2 and then oxidation by $O_2^{\bullet-}$.

3.4. Decomposition Pathways of the One-Electron Oxidation and Reduction of DMPO-O₂H

Cyclic voltammetry of tetra-alkyl- β -substituted nitroxides typically exhibit a reversible^{34,35} nitroxide/oxoammonium cation couple while their reduction to hydroxylamine is irreversible in organic solvents but show reversibility in aqueous solution.³⁵ The reversibility of the reduction process of tetra-alkyl- β -substituted nitroxides was previously demonstrated in the presence of Fe(II).³² This reversibility can be accounted to the stability of the nitroxide due to the alkyl substitution at the β -positions. However, it has previously³⁶ been shown that DMPO spin adducts upon reaction with $O_2^{\bullet-}$ results in their depletion and could not be restored through reoxidation with ferricyanide. Furthermore, the observed NO release⁴³ from DMPO-O₂H may also be a result of a ring opening mechanism. Decomposition of DMPO-O₂H may possibly proceed via the disproportionation of DMPO-O₂H to form the respective nitron 7 and hydroxylamine 17 (Scheme 2) via electron transfer mechanism forming, [DMPO-O₂H][–] (1) and [DMPO-O₂H]⁺ (9). Nitroxides bearing a β -H have been shown to disproportionate to their respective nitrones and hydroxylamine.^{58,59} Moreover, decomposition of DMPO-O₂H to form 1 and 9 from further reaction with $O_2^{\bullet-}$ could also be possible.

Since the general trends in energetics of redox reactions using PCM(water)/BHandHLYP/6-311G(d,p) (Table 1) are comparable to that at PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level (Table S1), the mechanisms of decomposition of the reduced and oxidized DMPO-O₂H were therefore theoretically explored using the latter level of theory. Scheme 3 shows the potential decomposition pathways for the one-electron reduced DMPO-O₂H. Protonation of the initially formed *N*-olate anion (1) to form the 5-hydroperoxy-2,2-dimethylpyrrolidin-1-ol

(7) is highly favorable giving a calculated pK_a for **7** of 9.9 and 12.2, for the “*cis*” and “*trans*” conformations, respectively, as shown in Figure 3. It is therefore expected that in neutral pH, the reduction of DMPO- O_2H to **1** will spontaneously lead to the formation of **7** in aqueous solution. Rearrangement of **7** via $HO^\bullet$ migration to form 5-(dihydroxyamino)-5-methylhexan-2-one (**8**) (see Figure 4) is highly favorable with free energy of reaction of -29.4 kcal/mol. Further decomposition of **8** to the aldehyde (**6**) with concomitant release of nitrous acid (HNO_2), which has been shown to decompose to NO ,⁶⁰ is also exoergic with free energy of decomposition of -18.4 kcal/mol. The predicted formation of **6** and NO has been experimentally demonstrated to be formed from DMPO- O_2H .⁴³ Although ring opening of the unprotonated **1** to form **2** (see Figure 4) was found to be exoergic by -29.2 kcal/mol, protonation of **2** is expected to be instantaneous due to the calculated pK_a of 11.5 for **8**. Since the radical pathway, (i.e., Reactions A→D) for the formation of **6** is endoergic with $\Delta G_{rxn,aq} = 8.9$ kcal/mol, the decomposition pathway via Reaction F→G seems the most plausible decomposition pathway for the one-electron reduction of DMPO- O_2H .

The potential decomposition pathways for the one-electron oxidized DMPO- O_2H and their respective energetics are shown in Scheme 4. Results show that the formation of the tautomeric form **13** from **9** is thermodynamically more favored by ~30 kcal/mol compared to the ring opening mechanism to form **10**. However, the formation of the nitron **17** via deprotonation of **13** and the nucleophilic addition of water to **13** to yield **14** (see Figure 5) are both endoergic with $\Delta G_{rxn,aq}$ of 27.7 and 37.6 kcal/mol, respectively. Elimination of H_2O_2 from **14** to give the hydroxamic acid **15** and its subsequent tautomerization to form **16** is exoergic with a total $\Delta G_{rxn,aq}$ for these two processes of -35.9 kcal/mol, while the radical pathway leading to the formation of **16** from **17** is less exoergic with $\Delta G_{rxn,aq}$ of -7.2 kcal/mol. Therefore, although the formation of **17** and **14** from **13** are endoergic, the pathway leading to the formation of the nitron **16** via elimination of H_2O_2 is the most preferred ($\Delta G_{rxn,aq} = -74.4$ kcal/mol) compared to the pathways leading to the formation of **3** and **12** via the ring opening mechanism with net free energies of reaction of -32.0 and -24.5 kcal/mol, respectively.

4. Conclusions

Using density functional theory approach, the redox properties of nitrones and their respective superoxide adducts was investigated. In general, nitrones with electron-withdrawing group substitution at the C-5 position results in higher calculated EAs and IPs making these nitrones more susceptible to reduction but are more difficult to oxidize compared to DMPO. Reduction of nitrones yielded elongated N-C₂ and N-O bonds but shorter C₅-N bonds, while their oxidation only results in a slight elongation of the N-C₂ bond. The *cis* and *trans* DEPMPO- O_2H gave the lowest EA but highest IP indicating that DEPMPO- O_2H is hardest to reduce and oxidize compared to the $O_2^{\bullet-}$ adducts of DMPO, EMPO and AMPO. In general, nitroxides gave higher EAs compared to nitrones making the nitroxides more susceptible to reduction. The IPs of the nitrones and of their respective $O_2^{\bullet-}$ adducts are not significantly different but in general are higher compared to the more stable nitroxides such as TEMPO, PROXYL, DMPO-OH and DMPO-CH₃ adducts indicating a more facile oxidation for these latter class of nitroxides. One-electron oxidation of nitroxides leads to elongation of the N-C₂ bond and shortening of N-O bond. Reduction of nitroxides leads only to elongation of the N-O bonds consistent with the formation of *N*-olate anion.

The order of preference for nitroxide decomposition is via reduction by $O_2^{\bullet-}$ followed by oxidation by O_2 and then oxidation by $O_2^{\bullet-}$. The thermodynamically preferred decomposition pathway for the one-electron reduction of DMPO- O_2H is via formation of the hydroxylamine and subsequent ring opening to form the aldehyde and concomitant elimination of HNO_2 . For the one-electron oxidation of DMPO- O_2H , the preferred decomposition is the formation of the hydroxamic acid via nucleophilic addition of water to the nitron **13**. This theoretical study

predicts the redox properties of the nitrones and of the $O_2^{\bullet-}$ adducts as well as their subsequent decomposition to form biological active species such as NO, H_2O_2 , and hydroxamic acid. Hydroxamic acid for example is widely employed as an iron chelator and enzyme inhibitors.^{61–63} The redox activity of nitrones and their spin adducts as well as the formation of biologically active by-products could play crucial roles in regulating cellular redox state and may be used as rationale for the pharmacological activities of nitrones against oxidative stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Cyclic nitrones and nitroxides.

Figure 2. Resonance structures for the radical anion (top) and cation (bottom) showing the location of the unpaired electron.

Figure 3. Relative free energy and enthalpy in kcal/mol of the two conformational isomers of the 5-hydroperoxy-2,2-dimethylpyrrolidin-1-ol (**7**) at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) showing the “*cis*” (left) and “*trans*” (right) conformations of the hydroxylamine-H relative the hydroperoxyl moiety.

Figure 4. Optimized geometries of the unprotonated (**2**, left) and protonated (**8**, right) 5-(dihydroxyamino)-5-methylhexan-2-one at the B3LYP/6-31G(d) level of theory.

Figure 5. Relative free energy and enthalpy in kcal/mol at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level of theory of the two conformations of **14** in which the *N*-hydroxy-H is oriented *cis* (right) and *trans* (left) to the hydroperoxyl moiety.

Scheme 1.
Redox reaction of PROXYL radical.

Scheme 2.
Disproportionation reaction of DMPO-O₂H.

Scheme 3. Potential Decomposition Pathways upon Reduction of DMPO-O₂H.^a

^aThe reaction free energies ($\Delta G_{\text{rxn, aq, 298K}}$) and enthalpies ($\Delta H_{\text{rxn, aq, 298K}}$ in parentheses) in aqueous phase in kcal/mol at the PCM/B3LYP/6-31G+(d,p)/B3LYP/6-31G(d) level for reactions: A -29.2 (-29.4); B -13.2 (1.2); C 25.6 (38.8); D 25.7 (23.8); E -37.5 (-21.1); F -29.4 (-28.2); G -18.4 (-3.4); A + B + C + D (net): 8.9 (34.4); A + E (net): -66.6 (-50.4); F + G (net): -47.8 (-31.6).

Scheme 4. Potential Decomposition Pathways upon Oxidation of DMPO-O₂H.^a

^aThe reaction free energies ($\Delta G_{\text{rxn, aq, 298K}}$) and enthalpies ($\Delta H_{\text{rxn, aq, 298K}}$ in parentheses) in the aqueous phase in kcal/mol at the PCM/B3LYP/6-31G+(d,p)//B3LYP/6-31G(d) level for reactions: H -44.7 (-44.3); I 18.2 (10.0); J -5.6 (6.8); K 20.2 (23.7); L -76.1 (-75.7); M 37.6 (25.3); N -26.5 (-13.3); O -9.4 (-9.3); P 27.7 (27.3); Q -23.9 (-12.5); R 53.3 (52.3); S -7.2 (-6.0); H + I + J (net): -32.0 (-27.4); H + K (net): -24.5 (-20.6); L + M + N + O (net): -74.4 (-73.0); Q + R (net): 29.4 (39.8).

Table 1

Electron Affinities and Ionization Potentials (in kcal/mol and eV) for Various Nitrones and Nitroxides at the PCM(water)/BHandLYP/6-311G(d,p)//B3LYP/6-31G(d) Level of Theory at 298 K.

compound	electron affinity		ionization potential	
	kcal/mol	eV	kcal/mol	eV
nitrones				
DMPO	27.37	1.19	131.11	5.69
AMPO	36.22	1.57	138.15	6.00
EMPO	25.26	1.10	134.60	5.84
DEPMPO	33.93	1.47	136.33	5.92
aminoxyls				
DMPO-O ₂ H	71.56	3.11	128.12	5.56
AMPO-O ₂ H <i>cis</i>	78.16	3.39	135.14	5.87
AMPO-O ₂ H <i>trans</i>	75.83	3.29	134.55	5.84
EMPO-O ₂ H <i>cis</i>	71.63	3.11	133.99	5.82
EMPO-O ₂ H <i>trans</i>	68.38	2.97	135.70	5.89
DEPMPO-O ₂ H <i>cis</i>	67.08	2.91	139.62	6.06
DEPMPO-O ₂ H <i>trans</i>	69.22	3.00	137.72	5.98
TEMPO	49.16	2.13	123.41	5.36
PROXYL	44.59	1.94	123.45	5.36
DMPO-OH	55.30	2.40	127.27	5.52
DMPO-CH ₃	50.20	2.18	123.32	5.35

Table 2

Bond Lengths (Å), NPA Charges and Spin Densities (e) (in parentheses) of Nitronyl C₂, N and O of Various Nitrones, Radical Anions and Cations at the PCM/BHandLYP/6-311G(d,p)//B3LYP/6-31G(d) Level of Theory.

	DMPO	AMPO	DEPMPO	EMPO
nitronyl N-C₂	1.31	1.30	1.31	1.31
[nitronyl]⁻ N-C₂	1.42	1.40	1.39	1.34
[nitronyl]⁺ N-C₂	1.33	1.34	1.34	1.34
nitronyl N-O	1.27	1.28	1.27	1.26
[nitronyl]⁻ N-O	1.33	1.36	1.44	1.29
[nitronyl]⁺ N-O	1.26	1.26	1.26	1.26
nitronyl C₅-N	1.53	1.52	1.51	1.52
[nitronyl]⁻ C₅-N	1.47	1.49	1.47	1.57
[nitronyl]⁺ C₅-N	1.54	1.52	1.51	1.52
nitronyl C₅-X₆	1.53	1.56	1.86	1.54
[nitronyl]⁻ C₅-X₆	1.55	1.56	1.89	1.49
[nitronyl]⁺ C₅-X₆	1.53	1.56	1.89	1.56
nitronyl N	0.07	0.05	0.06	0.07
[nitronyl]⁻ N	-0.07 (0.43)	-0.14 (0.30)	-0.25 (0.12)	-0.03 (0.18)
[nitronyl]⁺ N	-0.01 (-0.08)	-0.01 (-0.02)	-0.02 (-0.06)	-0.01 (-0.07)
nitronyl O	-0.66	-0.67 -0.82	-0.66 -0.76	-0.65 -0.77
[nitronyl]⁻ O	-0.81 (0.12)	(0.04)	(0.05)	(0.04)
[nitronyl]⁺ O	-0.14 (0.74)	-0.16 (0.73)	-0.15 (0.73)	-0.13 (0.74)
nitronyl C₂	0.07	0.12	0.10	0.10
[nitronyl]⁻ C₂	-0.33 (0.42)	-0.18 (0.62)	-0.02 (0.81)	-0.15 (0.42)
[nitronyl]⁺ C₂	0.39 (0.32)	0.42 (0.27)	0.41 (0.31)	0.41 (0.31)
nitronyl C₅	0.18	0.08	-0.18	0.07
[nitronyl]⁻ C₅	0.17 (-0.03)	0.07 (-0.00)	-0.14 (-0.01)	0.04 (0.03)
[nitronyl]⁺ C₅	0.18 (-0.00)	0.09 (-0.00)	-0.16 (-0.00)	0.08 (-0.00)
nitronyl X₆	-0.58	0.76	2.44	0.90
[nitronyl]⁻ X₆	-0.58 (0.02)	0.76 (0.02)	2.38 (0.00)	0.79 (0.19)
[nitronyl]⁺ X₆	-0.58 (0.00)	0.75 (0.00)	2.44 (0.00)	0.90 (0.00)

Table 3

Bond Lengths ($\text{\AA}$) of the Aminoxy Compounds and their Respective Oxidized and Reduced Forms at the B3LYP/6-31G(d) Level of Theory.

bond distances ($\text{\AA}$)	HO ₂ [•] adducts										miscellaneous			
	DMPO	AMPO <i>cis</i>	AMPO <i>trans</i>	DEPMPO <i>cis</i>	DEPMPO <i>trans</i>	EMPO <i>cis</i>	EMPO <i>trans</i>	TEMPO	PROXYL	DMPO -OH	DMPO -CH ₃			
[R ₂ N-O] [•] N-C ₂	1.47	1.47	1.47	1.46	1.47	1.48	1.47	1.50	1.49	1.47	1.48			
[R ₂ N-O] [•] N-C ₂	1.45	1.45	1.45	1.47	1.45	1.48	1.45	1.49	1.49	1.45	1.46			
[R ₂ N=O] [•] N-C ₂	1.54	1.55	1.55	1.52	1.59	1.57	1.55	1.54	1.52	1.60	1.51			
[R ₂ N-O] [•] C ₅ -N	1.50	1.49	1.48	1.48	1.48	1.47	1.48	1.50	1.49	1.49	1.49			
[R ₂ N-O] [•] C ₅ -N	1.48	1.49	1.48	1.48	1.50	1.49	1.51	1.49	1.51	1.48	1.49			
[R ₂ N=O] [•] C ₅ -N	1.52	1.45	1.47	1.48	1.45	1.46	1.48	1.54	1.52	1.51	1.51

bond distances (Å)	HO ₂ [•] adducts							miscellaneous			
	DMPO	AMPO <i>cis</i>	AMPO <i>trans</i>	DEPMPO <i>cis</i>	DEPMPO <i>trans</i>	EMPO <i>cis</i>	EMPO <i>trans</i>	TEMPO	PROXYL	DMPO -OH	DMPO-CH ₃
[R ₂ N-O] [•] N-O	1.28	1.29	1.28	1.28	1.28	1.27	1.28	1.29	1.28	1.28	1.28
[R ₂ N-O] [•] N-O	1.42	1.42	1.42	1.43	1.41	1.41	1.41	1.41	1.39	1.40	1.39
[R ₂ N=O] [•] N-O	1.19	1.20	1.20	1.20	1.19	1.19	1.19	1.20	1.19	1.19	1.19
[R ₂ N-O] [•] C ₂ -O _β	1.42	1.42	1.41	1.42	1.42	1.41	1.41	1.54 (C-C)	1.53 (C-C)	1.41	1.53 (C-C)
[R ₂ N-O] [•] C ₂ -O _β	1.44	1.44	1.42	1.43	1.44	1.40	1.44	1.54	1.53	1.41	1.53
[R ₂ N=O] [•] C ₂ -O _β	1.39	1.38	1.39	1.40	1.37	1.36	1.38	1.53	1.55	1.37	1.54
[R ₂ N-O] [•] O _β -O _γ	1.45	1.45	1.45	1.45	1.46	1.45	1.45	n/a	n/a	n/a	n/a
[R ₂ N-O] [•] O _β -O _γ	1.47	1.47	1.47	1.45	1.47	1.48	1.47	n/a	n/a	n/a	n/a

HO ₂ [•] adducts										miscellaneous			
bond distances (Å)		DMPO	AMPO <i>cis</i>	AMPO <i>trans</i>	DEPMPO <i>cis</i>	DEPMPO <i>trans</i>	EMPO <i>cis</i>	EMPO <i>trans</i>	TEMPO	PROXYL	DMPO -OH	DMPO- CH ₃	
[R ₂ N=O] ⁺ O _β -O _γ		1.45	1.46	1.45	1.43	1.47	1.46	1.45	n/a	n/a	n/a	n/a	
[R ₂ N-O] ⁺ O _γ -H		0.99	0.98	0.98	0.99	0.98	0.98	0.98	n/a	n/a	0.97	n/a	
[R ₂ N-O] ⁻ O _β -H		1.09	1.03	1.02	1.00	1.05	1.58	1.06	n/a	n/a	1.00	n/a	
[R ₂ N=O] ⁺ O _γ -H		0.98	0.98	0.98	0.99	0.98	0.98	0.98	n/a	n/a	0.97	n/a	
[R ₂ N-O] ⁺ C ₅ -X ₆		1.54	1.56	1.56	1.87	1.86	1.55	1.54	1.54	1.54	1.54	1.54	
[R ₂ N-O] ⁻ C ₅ -X ₆		1.54	1.56	1.56	1.90	1.84	1.55	1.53	1.54	1.53	1.55	1.55	
[R ₂ N=O] ⁺ C ₅ -X ₆		1.55	1.65	1.64	1.92	1.95	1.61	1.60	1.53	1.53	1.56	1.55	

Table 4

NPA Charges (e) on the Aminoxy Compounds and their Respective Oxidized and Reduced Forms at the PCM/BHandLYP/6-311G(d,p)//B3LYP/6-31G(d) Level of Theory.

HO ₂ [•] adducts										miscellaneous			
	DMPO	AMPO <i>cis</i>	AMPO <i>trans</i>	DEPMPO <i>cis</i>	DEPMPO <i>trans</i>	EMPO <i>cis</i>	EMPO <i>trans</i>	TEMPO	PROXYL	DMPO -OH	DMPO -CH ₃		
[R ₂ N-Q]• O	-0.473	-0.471	-0.469	-0.417	-0.427	-0.409	-0.414	-0.431	-0.497	-0.452	-0.494		
[R ₂ N-Q]• O	-0.838	-0.837	-0.842	-0.760	-0.827	-0.662	-0.828	-0.919	-0.914	-0.906	-0.921		
[R ₂ N=O] ⁺ O	-0.113	-0.139	-0.137	-0.119	-0.137	-0.104	-0.099	-0.145	-0.147	-0.121	-0.140		
[R ₂ N-Q]• N	-0.014	-0.009	-0.014	-0.043	-0.041	-0.044	-0.033	-0.052	-0.004	-0.035	0.007		
[R ₂ N-Q]• N	-0.294	-0.283	-0.287	-0.288	-0.303	-0.265	-0.274	-0.318	-0.294	-0.301	-0.284

	HO ₂ [•] adducts										miscellaneous			
	DMPO	AMPO <i>cis</i>	AMPO <i>trans</i>	DEPMPO <i>cis</i>	DEPMPO <i>trans</i>	EMPO <i>cis</i>	EMPO <i>trans</i>	TEMPO	PROXYL	DMPO -OH	DMPO -CH ₃			
[R ₂ N=O] ⁺ N	0.290	0.277	0.271	0.277	0.266	0.285	0.277	0.301	0.308	0.294	0.321			
[R ₂ N-O] [•] Oβ	-0.343	-0.338	-0.339	-0.354	-0.334	-0.331	-0.335	n/a	n/a	-0.777	n/a			
[R ₂ N-O] [•] Oβ	-0.408	-0.390	-0.370	-0.383	-0.401	-0.443	-0.403	n/a	n/a	-0.825	n/a			
[R ₂ N=O] ⁺ Oβ	-0.310	-0.315	-0.312	-0.310	-0.301	-0.299	-0.311	n/a	n/a	-0.728	n/a			
[R ₂ N-O] [•] Oγ	-0.471	-0.464	-0.465	-0.481	-0.486	-0.483	-0.485	n/a	n/a	n/a	n/a			
[R ₂ N-O] [•] Oγ	-0.574	-0.536	-0.527	-0.511	-0.549	-0.690	-0.556	n/a	n/a	n/a	n/a			
[R ₂ N=O] ⁺ Oγ	-0.450	-0.456	-0.450	-0.444	-0.473	-0.470	-0.449	n/a	n/a	n/a	n/a			
[R ₂ N-O] [•] C2	0.292	0.295	0.295	0.302	0.308	0.302	0.305	0.169	0.160	0.322	-0.018

HO ₂ [•] adducts										miscellaneous		
	DMPO	AMPO <i>cis</i>	AMPO <i>trans</i>	DEPMPO <i>cis</i>	DEPMPO <i>trans</i>	EMPO <i>cis</i>	EMPO <i>trans</i>	TEMPO	PROXYL	DMPO -OH	DMPO -CH ₃	
[R ₂ N-O] - C2	0.309	0.308	0.306	0.315	0.328	0.330	0.309	0.187	0.172	0.339	-0.001	
[R ₂ N=O] ⁺ C2	0.308	0.320	0.318	0.308	0.326	0.314	0.315	0.164	0.154	0.350	-0.035	
[R ₂ N-O] [•] C5	0.157	0.058	0.060	-0.193	-0.182	0.051	0.058	0.169	0.160	0.156	0.157	
[R ₂ N-O] - C5	0.175	0.068	0.074	-0.141	-0.168	0.068	0.061	0.187	0.172	0.172	0.178	
[R ₂ N=O] ⁺ C5	0.144	0.033	0.043	-0.197	-0.175	0.039	0.042	0.164	0.154	0.136	0.150	
[R ₂ N-O] [•] H	0.484	0.485	0.486	0.502	0.503	0.492	0.501	n/a	n/a	0.490	n/a	
[R ₂ N-O] - H	0.474	0.483	0.485	0.507	0.479	0.481	0.476	n/a	n/a	0.475	n/a	
[R ₂ N=O] ⁺ H	0.529	0.529	0.528	0.519	0.532	0.531	0.529	n/a	n/a	0.524	n/a	

Table 5

Reaction enthalpies ($\Delta H_{\text{rxn,aq}}$) and free energies ($\Delta G_{\text{rxn,aq}}$) for the reduction and oxidation of various aminoxyl compounds by superoxide radical anion to *N*-olate anions and oxoammonium cations, respectively, at the PCM/BHandL YP/6-311G(d,p)//B3LYP/6-311G(d,p) level of theory at 298 K.

aminoxyls	$\text{O}_2^{\cdot-} + [\text{nitroxide}]^{\cdot-} \rightarrow \text{O}_2 + [N - \text{olate}]^-$		$\text{O}_2^{\cdot-} + [\text{nitroxide}]^{\cdot-} \rightarrow \text{O}_2^{2-} + [\text{oxoammonium}]^+$		$\text{O}_2 + [\text{nitroxide}]^{\cdot-} \rightarrow \text{O}_2^{\cdot-} + [\text{oxoammonium}]^+$	
	$\Delta H_{\text{rxn,aq}}$	$\Delta G_{\text{rxn,aq}}$	$\Delta H_{\text{rxn,aq}}$	$\Delta G_{\text{rxn,aq}}$	$\Delta H_{\text{rxn,aq}}$	$\Delta G_{\text{rxn,aq}}$
DMPO-O ₂ H	-0.01	0.00	0.18	0.18	0.10	0.10
AMPO-O ₂ H <i>cis</i>	-0.02	-0.01	0.20	0.19	0.11	0.10
AMPO-O ₂ H <i>trans</i>	-0.01	-0.01	0.19	0.20	0.11	0.11
EMPO-O ₂ H <i>cis</i>	-0.01	0.00	0.19	0.19	0.11	0.10
EMPO-O ₂ H <i>trans</i>	0.00	0.00	0.20	0.20	0.11	0.11
DEPMPO-O ₂ H <i>cis</i>	0.00	0.01	0.20	0.20	0.11	0.11
DEPMPO-O ₂ H <i>trans</i>	0.00	0.00	0.20	0.20	0.11	0.11
TEMPO	0.03	0.03	0.18	0.18	0.09	0.09
PROXYL	0.04	0.04	0.18	0.18	0.09	0.09
DMPO-OH	0.02	0.02	0.18	0.18	0.09	0.09
DMPO-CH ₃	0.03	0.03	0.18	0.18	0.09	0.09