JOC The Journal of Organic Chemistry

Article

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Effect of Sterical Shielding on the Redox Properties of Imidazoline and Imidazolidine Nitroxides

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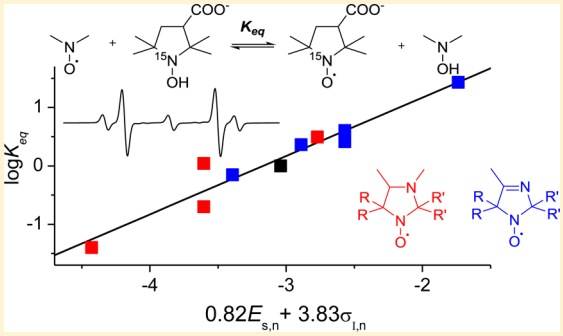
Supporting Information

ABSTRACT: The oxidant properties of the series of 2,2,5,5-tetraalkyl imidazoline and imidazolidine nitroxides were investigated. An increase in the number of bulky alkyl substituents leads to a decrease in the rate of reduction with ascorbate, which makes the electrochemical reduction potential more negative and shifts the equilibrium in the mixture of nitroxide and reference hydroxylamine (3-carboxy-1-hydroxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl-1-^{15}N) toward the starting compounds. The effect of structural factors on these reactions was analyzed by means of multiple regression using the Fujita steric constant E_s and the inductive Hammett constant σ_I. Satisfactory statistical outputs were obtained in all of the biparameter correlations, denoting that the oxidant properties of the nitroxides are determined by steric and electronic effects of the substituents. The data imply that bulky substituents can stabilize nitroxide and/or destabilize hydroxylamine.



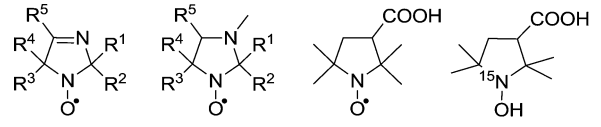
INTRODUCTION

The terms "sterically shielded" and "sterically hindered" are commonly applied to nitroxides with several substituents larger than methyl at α-carbons of the nitroxide group. These radicals are currently attracting growing interest because bulky alkyl substituents or spirocyclic moieties have been shown to increase nitroxide stabilty to reduction, which is of great importance for a variety of biological applications.[1−3] The versatility of this effect has been clearly demonstrated for various types of nitroxides, including those of isoindoline,[4] imidazoline, imidazolidine,[5] piperidine,[6] pyrroline, and pyrrolidine[7,8] series. The 2,2,5,5-tetraethyl-substituted pyrrolidine nitroxides[9] demonstrate the highest stability inside cells, exceeding that of trityl radicals.[10]

The physicochemical studies of sterical shielding effect were mainly focused on the kinetics of nitroxide decay.[4,5,9,11] Recently, attempts have been made to study the effect of bulky substituents on the thermodynamic parameters of nitroxide reduction.[10,12] The obtained data on chemical equilibrium constants in the reaction of nitroxides with ascorbate imply that the effect of bulky substituents is not limited to "sterical shielding" or hindering the access of a reductant to the nitroxide group.[10] However, the equilibrium constants in these studies were obtained indirectly from electrochemical potentials or from deviations in the kinetics of nitroxide reduction from the second-order law, and the accuracy of these measurements is questionable. Moreover, none of these data provide clear separation of electronic and steric effects.

In this study, we performed direct measurements of equilibrium parameters in the mixture of the reference isotopically labeled (^{15}N) hydroxylamine (**CPH-^{15}N**, see Chart 1) and the nitroxides of the imidazoline (**Im1−5**) and imidazolidine (**In1−11**) series with varied numbers of bulky alkyl substituents adjacent to the N−O group. The kinetics of the nitroxide reduction with ascorbate and electrochemical reduction was also studied. The data obtained were subjected

Chart 1. Structures of the Compounds Used in This Study[a]



Im1-5 **In1-11** **PCA** **CPH-^{15}N**

[a]The substituents are listed in the Table 1.

Received: July 1, 2015
Published: August 24, 2015

DOI: 10.1021/acs.joc.5b01494
J. Org. Chem. 2015, 80, 9118−9125

Table 1. Parameters of the Kinetics of Nitroxide Reduction with Ascorbate, Corresponding Values of Stabilization/Polar ($\sigma_{I,n}$) and Steric ($E_{s,n}$) Molecular Descriptors, Equilibrium Constants in the Mixtures of the Nitroxides, and the Reference Hydroxylamine CPH-^{15}N, and ΔG Values for This Reaction

	nitroxide					kinetics of the reduction with ascorbate						
no.	R^1	R^2	R^3	R^4	R^5	k_1, M^{-1} s^{-1}	k_{-1}, M^{-1} s^{-1}[14]	pH	$\sigma_{I,n}$	$E_{s,n}$	equilibrium with **CPH-^{15}N**, K_{eq}	ΔG, kJ/mol
In1	Me	Me	Me	Me	Me	0.85 ± 0.05[5]		7.5	0.18		3.1 ± 0.5	−2.8
						0.85 ± 0.05[14]	(5 ± 1) × 10^3	7.6		−4.20		
In2	Et	Et	Me	Me	Me	0.09 ± 0.01		7.5	0.18	−5.21	1.1 ± 0.2	−0.24
In3	Me	Me	Et	Et	Me	0.08 ± 0.01		7.5	0.18	−5.21	0.20 ± 0.4	4.0
In4	Et	Et	Et	Et	Me	0.020 ± 0.005[14]	(2.3 ± 0.5) × 10^3	7.6	0.18	−6.21	0.04 ± 0.01[d]	8.0
In5	$(CH_2)_5$	$(CH_2)_5$	Et	Et	Me	0.08 ± 0.01		7.5	0.18	−5.60[a]	nd	
In6	$(CH_2)_5$	$(CH_2)_5$	Me	Me	Me	0.50 ± 0.05		7.5	0.18	−4.60[a]	nd	
In7	$(CH_2)_4$	$(CH_2)_4$	Me	Me	Me	0.85 ± 0.05		7.5	0.18	−4.31	nd	
In8	*n*-Bu	*n*-Bu	Me	Me	Me	0.08 ± 0.01		7.5	0.18	−6.05[a]	nd	
In9	Me	Me	Me	$(CH_2)_4$	$(CH_2)_4$	0.50 ± 0.05		7.5	0.18	−4.20	nd	
In10	$(CH_2)_5$	$(CH_2)_5$	Me	$(CH_2)_4$	$(CH_2)_4$	0.50 ± 0.05		7.5	0.18	−4.60	nd	
In11	$(CH_2)_2COO^-$	$(CH_2)_2COO^-$	Et	Et	Me	0.040 ± 0.005		7.5	0.14[b]	−5.97[c]	nd	
Im1	Me	Me	Me	Me	Me	5.6 ± 0.3		7.5	0.45	−4.20	27 ± 5	−8.2
Im2	Et	Et	Me	Me	Me	nd			0.45	−5.21	4 ± 1	−3.4
Im3	Me	Me	Et	Et	Me	nd			0.45	−5.21	2.6 ± 0.5	−2.4
Im4	Et	Et	Et	Et	Me	0.5 ± 0.3[5]		7.5	0.45	−6.21[a]	0.7 ± 0.2	0.88
						0.5 ± 0.05[14]	(1.1 ± 0.2) × 10^5	7.6				
Im5	$(CH_2)_5$	$(CH_2)_5$	Et	Et	Me	0.5 ± 0.05[14]	(1.9 ± 0.3) × 10^5	7.6	0.45	−5.60[a]	2.3 ± 0.6	2.0
PCA	Me	Me	Me	Me	CO_2H	0.1 ± 0.01[14]	(1.1 ± 0.2) × 10^3	7.6	0.11	−4.20	1.00 ± 0.05	0

[a]Recalculated from the data listed in Table 2 of ref 16. [b]Assuming $\sigma_{I,(CH_2CH_2COO^-)} = -0.03$ with $\sigma_{I,COO^-} = -0.19$. [c]Assuming $r_{(CH_2CH_2COO^-)} = r_{(n\text{-Pr})} = 0.67$. [d]The measurements were made for partially deuterated compound $R^1 = R^2 = CD_3CD_2$ (2/3) or CH_3CD_2 (1/3) and $R^3 = R^4 = CH_3CD_2$. The imidazolidine nitroxides are bases capable of protonation onto the nitrogen atom at position 3 of the heterocycle. The contribution of the protonated form was neglected because the p*K* was reported to be below 5.[5]

DOI: 10.1021/acs.joc.5b01494
J. Org. Chem. 2015, 80, 9118−9125

to multiparameter analysis using the Fujita steric constant E_s and the inductive Hammett constant σ_I of the substituents, clearly showing that all of the redox characteristics described above linearly depend on steric and inductive effects. The data support previously made statements that stabilization of nitroxide and/or destabilization of hydroxylamine by bulky substituents rather than steric shielding of the nitroxide group determine the oxidative properties of the nitroxides.

RESULTS AND DISCUSSION

Reduction with Ascorbate. The reaction of nitroxides with ascorbate have been thoroughly studied.[13,14] In most cases, it follows the second-order kinetics law with the only product of nitroxide reduction being hydroxylamine. When the rate constant of this reaction is low, the reversibility of the first steps may contribute to the kinetics, making the hydrolysis of dehydroascorbate a rate-limiting step. In these cases, the rate constants of the direct and reverse reactions were determined using a complete reaction scheme, or glutathione was added to suppress the reverse reaction.[14] It should be noted that the rate constants measured in the presence of glutathione coincide, within measurement accuracy, with those obtained using simulations of experimental kinetics taking into account the reversibility of some steps.[14] The obtained reduction rate constants are listed in Table 1.

The data on the reduction with ascorbate confirm the earlier statements[4−6] that an increase in the number of substituents larger than methyl at the α-carbons of the nitroxide group makes the reduction slower. Comparison of the reduction kinetics for nitroxides of the 2,5-dihydroimidazol and perhydroimidazol series shows that replacement of four methyl groups with ethyls is sufficient to compensate for the electronic effect of the C═N fragment in the heterocycle. Surprisingly, in 2,5-dihydroimidazol and the perhydroimidazol series, the difference between the spiro-cyclohexane ring and geminal ethyl groups is not so large compared to piperidine and pyrrolidine nitroxides.[6,9] Moreover, the lengths of *n*-alkyl substituents seem to play a minor role.

It has been shown that the rate constants of nitroxide reduction with ascorbate correlate with the inductive constants of the substituents,[15] but attempts to quantify the influence of steric effects upon redox properties were not successful.[6,9] Recently, a pattern of multiparameter correlation analysis of the rate constants of carbon-centered radical trapping for a large number of nitroxides of different families using inductive Hammett constant σ_I and modified Taft steric constant E_s was published.[16,17] The authors used the correlations to unveil different effects involved in nitroxide reactivity. In this work, we used the same approach; for details of the calculations, see the Supporting Information. The estimated parameters $\sigma_{I,n}$ and $E_{s,n}$ are listed in Table 1; the plot is shown in Figure 1, and the multiple regression data are shown in eq 1. For the nitroxides **In4** and **Im4**, the data from the more recent publication[14] were used in the correlation.

$$\log(k_1) = 1.58(\pm0.42) + 3.83(\pm0.56) \times \sigma_{I,n} + 0.60(\pm0.08) \times E_{s,n} \quad (1)$$

$$R^2 = 0.88\ s = 0.24\ N = 14\ F_{99.99\%} = 43\ t_{E_s} = t_{\sigma_I} = 99.99\%$$

The positive slope for σ_I denotes an increase in the reduction rate with an electron-withdrawing effect of the substituents, whereas the positive slope for E_s denotes that sterical shielding of the N−O group makes the reduction slower.

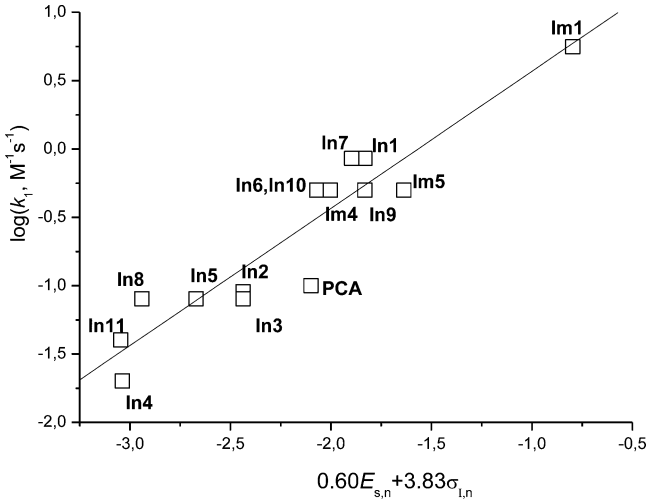


Figure 1. Correlation of $\log(k_1, M^{-1}\cdot s^{-1})$ vs $f(E_s,\sigma_I)$.

Because both rate constants of the reduction of nitroxides and the rates of their reactions with carbon-centered radicals are satisfactorily described using the same molecular descriptors, we tried to plot the rate constant of 1-(*tert*-butoxycarbonyl)-1-methylethyl radical trapping with nitroxide, k_c (see ref 16) against the reduction rate constant k_1 (Table 1) for imidazolidine nitroxides (Figure 2). The selected nitroxides

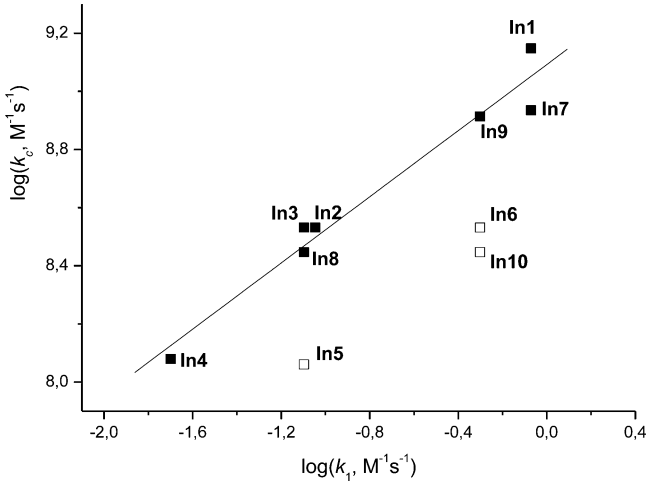
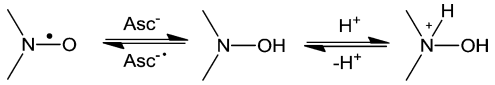


Figure 2. Correlation of $\log(k_c)$[16] for 1-(*tert*-butoxycarbonyl)-1-methylethyl radical (Table 1) vs $\log(k_1)$; the empty squares denote the data not counted in the correlation.

do not differ significantly in electronic effects of the substituents. The data showed poor correlation when all of the points were taken into consideration (R = 0.80, Figure S1); however, the correlation was much better (R = 0.98) when the data for nitroxides **In5**, **In6**, and **In10**, containing a 2-spiro-cyclohexane moiety, were omitted. This effect may result from a difference in cyclohexane ring conformation occupancy, which is likely to be dependent on the solvation. Note that k_c and k_1 were measured in different solvents. We have recently shown that conformation of the cyclohexane ring at the carbon atom adjacent to the N−O group may significantly affect the nitroxide reduction rate.[7]

DOI: 10.1021/acs.joc.5b01494
J. Org. Chem. 2015, 80, 9118−9125

Scheme 1. Redox Equilibrium of the Nitroxide/Hydroxylamine Couple in the Presence of Ascorbate and Protonation Equilibrium for the Hydroxylamine Group

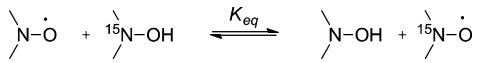


$$pK = -\log\left(\frac{[RR'NOH]\times[H^+]}{[RR'N^+HOH]}\right)$$

It has been mentioned above that the reversibility of the initial steps of the reaction of nitroxides with ascorbate can contribute to the reduction kinetics. Analysis of the calculated rate constants of the reverse reaction k_{-1}[14] (Table 1) shows that their dependence on steric demand of bulky substituents is more complex than that of k_1. For instance, the substitution of four methyl groups in imidazolidine nitroxide **In1** to ethyl groups (**In4**) leads to a decrease in the value of k_1 by a factor of 40 and only a 2-fold decrease of k_{-1}. The rate constants k_{-1} of the imidazoline nitroxides **Im4** and **Im5** are surprisingly high relative to those for imidazolidine and pirrolidine nitroxides (**In1**, **In4**, and **PCA**, see Table 1). This observation may result from a difference in the corresponding hydroxylamine basicity. Because of the electron withdrawing effect of the C═N group, 1-hydroxy-imidazolines are much weaker bases than imidazolidines or pyrrolidines. Indeed, the pK value of the hydroxylammonium group of **CPH** is 7.7[18] wheras the pK reported for 1-hydroxy-2,2,4,5,5-pentamethyl-2,5-dihydroimidazole (**Im1H**) is 3.8.[19] Note that this value is likely to correspond to protonation of the N-3 nitrogen; therefore, the hydroxylamino group may be even less basic. Potentiometric titration of imidazolidines is complicated because of their susceptibility to hydrolysis; the pK can be roughly estimated as 7, taking into account that the pK of imidazolidine nitroxides (4.5−4.9) is decreased by ∼2.5 units due to the electronic effect of the nitroxide group.[19] Obviously, protonation of the heterocycle onto either the hydroxylamine nitrogen atom (Scheme 1) or another nitrogen atom in the ring should lead to stabilization of the hydroxylamine, decreasing the rate of reoxidation to nitroxide. Similarly, in biological systems, both reduction of nitroxides and reoxidation of the hydroxylamines contribute to concentration of the spin probe; therefore, the spin probe lifetime in the biological environment depends on the pK of the corresponding hydroxylamine.

Hydrogen Atom Transfer between Nitroxide and Hydroxylamine. The reaction of a nitroxide and a reference hydroxylamine-^{15}N leads to an equilibrium due to reversible hydrogen atom transfer between N−O· and ^{15}N−OH groups (Scheme 2). The EPR spectra of ^{15}N-labeled nitroxide consists

Scheme 2. Redox Equilibrium between Nitroxide and ^{15}N-Labeled Hydroxylamine



of two lines with splitting ∼1.4× larger than that for the triplet of ^{14}N-nitroxides. The ratio of ^{14}N and ^{15}N nitroxides can be easily followed by EPR because triplet and doublet spectra of these nitroxides do not overlap. The equilibrium constants, K_{eq}, are related by the Gibbs free energy of the reaction: $\Delta G^\circ = -RT\ln K_{eq}$. The difference between ΔG for each pair of nitroxides (or the ratio of K_{eq}) is independent of the reference hydroxylamine and characterizes relative oxidant capacity of the nitroxides. The equilibrium constants with different reference hydroxylamines were measured for a broad series of nitroxides and used to reveal the structural factors affecting the properties of the nitroxides[20,21] or hydroxylamines.[22]

We chose **CPH-^{15}N** as a reference hydroxylamine because the corresponding nitroxide **PCA** has a medium rate of reduction with ascorbate in the series of nitroxides studied (see Table 1). This allowed us to avoid large differences between ^{14}N and ^{15}N nitroxide final concentrations and to thereby minimize experimental error of the measurements. Because of significant line broadening of the EPR spectra of sterically hindered imidazolidine nitroxide **In4**,[23] partially deuterated nitroxide **In4D** was used in the measurements to prevent the spectral lines from overlapping. The K_{eq} values (Scheme 2) can be influenced by acid−base equilibriums, e.g., due to differences in the pK of the hydroxylamines. To exclude this effect, we performed the measurements in a 15 mM solution of NaOH in methanol. Under these conditions, the reaction should follow Scheme 2 with no significant interference of other processes. The solutions were mixed under anaerobic conditions, and the EPR spectra were recorded. The spectra showed growth of the doublet of **PCA-^{15}N** (Figure 3). After a 24 h incubation at 25

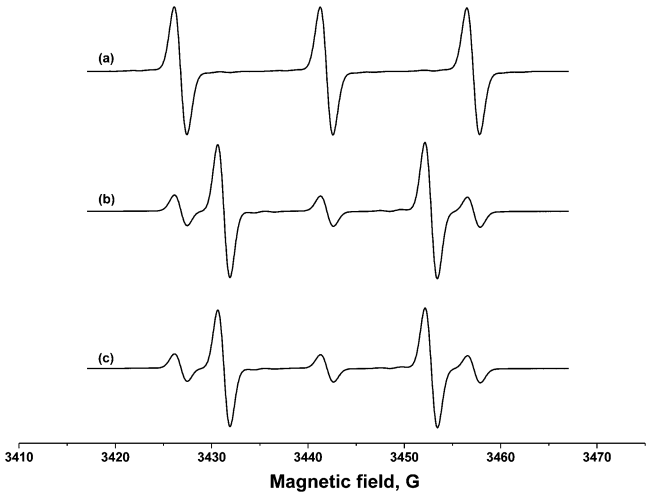


Figure 3. EPR spectra of the reaction mixture containing nitroxide **In1** (0.25 mM), **CPH-^{15}N** (0.25 mM), NaOH (0.15 mM), and DTPA (0.1 mM) in methanol under anaerobic conditions recorded immediately after mixing (a), after 24 h (b), and after 72 h (c) of incubation at 25 °C.

°C, double integral intensities of the doublet and triplet in the EPR spectra showed no more changes, indicating that this amount of time was sufficient to reach the equilibrium state.

The K_{eq} and ΔG values calculated from the final double integral intensities of the doublet (I_d) and triplet (I_t), using the eq 2, are listed in Table 1. The higher values of K_{eq} correspond to stronger oxidants. The equilibrium in the mixture of **PCA** and **CPH-^{15}N** was studied to estimate the accuracy of measurements, yielding a K_{eq} value of 1.00 ± 0.05.

DOI: 10.1021/acs.joc.5b01494
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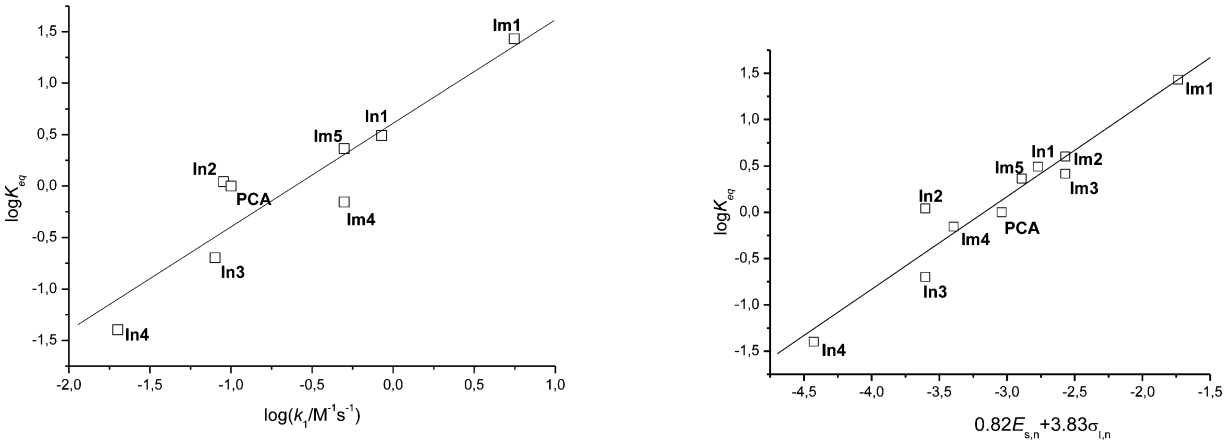


Figure 4. Correlation of $\log(K_{eq})$ vs $\log(k_1)$ and multiple regression plot of $\log(K_{eq})$ vs $f(E_s,\sigma_I)$ (Table 1).

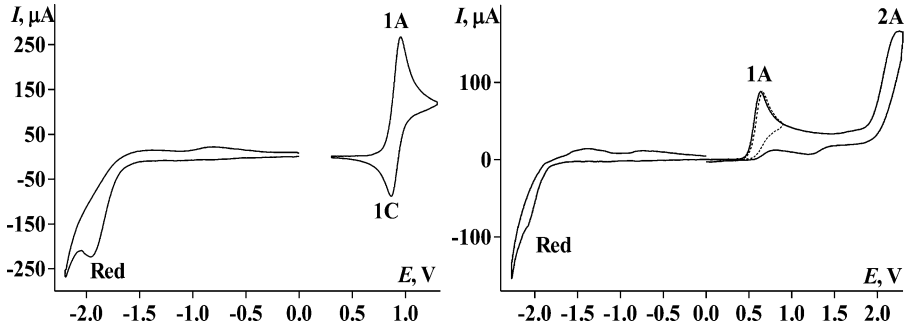


Figure 5. Cyclic voltammograms of 5.2 mM **Im4** (left) and 2.1 mM **In3** (right) in MeCN at ν = 0.1 V/s.

$$K_{eq} = \frac{[RR'N\text{-}OH][PCA\text{-}^{15}N]}{[RR'N\text{-}O\bullet][CPH\text{-}^{15}N]} = \left(\frac{I_d}{I_t}\right)^2 \quad (2)$$

The effect of bulky alkyl substituents on equilibrium in the mixtures of nitroxides and **CPH-^{15}N** is similar to that on the kinetics of reduction with ascorbate (Table 1). Multiple regression data are shown in Figure 4 (right) and eq 3. It is remarkable that the coefficients δ' and ρ_I' at the parameters E_s and σ_I in eqs 1 and 3 are close. This means that both k_1 and K_{eq} are determined by the same structural factors (steric and electronic effects of the substituents) in similar proportions. Indeed, the equilibrium constants correlate with the rates of reduction with ascorbate (Figure 4, left) $R^2 = 0.92$ ($R < 0.96$).

$$\log(K_{eq}) = 3.17(\pm 0.54) + 3.83(\pm 0.54) \times \sigma_{I,n} + 0.82(\pm 0.11) \times E_{s,n} \quad (3)$$

$$R^2 = 0.93\ s = 0.24\ N = 10\ F_{99.99\%} = 44$$

Electrochemical Oxidation and Reduction of Imidazoline and Imidazolidine Nitroxides. The electrochemical behavior of the imidazoline and imidazolidine nitroxides was studied in acetonitrile. Electrochemical oxidation usually gives reproducible data, which demonstrates a minor dependence on solvent polarity and anode material.[20] The oxidation of imidazoline nitroxides **Im1**, **Im2**, **Im3**, and **Im4** is characterized by one reversible and diffusion-controlled peak (the ratio of peak currents $I_p^{1A}/I_p^{1C} \approx 1$ and $\Delta E = E_p^{1A} - E_p^{1C} = 0.06$ V and $I_p^{1A} \times \nu^{-1/2}$ = const, see the left side of Figure 5 for an example) with the values of potentials shown in Table 2. The nitroxides **In1**, **In2**, **In3**, and **In4** showed two irreversible diffusion-controlled peaks at 0.59−0.66 V and at 2.17−2.24 V. The I_p value for the second peak was approximately two times higher then for the first. This second peak is likely to correspond to an ECE (electron transfer, chemical reaction, electron transfer) process (two reaction stages of one-electron transfer separated by chemical reaction of deprotonation). Previously, irreversibility of the electrochemical oxidation was observed for 2,2,3,5,5-pentamethyl-4-phenylimidazolidine-1-oxyl.[24] It was also shown that chemical oxidation of **In1** and **In4** can lead to irreversible destruction.[25] The irreversibility of the oxidation of imidazolidines may result from instability of the corresponding oxoammonium cation. However, oxidation potentials of **In1**−**4** are close to those of some cyclic akylamines and alkyldiamines (0.6−0.75 V).[26] Moreover, it was demonstrated

Table 2. Cyclic Voltammetry Data for the Nitroxides Im1−4 and In1−4 within the potential range from −2.3 to +2.50 V at ν = 0.1 V/s

	oxidation			reduction
	E_p^{1A}, V	E_p^{1C}, V	E_p^{2A}, V	$-E_p^{red}$, V
Im1	1.01[a]	0.90		1.88
Im2	0.96	0.88	2.60	1.97
Im3	0.99	0.90		1.98
Im4	0.95	0.87		1.96
In1	0.59[a]		2.20	2.03
In2	0.62		2.24	2.08[b]
In3	0.66		2.24	2.10[b]
In4	0.58[a]		2.17	2.16

[a]Data previously reported for **Im1**, **In1**, and **In4** were 0.95, 0.64 and 0.60.[21] [b]Values measured after the background current subtraction.

DOI: 10.1021/acs.joc.5b01494
J. Org. Chem. 2015, 80, 9118−9125

using EPR that electrochemical oxidation of some cyclic alkyldiamines may lead to corresponding radical cation formation.[27] Therefore, the nature of the first oxidation peak for imidazolidine nitroxides **In1−4** remains unclear because it might correspond to the formation of an unstable diradical cation.

The outcome of electrochemical reduction of nitroxides depends on numerous factors, including pH, the electrode material, and the solvent.[20] During cathode reduction, the nitroxides are converted to corresponding anions. The later can then undergo protonation with the formation of corresponding hydroxylamines or hydroxylammonium cations.[28] A single diffusion-controlled irreversible reduction peak ($I_p^{1Red} \times \nu^{-1/2}$ = const up to ν = 10 V/s for **Im2**, **Im4**, **In1**, **In3**, and **In4** and up to ν = 5 V/s for **Im1** and **Im3**) was observed for all compounds (except for **In2**, whose reduction peak was not detectable on cyclic voltammograms and manifested only after subtraction of the background current) in the region of negative potentials from −1.88 to −2.16 V (for the electrochemical data, see Table 2).

Similarly to k_1 and K_{eq}, the electrochemical reduction potentials E_p^{Red} are sensitive to steric and electronic effects of the substituents. The values of the reduction potentials of **Im1−4** are more positive by 0.1−0.2 V as compared to **In1−4** due to the inductive effect of the C═N fragment of the imidazoline cycle. Replacement of each pair of geminal methyl groups in imidazolidines **In1−4** to ethyls shifts the value of the reduction potential to the negative region by 0.05−0.07 V. The difference between reduction potentials of 2,2,5,5-tetramethyl-3-imidazoline **Im1** and ethyl-substituted imidazolines **Im2−4** is smaller and does not exceed 0.1 V. It is remarkable that the potentials E_p^{Red} are less dependent on sterical factors compared to k_1 and K_{eq} and that the areas of reduction potentials of imidazoline and imidazolidine nitroxides do not overlap.

The effects of substituents upon the reduction potential were studied using σ_I and E_s constants. The multiple regression data are shown in Figure 6 and eq 4.

$$E_p^{red} = -1.917(\pm 0.077) + 0.537(\pm 0.073) \times \sigma_{I,n} + 0.0523(\pm 0.0139) \times E_{s,n} \quad (4)$$

$$R^2 = 0.90\ s = 0.28\ N = 7\ F_{99.99\%} = 34$$

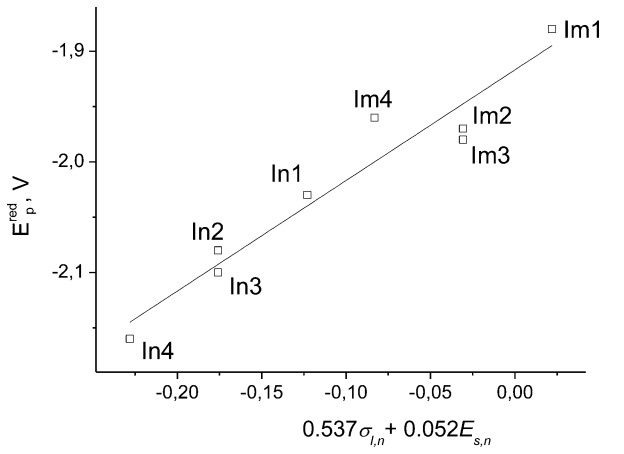


Figure 6. Multiple regression plot of E_p^{red} (Table 2) vs $f(E_s,\sigma_I)$ (Table 1).

In this work, we performed multiparameter analysis of different sets of experimental data, characterizing redox properties of nitroxides in different reactions and under different conditions. However, it is remarkable that all these data, namely, the logarithm of the rate constants of nitroxide reduction with ascorbate ($\log(k_1)$), the logarithm of the equilibrium constant in the mixture of nitroxides with the reference hydroxylamine ($\log(K_{eq})$), and the electrochemical reduction potential (E_p^{ted}), demonstrate satisfactory correlation with the same parameters that characterize electronic and steric effects of the substituents. According to eqs 1, 2, and 4, the weights of the steric effect in total variation of $\log(k_1)$, $\log(K_{eq})$, and E_p^{red} (determined as $\delta\Delta E_{s,n}/(\delta\Delta E_{s,n} + \rho_I\Delta\sigma_I)$ within the select) are 48, 55, and 36%, respectively.

It has been supposed that the effect of bulky substituents on the kinetics of nitroxide reduction results from sterical shielding of the nitroxide group.[4,5,9] Bulky substituents should make the nitroxide group less accessible to reductants, increasing the activation energy. However, attempts to find correlation between solvent accessible surface areas of the N−O group and nitroxide reduction rates were not successful.[6,9] Unlike the kinetic data, the thermodynamic parameters of equilibrium are dependent neither on accessibility of reaction centers nor on the reaction pathway and mechanism. They only reflect the relative Gibbs free energy difference (ΔG) between corresponding nitroxides and hydroxylamines. Thus, bulky substituents can stabilize nitroxide and/or destabilize hydroxylamine. This statement is in agreement with a previously made conclusion that the electronic factors (singly occupied molecular orbital-lowest unoccupied molecular orbital energy gap) largely determine the radicals' stability.[6] Hypothetically, this effect may result from a difference in the geometry of nitroxide and hyroxylamine due to different hybridization of nitrogen: steric interactions of tetrahedral hydroxylamine group with bulky substituents in neighboring positions should be stronger than those of the planar nitroxide group. Meanwhile, the difference in redox parameters of 5-membered and 6-membered cyclic nitroxides was attributed to similar reasoning.[29]

The statement above on the thermodynamic stabilization of nitroxide due to bulky substituents implies that similar effects should be observed for various reductants. However, this should be carefully applied to biological systems, where cell permeability, localization, or accumulation in certain cellular compartments and accessibility to enzymatic reaction centers may play a crucial role.

■ CONCLUSIONS

The investigation of oxidant properties of the series of imidazoline and imidazolidine nitroxides showed that an increase in the size and number of alkyl substituents (larger than methyl) at positions 2 and 5 leads to a decrease in the rate of reduction with ascorbate, which makes the electrochemical reduction potential more negative and shifts the equilibrium in the mixture of nitroxide and reference hydroxylamine (CPH-^{15}N) to the formation of the starting compounds. The multiparameter analysis using Fujita steric constant E_s and inductive Hammett constant σ_I clearly showed that variation of all of the above-mentioned characteristics of oxidant activity of nitroxides are fully determined by steric and inductive effects of substituents. Strong dependence of the equilibrium constant K_{eq} on the steric effect of the substituents implies stabilization

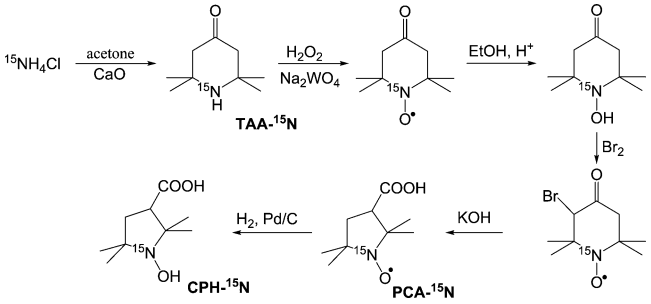
DOI: 10.1021/acs.joc.5b01494
J. Org. Chem. 2015, 80, 9118−9125

of nitroxide and/or destabilization of hydroxylamine by bulky substituents.

EXPERIMENTAL SECTION

Synthesis. Nitroxides **In1**,[30] **In2**, **In3**, **In5**, **In7**−**In10**, **Im2**, **Im3**,[31] **In4**,[5] **In11**,[32] **Im1**,[33] **Im5**,[14] **PCA**,[34] **In4D**, and **Im4D**[23] were prepared according to the procedures described in the corresponding literature; **CPH-^{15}N** hydrochloride was prepared via hydrogenation of **PCA-^{15}N** using the procedure developed for the ^{14}N-containing compound[32] (see below); **PCA-^{15}N** was prepared from triacetonamine-^{15}N (**TAA-^{15}N**) according to a literature procedure;[35] **TAA-^{15}N** was prepared from $^{15}NH_4Cl$ (99.9% isotope enrichment) according to the method by Pirrwitz and Schwarz[36] with minor modifications (Scheme 3).[37]

Scheme 3. Synthetic Scheme of CPH-^{15}N Preparation



*1-Hydroxy-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid hydrochloride (**CPH**).* This compound was incorrectly described previously;[34] for the characteristics of the zwitterion, see ref 35. The samples of **CPH** were prepared using two different methods, both giving equal yields, and the samples have identical spectral characteristics.

Method A (analogous to that in ref 32). A solution of PCA (1.87 g, 10 mmol) in MeOH (50 mL) was placed in a 200 mL Erlenmeyer flask equipped with an adapter for the gas supply with a valve at the outlet, and then the wet catalyst (Pd/C, 1%, 200 mg) was added. A magnetic stirring bar was placed in the flask. Then, the flask was purged with nitrogen followed by hydrogen and connected to a gasometer. The reaction mixture was vigorously stirred at ambient temperature until gas absorption stopped (usually the amount of the gas slightly exceeded the calculated value of 112 mL). The flask was opened, and the reaction mixture was acidified with HCl to pH 2−3. The catalyst was filtered off; the solvent was distilled off under reduced pressure, and the residue was crystallized from concentrated hydrochloric acid to give **CPH** (1.8 g, 80%): mp 190−192 °C dec (from aq concentrated HCl); IR (KBr) ν_{max} (cm^{-1}) 1724, 1500, 1466, 1417, 1406, 1391, 1379, 1309, 1292, 1274, 1242, 1202, 1180, 1142, 1049, 986, 853, 764, 721, 650, 601; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 1.24 (br s, 3H), 1.38 (br s, 3H), 1.41 (br s, 3H), 1.53 (br s, 3H), 2.08 (dd, J_1 = 7.7 Hz, J_2 = 13.4 Hz, 1H), 2.19 (br m, 1H), 3.13 (br m, 1H), 11.6 (br s, 1H); ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CF_3COOH, a mixture of two isomeric cations, slow exchange in NMR time scale) major isomer (60%) 1.42 (s, 3H), 1.53 (s, 3H), 1.60 (s, 3H), 1.72 (s, 3H), 2.30 (dd, J_1 = 7.7 Hz, J_2 = 13.7 Hz, 1H), 2.54 (dd, J_1 =12.1 Hz, J_2 = 13.7 Hz, 1H), 3.41 (dd, J_1 = 7.7 Hz, J_2 = 12.1 Hz, 1H), 10.49 (s, 1H), minor isomer (40%) 1.49 (s, 3H), 1.59 (s, 3H), 1.63 (s, 3H), 1.64 (s, 3H), 2.38 and 2.42 (ABd, J_{AB} = 14.5 Hz, J_d = 8.5 Hz, 2H), 3.30 (t, J = 8.5 Hz, 1H), 10.41 (s, 1H); ^{13}C NMR (75 MHz; $CDCl_3$ + 10% CF_3COOH) major isomer 16.0 (CH_3), 23.6 (CH_3), 25.0 (CH_3), 27.1 (CH_3), 37.4 (CH_2), 47.3 (CH), 72.2 (C), 74.4 (C), 174.3 (C), minor isomer 21.9 (CH_3), 22.2 (CH_3), 23.8 (CH_3), 25.3 (CH_3), 36.5 (CH_2), 49.7 (CH), 72.4 (C), 74.7(C), 175.5 (C); Anal. Calcd for $C_9H_{18}NClO_3$ (%) C 48.32, H 8.11, N 6.26, Cl 15.85; found (%) C 48.17, H 8.12, N 6.26, Cl 15.78.

CPH-^{15}N: mp 189−192 °C dec (from aq concentrated HCl); IR (KBr) ν_{max} (cm^{-1}) 1724, 1495, 1466, 1408, 1391, 1381, 1292, 1202, 1180, 1142, 853, 754, 716, 650, 605; ^{1}H NMR (500 MHz, $CDCl_3$ + 15% CF_3COOH, a mixture of two isomeric cations, slow exchange in NMR time scale) major isomer (60%) 1.38 (d, J = 1.6 Hz, 3H), 1.50 (d, J = 1.6 Hz, 3H), 1.54 (m, 3H), 1.67 (d, J = 2.3 Hz, 3H), 2.27 (ddd, J_1 = 7.7 Hz, J_2 = 13.7 Hz, J_3 = 2.6 Hz, 1H), 2.50 (dd, J_1 = 12.1 Hz, J_2 = 13.7 Hz, 1H), 3.38 (dd, J_1 = 7.7 Hz, J_2 = 12.1 Hz, 1H), 10.64 (d, 0.5H (one-half of the multiplet)), minor isomer (40%) 1.45 (d, J = 1.3 Hz, 3H), 1.55 (m, 3H), 1.60 (m, 6H), 2.35 (ddd, J_1 = 14.4 Hz, J_2 = 8.8 Hz, 1H) 2.39 (dd, J_1 = 14.4 Hz, J_2 = 7.3 Hz, J_3 = 4.7 Hz, 1H) 3.27 (br t, J = 8.2 Hz, 1H), 10.46 (d, J = 80.5 Hz, 1H); ^{13}C NMR (125.77 MHz; $CDCl_3$ + 10% CF_3COOH) major isomer 16.1 (CH_3), 23.7 (CH_3), 25.0 (CH_3), 27.1 (CH_3), 37.4 (d, J =1.9 Hz, CH_2), 47.3 (d, J = 4.7, CH), 72.2 (d, J = 1.6 Hz, C), 74.4 (d, J = 3.2 Hz, C), 174.3 (br, C), minor isomer 21.9 (CH_3), 22.3 (CH_3), 23.9 (CH_3), 25.3 (CH_3), 36.6 (d, J = 3.1 Hz, CH_2), 49.8 (d, J = 3.1 Hz, CH), 72.4 (d, J = 2.1 Hz, C), 74.7(d, J = 2.9 Hz, C), 175.5 (C).

Method B (analogous to that in ref 34). Hydrochloric acid (36%, 1 mL, 11.6 mmol) was added to a solution of PCA (210 mg, 1.13 mmol) in ethanol (2 mL). The mixture was heated to slow boiling and stirred for 20 min to form a nearly colorless solution, which was allowed to cool to ambient temperature and evaporate to dryness under reduced pressure. Hydrochloric acid (36%, 2 mL) was added to the residue, and the mixture was heated to slow boiling and stirred for 2 h, evaporating to approximately one half of the initial volume. The solution was allowed to cool to ambient temperature and placed in a refrigerator (−18 °C) for 12 h. The crystalline precipitate of CPH was filtered off and washed with cold hydrochloric acid to yield 206 mg (80%).

Rate Constants of Nitroxide Reduction with Ascorbate. The solutions of nitroxide (0.1 mM) were mixed under anaerobic conditions in a glovebox at an oxygen level of <1 ppm with various concentrations of ascorbate (1−100 mM) in 0.1 M Na-phosphate buffer, pH 7.6. The mixture was transferred to the 50 μL glass capillary tube, and the EPR spectrum was recorded using an X-band spectrometer within 1−2 min after mixing. For the kinetics studies, the double integral of the low-field component of the EPR spectrum was monitored.

Cyclic Voltammetry. Cyclic voltammograms (CVs) of nitroxides (2.0×10^{-3}−10^{-2} M solutions in dry MeCN, 0.1 M Et_4NClO_4) were performed at 295 K under an argon atmosphere with an SVA-1BM electrochemical system (Bulgaria) equipped with a digital interface. A stationary working cylindrical Pt electrode (S = 0.08 cm^2) was used. A Pt spiral was used as an auxiliary electrode. The peak potentials were quoted with reference to saturated aq Hg/Hg_2Cl_2. Measurements were carried out in a mode of triangular pulse potential with the sweep rate, v, varied from 0.05 to 10 V/s. Measurements were performed in the voltage range from −2.3 to +2.5 V.

Equilibrium in CPH-^{15}N−Nitroxide Mixtures. Fresh stock solutions (0.5 mM) were prepared by dissolving the nitroxides and **CPH-^{15}N** in a 15 mM solution of KOH in methanol containing 100 μM DTPA under anaerobic conditions in a glovebox filled with argon (oxygen concentration below 5 ppm). The aliquots of each nitroxide solution were mixed with an aliquot of **CPH-^{15}N**, sealed, and left for 24 h or more in the glovebox at 25 °C. The solutions were then transferred into 50 μL glass capillary tubes and sealed. The EPR spectra were recorded on a spectrometer using a modulation amplitude of 0.5 G and microwave power of 6.36 mW.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01494.

Copies of the ^{1}H and ^{13}C NMR spectra of **CPH** and **CPH-^{15}N**, EPR spectrum of **PCA-^{15}N**, and calculation of parameters $E_{s,n}$ and $\sigma_{I,n}$ for multiple regression (PDF)

DOI: 10.1021/acs.joc.5b01494
J. Org. Chem. 2015, 80, 9118−9125

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work has been supported by the Russian Science Foundation (No. 14-14-00922). We thank the personnel of the Collective Service Center of SB RAS for recording the IR and NMR spectra and performing elemental analyses. The contribution by D.A.K. was supported by RFBR (Grant No. 13-04-1258).

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DOI: 10.1021/acs.joc.5b01494
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