ESR Study of Free Radical Decomposition of *N*,*N*-Bis(arylsulfonyl)hydroxylamines in Organic Solution

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Decomposition of *N*,*N*-bis(*p*-tolylsulfonyl)hydroxylamine (BTH) in chloroform and benzene solutions has been studied and was found to involve the formation of several radical intermediates. This process has been found to be accelerated by oxygen, resulting in the formation of *p*-toluenesulfonic acid and *N*,*N*,*O*-tris(*p*-tolylsulfonyl)hydroxylamine (TTH) as the main decay products. In addition, a small amount of *p*-toluenesulfonyl chloride has been isolated from chloroform solution, suggesting the chlorine abstraction from solvent. The formation of nitric oxide (NO) from BTH has been shown by mass spectrometry in gaseous phase and using nitronyl nitroxide as an NO trap in solution. It was proposed that liberation of NO proceeds through the homolytic cleavage of the S−N bond of *p*-tolylsulfonyl nitrite existing in equilibrium with BTH in solution. The formation of *p*-tolylsulfonyl radicals has been proved by spin trapping using 2-methyl-2-nitrosopropane (MNP) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). The rate of NO production in the presence of nitronyl nitroxide and the rate of oxygen consumption revealed linear plots in BTH concentration with the rate constants 0.0044 s^{-1} and 0.0016 s^{-1}, respectively. It was found also that nitrogen dioxide formed during NO oxidation reacts readily with BTH to produce the organic analog of Fremy's radical. This radical recombines with *p*-tolylsulfonyl radical yielding *N*,*N*,*O*-trisubstituted hydroxylamine TTH.

Introduction

During the past few years the enormous interest in the chemistry of prodrug analogs which are able to produce NO or its redox forms was stimulated by the universal biological importance of nitric oxide. At the present time it seems there are no vital physiological processes for which the participation of NO has not been found. There is smooth muscle relaxation, platelet inhibition, neurotransmission, immune regulation, penile erection, etc.[1] The wide spectrum of NO_x prodrugs used in the pharmacology is in agreement with the multiplicity of NO functions[2] and includes well known organic nitrates, nitroprusside, sidnonimines, authentic NO, various C-, N-, O-, S-nitroso compounds,[3] polyamine based N_2O_2 anions,[4] iron-nitrosyls,[5] and nitroxyl (HNO) releasing agents.[6−8] Some of them need metabolic activation for their action; others generate NO redox forms spontaneously.

N,*N*-disulfonated hydroxylamines might be of interest for nitric oxide pharmacology due to several reasons. First of all, it has been shown that the decomposition of these compounds is followed by the formation of brown fumes indicating the appearance of nitrogen oxides.[9,10] Recently, the similar compounds *N*-hydroxybenzenesulfonamide (Piloty's acid)[6,7] and some derivatives of *N*-(arylsulfonyl)carbamic acid[8] have been proposed as prodrugs of nitroxyl, another possible candidate of the endothelium-derived relaxing factor (EDRF).[11] Moreover the formation of *N*,*N*-disulfonated hydroxylamines seems to be possible in vivo. Indeed, these compounds are readily formed in S-nitrosation reaction of sulfinic acids,[9,10] which are the most reactive substances toward nitrosation.[12] On the other hand, sulfinic acids are the stable metabolites of thiol oxidation appearing in many biochemical processes.[13]

Preliminary results revealed that the decomposition of some *N*,*N*-bis(arylsulfonyl)hydroxylamines proceeds hydrolytically in aqueous solution,[14] as was found for the *N*-(arylsulfonyl)carbamic acids.[8] In the present paper the

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decomposition of *N*,*N*-bis(*p*-tolylsulfonyl)hydroxylamine in organic solution has been studied. It has been shown that this process is followed by intermediate free radical formation, nitric oxide included. This result seems to be of interest due to possible application of such compounds in pharmacology. Indeed, the observed radical decomposition of *N*,*N*-bis(arylsulfonyl)hydroxylamines can occur in vivo as a result of partial localization of these lipophilic prodrugs in the hydrophobic biomembrane phase.

Results and Discussion

Analysis of the Products of BTH Decomposition. Decomposition of BTH was examined in chloroform and benzene solutions at room temperature. In the absence of oxygen ca. 90% of BTH was recovered from solution after 3 days of incubation. The residue was a mixture of *p*-toluenesulfonic and *p*-toluenesulfinic acids based on the basis of TLC and IR spectroscopy. In the presence of oxygen complete decomposition of 3 mmol of BTH in 50 mL of solution took about one day. The main isolated products were *p*-toluenesulfonic acid (60−80%) and TTH (15−25%). The formation of *N*,*N*,*O*-trisubstituted hydroxylamine, TTH, rather then *N*,*N*,*N*-trisubstituted amine oxide was confirmed by NMR spectroscopy (see Experimental Section). This is in agreement with data obtained for other *N*,*N*,*O*-tris(arylsulfonyl)hydroxylamines.[10,15] In air-saturated chloroform solution a small amount (ca. 5%) of *p*-toluenesulfonyl chloride was isolated from BTH decomposition products. Thus, this decomposition can be described by eq 1:

$$\underset{}{Ts_2NOH} \xrightarrow{CHCl_3/air} \underset{(70\%)}{TsOH} + \underset{(20\%)}{Ts_2NOTs} + \underset{(5\%)}{TsCl} \quad (1)$$

Since the starting hydroxylamine was of analytical purity, the formation of TsCl is the result of the reactions with solvent. It seems to be possible that these reactions include the participation of *p*-tolylsulfonyl radicals. The formation of trisubstituted hydroxylamine (eq 1) can also proceed via a *p*-tolylsulfonyl radical-mediated process. Note that similar trisubstituted hydroxylamines are formed in free radical decomposition of nitrosocompounds.[15,16]

The headspace gas of the deoxygenated BTH solution was examined by mass spectrometry. The presence of nitric oxide (*m*/*z* 30) under 10 mL of 3 mM BTH solution was determined after one day of incubation. The signal intensities corresponded to the formation of 0.4−1.0 μmol of NO indicating 1−3% of hydroxylamine conversion. To confirm the assignment of peak *m*/*z* 30 to nitric oxide ^{15}N-BTH was used. The signal *m*/*z* 31 instead of the peak *m*/*z* 30 was observed. The signal from nitrous oxide was not found in both cases indicating that nitroxyl was not formed during the decomposition. The release of NO from deoxygenated solution of BTH can be explained by the homolysis of S−N bond of *p*-tolylsulfonyl nitrite (eq 3)[15] existing in equilibrium with BTH (eq 2):

$$Ts_2NOH \leftrightarrows TsNO + TsH \quad (2)$$

$$TsNO \leftrightarrows Ts^{\bullet} + NO^{\bullet} \quad (3)$$

Analogous equilibria have been proposed for the reactions of sulfinic acids[17] and thiols[18] with nitrosoarenes as well as for the decomposition of *N*-(arylsulfonyl)-carbamic acids.[8] To support the role of equilibrium 2,O-acylation of BTH was done to prevent this reaction. It was found that O-acetyl BTH is stable in organic solutions for days and does not release detectable level of NO.

Spin Trapping of *p*-Tolylsulfonyl Radicals. To prove the formation of *p*-tolylsulfonyl radicals in the solution of BTH (eq 3) the spin trapping method was used. Unpaired spin density in sulfonyl radicals is mainly located on the sulfur atom (ca. 40%) based on ESR[19] and multiply scattering X_α calculation.[20] To scavenge these S-centered radicals in solution MNP and DMPO spin traps have been previously recommended.[21] Indeed, a strong ESR signal developed during the first 10 min after the addition of 10 mM MNP to 1 mM deoxygenated solution of BTH and remained stable up to a few hours (Figure 1A). This signal was obtained both in chloroform and benzene solution. The trace of di-*tert*-butylaminoxyl (a_N = 15.45 G in benzene)[22] was also registered indicating the formation of *tert*-butyl radicals (Figure 1A). In the presence of oxygen the life time of the spin adduct formed decreased to 1 h. The triplet ESR spectrum of this spin adduct was tentatively assigned to Ts$^{\bullet}$/MNP radical due to the relatively small hyperfine splitting constant (a_N = 12.57 G in benzene).[21] To confirm this assignment the formation of sulfonyl radicals was induced by heterophase oxidation of *p*-toluenesulfinic acid with Ce(IV)/ammonium sulfate[23] in the presence of MNP in benzene (eq 4):

$$TsH + Ce(IV) \rightarrow Ts^{\bullet} + Ce(III) + H^{+} \quad (4)$$

The same ESR spectrum assigned to Ts$^{\bullet}$/MNP spin adduct was observed (Figure 1B). The reaction of 50 mM *p*-toluenesulfinic acid with 30 mM dinitrogen tetraoxide in the presence of 10 mM MNP was also followed by ESR spectroscopy and assigned to Ts$^{\bullet}$/MNP adduct both in chloroform and in benzene solution (Figure 1C). This reaction leads to formation of *p*-tolylsulfonyl nitrite and can take place during oxidative decomposition of BTH (eq 5):[15]

$$TsH \xrightarrow{N_2O_4} TsNO + TsOH \quad (5)$$

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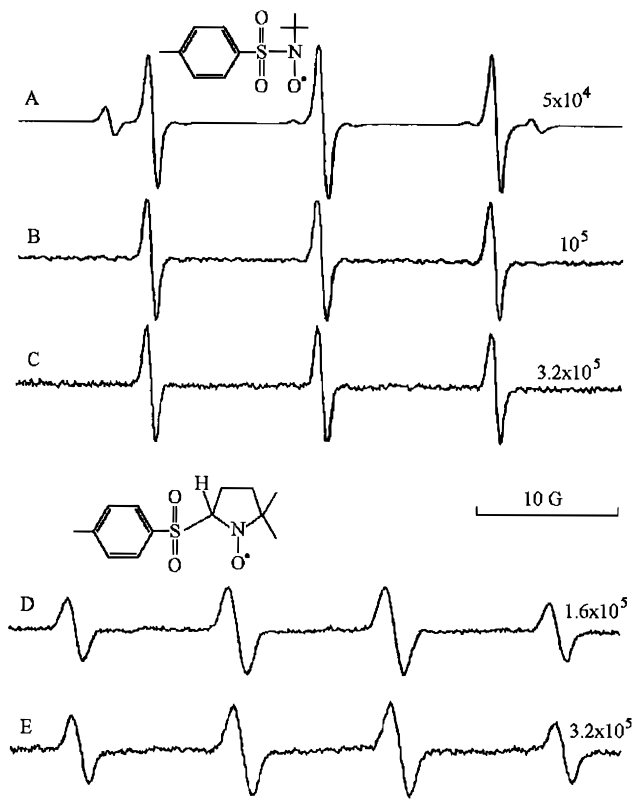

Figure 1. ESR spectra in benzene after 10 min of incubation obtained from the following: A, 10 mM MNP plus 1 mM BTH; B, 10 mM MNP, 50 mM TsH, and excess of Ce/ammonium sulfate; C, 10 mM MNP, 50 mM TsH, and 30 mM N_2O_4; D, 100 mM DMPO plus 2 mM BTH; E, 100 mM DMPO, 50 mM TsH, and excess of Ce/ammonium sulfate. ESR parameters of Ts•/MNP spin adduct (spectra A, B, C) are $a_N = 12.57$ G, $g = 2.00608$ ($a_N = 12.53$ G, $g = 2.00610$ in $CHCl_3$) and $a_N = 12.88$ G, $a_H = 13.03$ G, $g = 2.00617$ ($a_N = 12.59$ G, $a_H = 12.68$ G, $g = 2.00621$ in $CHCl_3$) for Ts•/DMPO spin adduct (spectra D, E). Spectrometer settings: microwave power 15 mW, modulation amplitude 0.5 G, and spectrometer gains are shown under spectra.

The formation of Ts•/MNP can be described by the spin trapping reaction in equation 6 as well as by the intermolecular addition reaction of *p*-tolylsulfonyl nitrite with MNP (eq 7):

$$Ts^{\bullet} + MNP \rightarrow Ts^{\bullet}/MNP \qquad (6)$$

$$TsNO + MNP \rightarrow Ts^{\bullet}/MNP + NO^{\bullet} \qquad (7)$$

To avoid the reactions of type 7 a different spin trap, DMPO, was also used for sulfonyl radical detection. A substantially higher concentration of spin trap is needed to obtained a satisfactory ESR signal in this case. Thus, when 0.1 M DMPO in deoxygenated solution was mixed with 2 mM BTH, the four-line ESR spectrum was observed (Figure 1D). Again, the reaction of *p*-toluenesulfinic acid with Ce(IV) (eq 4) produced the same spectrum in the absence of oxygen (Figure 1E). The formation of spin adducts was not observed in the mixture of *O*-acetyl-BTH and spin trap. In air saturated solutions only the products of DMPO oxidation (DMPOX, etc.)[24] were found. The spin adduct of DMPO with superoxide radicals was not observed indicating a negligible role for direct oxidation of BTH by molecular oxygen. Since the OH•/DMPO radical is a common artifact in any studies using DMPO and has a similar (Figure 1D) quartet ESR spectrum in water solution,[24] the possible formation of this spin adduct was examined. The extraction of OH•/DMPO adduct from Fenton/DMPO reaction mixture by chloroform revealed a six-line ESR spectrum ($a_H = 12.86$ G, $a_N = 13.76$ G) distinguished from that presented at Figure 1D. Therefore, the latter one was assigned to Ts•/DMPO adduct. Carbon-centered radicals were not detected by either MNP or DMPO suggesting negligible *p*-tolylsulfonyl radical fragmentation (eq 8):

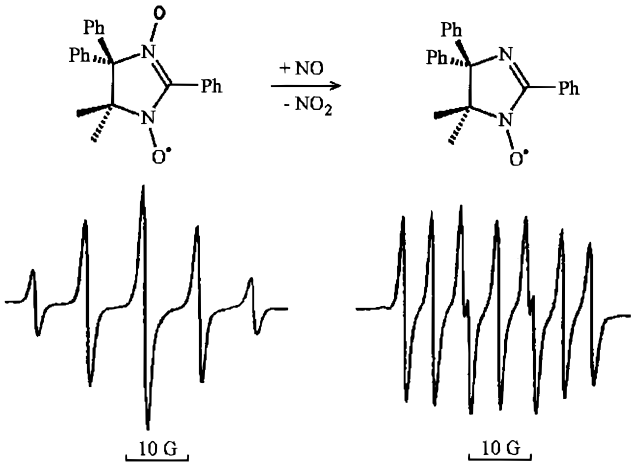

Figure 2. Scheme of the NO detecting reaction. Nitronyl nitroxide reacts with NO to produce imino nitroxide and NO_2 with the corresponding changes in ESR spectra from a five-line pattern to a nine-line pattern. Since the low field components of the ESR spectra of NNR and INR did not overlap, the kinetics of the reaction can be followed by time evolution of one of this components.

$$4\text{-}CH_3PhSO_2^{\bullet} \Longleftarrow\!/\!/\Longrightarrow 4\text{-}CH_3Ph^{\bullet} + SO_2 \qquad (8)$$

Such fragmentation is a well known intramolecular reaction of alkylsulfonyl radical[13b,23a] and may be explained by a weaker carbon-sulfur bond in aliphatic compounds ($\Delta D \sim 10$ kcal/mol).[25] The absence of fragmentation reactions have been proved earlier for arylsulfonyloxyl radicals.[26]

Liberation of NO in Solution. The kinetics of NO formation in a chloroform solution of BTH was followed by trapping of nitric oxide using nitronyl nitroxide (NNR). The trapping proceeds in a stoichiometric manner (NNR/NO = 1) to produce imino nitroxide (INR) with the rate constant ca. 10^4 M^{-1} s^{-1}.[27] The reaction is accompanied by characteristic changes in ESR spectrum: from five-line pattern (2N, $a_N = 7.92$ G) to nine-line pattern (2N, $a_{N1} = 4.29$ G, $a_{N2} = 9.68$ G) (Figure 2). It has been shown that this reaction is specific toward nitric oxide and did not occur with nitrite, nitrogen dioxide, and oxygen radicals.[27b] The time course of the ESR spectrum (low

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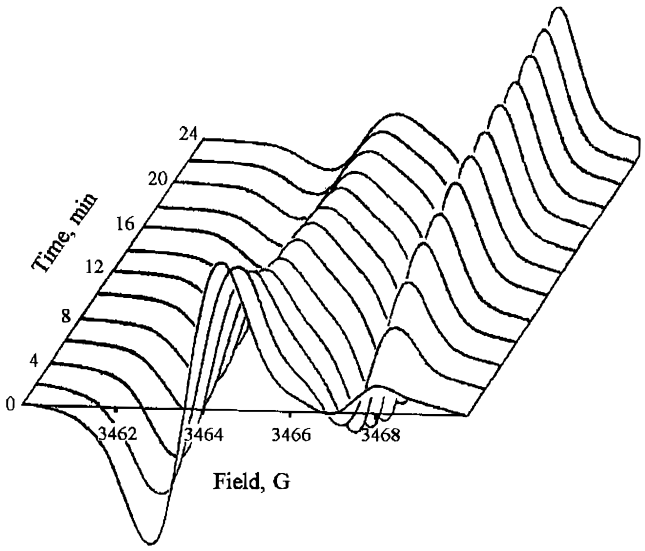

Figure 3. Time course of ESR spectra (low field components) of NNR and INR obtained from chloroform solution of 0.50 mM NNR and 0.41 mM BTH. ESR registration started ($t = 0$) 0.5 min after NNR addition to the deoxygenated BTH solution. Spectrometer settings: microwave power 15 mW and modulation amplitude 0.5 G.

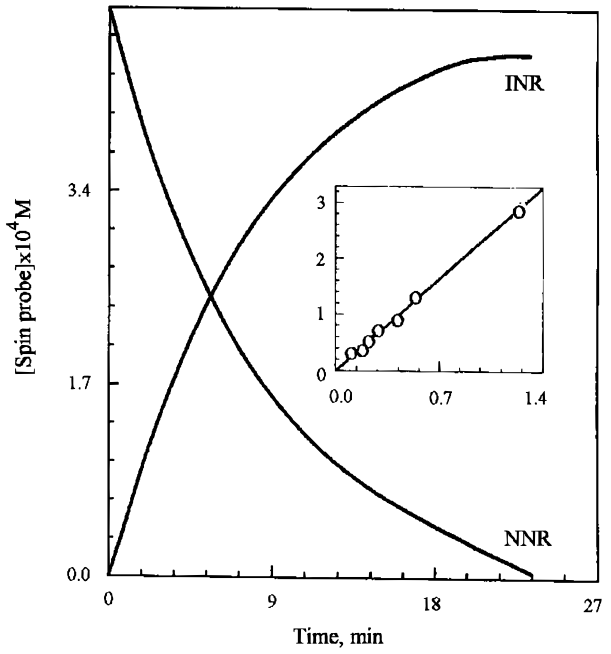

Figure 4. The kinetics of the low field component intensities of ESR spectra of NNR and INR in chloroform solution of 0.50 mM NNR and 0.53 mM BTH (shown in concentration scale). ESR double integral was checked and remained constant up to 15 min. Spectrometer settings: microwave power 15 mW and modulation amplitude 2 G. Inset: The dependence of initial rate of NNR decrease (ordinate, μM/s) on BTH concentration (abscissa, mM); the slope is 2.2×10^{-3} s^{-1}.

field components) in the deoxygenated solution of 0.5 mM 2,4,4-triphenyl-5,5-dimethylimidazoline-1-oxyl 3-oxide (see Figure 2) and 0.41 mM BTH is shown in Figure 3. Nitric oxide production results in a decrease of NNR spectrum intensity and in an increase of INR spectrum intensity (Figures 3 and 4). The ESR double integral remained constant at least at initial part of the kinetics up to 15 min, suggesting the absence of side reactions of the paramagnetic probe. The simplified kinetic scheme was proposed including the overall NO production by BTH (eq 9), the reaction of nitric oxide with NNR (eq 10, $k_{10} \sim 10^4$ M^{-1} s^{-1}), and recombination of NO with nitrogen dioxide (eq 11, $k_{11} \sim 10^9$ M^{-1} s^{-1}):[28]

$$\mathrm{BTH} \xrightarrow{K_9} \mathrm{NO} \tag{9}$$

$$\mathrm{NNR} + \mathrm{NO} \xrightarrow{k_{10}} \mathrm{INR} + \mathrm{NO_2} \tag{10}$$

$$\mathrm{NO} + \mathrm{NO_2} \xrightarrow{k_{11}} \mathrm{N_2O_3} \tag{11}$$

The characteristic time of spectral changes (Figures 3 and 4) is much longer than that expected for the reactions 10 and 11. Assuming that the reaction 9 is the rate-determining step, the initial slope of NNR decrease should be nearly proportional to the rate of nitric oxide production by BTH. The observed rates give a linear plot in BTH concentration (Figure 4, inset). The rate constant K_9 estimated from this concentration dependence with taking into account the overall stoichiometry NO/NNR = 2 (eqs 10 and 11) is 0.0044 ± 0.0008 s^{-1}. Additional kinetic studies are necessary to elucidate the detailed mechanism responsible for NO liberation with apparent rate constant K_9.

Oxygen Consumption. In air-saturated solution nitric oxide[29] and *p*-tolylsulfonyl radicals[19a,30] react readily with dissolved oxygen (eqs 12 and 13):

$$2\ \mathrm{NO} + \mathrm{O_2} \rightarrow 2\ \mathrm{NO_2} \tag{12}$$

$$\mathrm{Ts^\bullet} + \mathrm{O_2} \rightarrow \mathrm{TsO_2^\bullet} \rightarrow \rightarrow \mathrm{TsOH} \tag{13}$$

Spin-label oxymetry was used to determine the rate of oxygen consumption during BTH decomposition in chloroform solution. Since the oxygen concentration in organic phase is about 10 times higher relative to water (ca. 2 mM)[31] the simple line width-sensitive ESR technique can be employed.[32] The method is based on the ESR line broadening of a paramagnetic probe due to spin exchange with molecular oxygen.[33] Thus, for Lorentzian line shape, the line width (ΔH) is proportional to oxygen concentration (eq 14):

$$\Delta H\ (\text{with } \mathrm{O_2}) = \Delta H\ (\text{no } \mathrm{O_2}) + \text{const} \times [\mathrm{O_2}] \tag{14}$$

2,2,5,5-Tetramethyl-4-phenyl-3-imidazoline-1-oxyl radical used in this study has shown fairly linear dependence of ESR peak to peak line width on oxygen concentration (see Experimental Section) and sufficient chemical stability. When BTH (3.2 mM) was added to the air-saturated solution of 0.5 mM spin probe, the ESR line width decreased gradually from 2.25 to 0.85 G, indicating the change in oxygen concentration from 2.1 mM to nonde-

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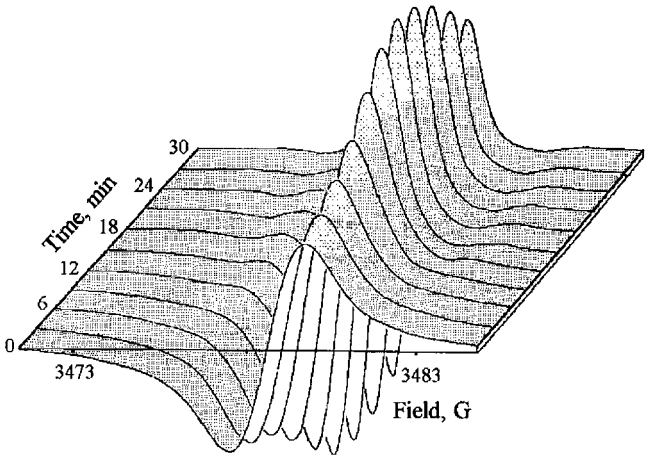


Figure 5. Time course of the central component of ESR spectrum of 2,2,5,5-tetramethyl-4-phenyl-3-imidazoline-1-oxyl radical obtained from air-saturated chloroform solution of 0.5 mM spin probe and 3.2 mM BTH. ESR registration started (t = 0) 0.5 min after BTH addition to the solution of spin probe. It can be seen that initial rise of ESR intensity due to line narrowing replaced by decrease of the signal amplitude due to spin probe destruction. Spectrometer settings: microwave power 15 mW and modulation amplitude 0.5 G.

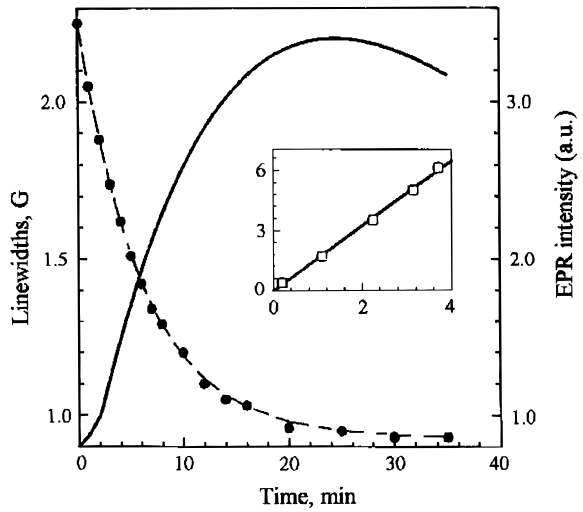


Figure 6. Oxygen consumption in BTH solution measured by ESR oxymetry. The kinetics of line width (symbols with dotted line) and ESR peak intensity (solid line) of the spin probe (0.5 mM) in air-saturated chloroform solution of 3.2 mM BTH. ESR double integral was checked and remained constant up to 8 min. The initial rates of oxygen consumption were estimated from ESR line width (ΔH) kinetics using the calibration curve $[O_2] = (\Delta H - 0.85\ \text{G})/0.71\ \text{G/mM}$ (see Experimental Section). Spectrometer settings: microwave power 15 mW and modulation amplitude 0.5G. Inset: The dependence of initial rates of oxygen consumption (ordinate, μM/s) on BTH concentration (abscissa, mM); the slope is $1.6 \times 10^{-3}\ s^{-1}$.

tectable level (Figures 5 and 6). Nitrogen oxides formed in the reaction appeared to have negligible effect on the ESR line width. The destruction of the aminoxyl entity was negligible at the initial part of the kinetics up to 8 min but was noticeable after oxygen was depleted (Figure 6). It was concluded that at the beginning of the reaction the inserted probe did not interfere with the decomposition process. The kinetics can be followed either by line width measurements and by continuous registration of ESR intensity, which is approximately square proportional to the line width (Figure 6). The line width measurements were employed since this method is independent of the spin probe concentration. The initial rates of oxygen consumption show a linear dependence on BTH concentration with a pseudo-first-order rate constant for the total oxygen consumption process (eq 15) equal to $0.0016 \pm 0.0006\ s^{-1}$ (Figure 6, inset).

$$O_2 + \text{BTH} \rightarrow \text{products} \qquad (15)$$

Assuming the stoichiometry coefficient for the nitric oxide oxidation is $NO/O_2 = 4$ (eqs 11 and 12), more than half of observed rate of oxygen consumption, $K_{15} \times [\text{BTH}]$, should be due to NO generation, ($K_9 \times [\text{BTH}]$). The close values of the rates of NO production and oxygen consumption as well as linear dependence on BTH concentration suggest that these two processes follow the same kinetics of BTH decomposition. In both cases *O*-acetyl BTH revealed the stability toward decomposition.

Formation of *N,N*-Bis(*p*-tolylsulfonyl)aminoxyl Radical. In the presence of oxygen the decomposition reactions of BTH lead to the formation of *N,N,O*-trisulfonated hydroxylamine (see eq 1). Similar compounds are also formed during the oxidation of *N,N*-disulfonated hydroxylamines by nitrous acid[9] or by metal ions.[10] It has been suggested without experimental proof that the latter reaction includes one-electron oxidation of the started compounds to form organic analogs of Fremy's radical (eq 16):[10]

$$(RSO_2)_2NOH \xrightarrow{-e} (RSO_2)_2NO^{\bullet} + H^+ \qquad (16)$$

In order to detect the appearance of these radicals the decomposition of BTH in air contacting solution was followed by ESR spectroscopy. After one day of incubation of the excess of undissolved BTH in organic solution the triplet ESR spectrum ($a_N = 10.50$ G in benzene) has been detected. This spectrum was replaced by a doublet ($a_N = 14.79$ G in benzene) when ^{15}N-BTH was used (Figure 7). These spectra were assigned to *N,N*-bis(*p*-tolylsulfonyl)aminoxyl radical in agreement with small hyperfine splitting constants resulting from uncompensated electron-withdrawing effect of two tosyl groups. The aminoxyl is unstable and has a lifetime of a few minutes. This is, probably, the result of the fragmentation reaction (eq 17):

$$Ts_2NO^{\bullet} \Leftrightarrow TsNO + Ts^{\bullet} \qquad (17)$$

It was proposed that the oxidant of the reaction 16 is not oxygen itself but formed during the oxidative decomposition of BTH since a long time is necessary to generate the observable concentration of *N,N*-bis(*p*-tolylsulfonyl)-aminoxyl radical. This oxidant might be nitrogen dioxide or persulfonic acid forming in the reaction of nitric oxide and sulfonyl radicals with oxygen, while the undissolved fraction of BTH serves as the source of BTH in solution. Indeed, when nitrogen dioxide was added to the solution of BTH, the spectrum of *N,N*-bis(*p*-tolylsulfonyl)aminoxyl radical was obtained immediately. It was also observed in agreement with earlier assumptions[10] that Ce(IV) salt oxidized BTH through the formation of aminoxyl giving the TTH with 85% yield (Figure 7 and Experimental Section). When BTH decomposition was catalyzed by Fe(II)/Fe(III) ions in the presence of oxygen the ESR signal was developed in the first hours (Figure 7). The catalysis seems to include the complexation of iron ions

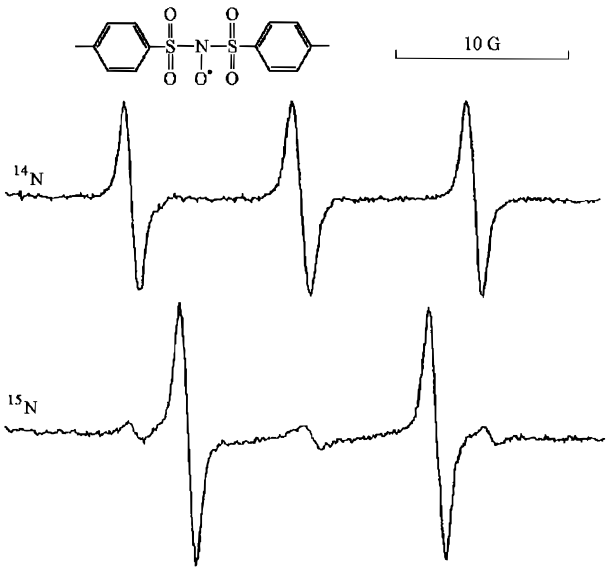
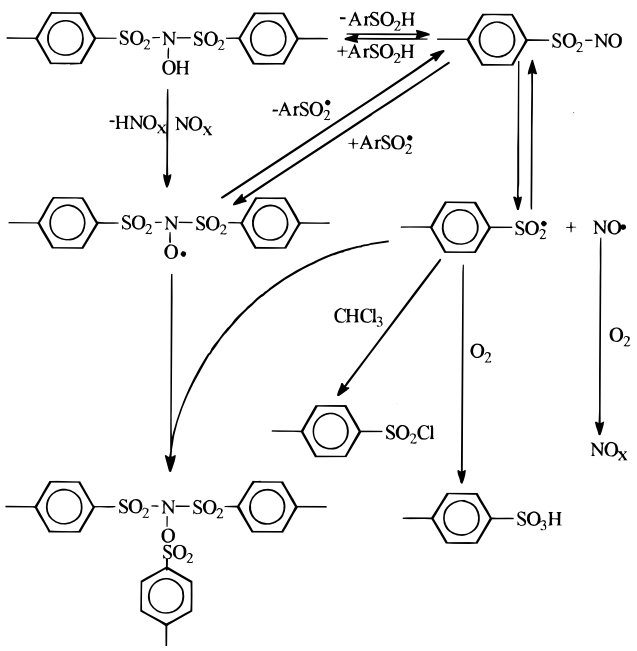


Figure 7. ESR spectra of ^{14}N- and ^{15}N- *N*,*N*-bis(*p*-tolylsulfonyl)aminoxyl radical obtained from air contacting benzene solution over the undissolved fraction of BTH. The spectra were taken after one day of incubation and have ESR parameters as follows: $a_N = 10.50$ G (^{14}N), $a_N = 14.79$ G (^{15}N), $g = 2.00635$ ($a_N = 10.45$ G (^{14}N), $a_N = 14.73$ G (^{15}N), $g = 2.00640$ in $CHCl_3$). The same spectra were obtained after 2 h when the reaction was catalyzed by 10^{-5} M $FeCl_3$, or immediately when the saturated solution of BTH was oxidized either by 30 mM N_2O_4 or an excess of Ce/ammonium sulfate. Spectrometer settings: microwave power 15 mW, modulation amplitude 0.5 G, and spectrometers gains 3.2×10^5.

Scheme 1. Mechanism of *N*,*N*-Bis(arylsulfonyl)hydroxylamine Decomposition in Organic Solution



by hydroxylamine with further acceleration of the formation of *p*-tolylsulfonyl nitrite by reaction 2. Another possibility is the formation of the aminoxyl radical as a result of electron transfer from hydroxylamine to Fe(III) ion in complex.

Decomposition Mechanism. Scheme 1 is proposed to account for the formation of the observed reaction products and free radical intermediates. Thus, the fact that a deoxygenated organic solution of *N*,*N*-bis(*p*-tolylsulfonyl)hydroxylamine produces nitric oxide and *p*-tolylsulfonyl radicals indicates, apparently, the existence of an equilibrium between BTH and a radical species (eqs 2 and 3). O-Acetylation of hydroxylamine prevents these reactions. Free radical intermediates formed in reactions 2 and 3 react readily with oxygen and other radical scavengers to promote decomposition (eqs 12 and 13). When nitrogen oxides are accumulated in the solution, they give rise to further oxidative reactions (eqs 5 and 16) leading to the formation of *N*,*N*-bis(*p*-tolylsulfonyl)-aminoxyl radicals. Fragmentation of these radicals proceeds likely through the formation of *p*-tolylsulfonyl nitrite and sulfonyl radicals (eq 17). The latter recombine with aminoxyl radicals to produce *N*,*N*,*O*-tris(*p*-tolylsulfonyl)hydroxylamine (see Scheme 1). Therefore, the main stable products formed during the BTH decomposition in the presence of oxygen are nitrogen dioxide, *p*-toluenesulfonic acid, *N*,*N*,*O*-tris(*p*-tolylsulfonyl)hydroxylamine and *p*-toluenesulfonyl chloride (in chloroform). The determined transient paramagnetic species are nitric oxide, *p*-tolylsulfonyl radicals and *N*,*N*-bis(*p*-tolylsulfonyl)aminoxyl.

Experimental Section

Benzene and chloroform used in this study were purified and dried as recommended,[34] acetic anhydride was distilled over phosphorus pentoxide. *p*-Toluenesulfinic acid was synthesized from *p*-toluenesulfochloride (Sigma) and p-thiocresol (Sigma) according to ref 35. BTH and it's derivatives were synthesized as described below. Ceric ammonium sulfate, sodium nitrite, MNP, and DMPO were from Sigma, and ferric chloride (anhydrous) was from Aldrich. Dinitrogen tetraoxide was obtained according to ref 35. 2,4,4-Triphenyl-5,5-dimethyl-2-imidazoline 3-oxide-1-oxyl and 4-phenyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl were synthesized according to ref 27c and kindly donated by Dr. Igor Kirilyuk, ^{15}N-sodium nitrite was kindly donated by Prof. Igor Grigor'ev. Argon (99.5%) was from "Biopol" (Russia). Melting points were determined on a Koffler apparatus and were uncorrected. Microanalyses were performed by Microanalyses Laboratories of the Novosibirsk Institute of Organic Chemistry.

***N*,*N*-Bis(*p*-tolylsulfonyl)hydroxylamine.** The modification of the previous procedure[9,10] was used. To a cooled (ice bath) stirred solution of *p*-toluenesulfinic acid (1.1 g, 7 mmol) in a mixture of ethanol (10 mL) and concentrated HCl (1 mL) was added a solution of sodium nitrite (0.5 g, 7 mmol) in 10 mL ice-cold water dropwise during 5 min. After addition the precipitate was filtered, washed by cold water and dried in vacuum over KOH. Yield of fairly pure product was 1.1 g (92%). For decomposition studies the compound was recrystallized from methanol giving the analytically pure sample (C,H,N,S ± 0.2%, mp 124−125 °C); IR (KBr, cm^{-1}) 3280 (OH), 1385 and 1190 (SO_2); ^{1}H-NMR (300 MHz, δ) 2.45 (s, 6H, CH_3), 7.25, 7.28, 7.76, 7.79 (dd, 8H, 1,4-disubstituted benzene). ^{15}N-BTH was synthesized by following the same procedure. The samples were stored in dried container in refrigerator no more than one month.

***O*-Acetyl-*N*,*N*-bis(*p*-tolylsulfonyl)hydroxylamine.** The solution of BTH (1 g, 3 mmol) in 25 mL of acetic anhydride was stirred under argon during the 0.5 h at room temperature and then 0.5 h at 60 °C. Then, the reaction mixture was cooled (ice bath) and poured on to ice-cold water. The precipitate was filtered and recrystallized from methanol/chloroform (1:1). Yield of analytically pure compound was 0.7 g (60%); mp 134−136 °C; analysis C,H,N,S ± 0.2%; IR (KBr, cm^{-1}) 1830 (C═O), 1385 and 1190 (SO_2); ^{1}H-NMR (300 MHz, δ) 2.19 (s, 3H,

(34) Gordon, A. J.; Ford, R. A. *The Chemist's Companion, a Handbook of Practical Data, Techniques, and References*; Wiley: New York, 1972.
(35) Lee, C.; Field, L. *Synthesis* **1990**, 391−398.

$OCOCH_3$), 2.45 (s, 6H, CH_3), 7.30, 7.33, 7.77, 7.80 (dd, 8H, 1,4-disubstituted benzene).

***N,N,O*-Tris(*p*-tolylsulfonyl)hydroxylamine.** To a stirred solution of BTH (1 g, 3 mmol) in benzene (50 mL) the thin powder of ceric ammonium sulfate (5 g, 8 mmol) was added under argon. The mixture was stirred overnight and filtered, and a salt residue was washed by benzene (2 × 25 mL). Collective filtrate was evaporated, the residue was washed by cold methanol giving 0.83 g (85%) of product. Analytically pure compound was obtained after recristallisation from methanol/chloroform (1:1); mp 189−190 °C; analysis C,H,N,S ± 0.2%; IR (KBr, cm^{-1}) 1390, 1195 and 1170 (SO_2); ^{1}H-NMR (300 MHz, δ) 2.458 (s, 6H, CH_3), 2.472 (s, 3H, CH_3) 7.30, 7.33, 7.78, 7.81 (dd, 8H, 1,4-disubstituted benzene), 7.36, 7.39, 7.85, 7.88 (dd, 4H, 1,4-disubstituted benzene).

Analysis of the Decay Products. The solution of BTH (1 g, 3 mmol) in 50 mL of chloroform was stirred overnight under dry air. The mixture was cooled in a refrigerator, and the formed precipitate was filtered. The precipitate was *p*-toluenesulfonic acid (0.7 g, 70%, satisfactory IR, mp 100−104 °C, monohydrate, after recrystallization). The filtrate was evaporated, and the residue was extracted by methanol. The undissolved fraction was fairly pure TTH (0.2 g, 20%, satisfactory IR, mp 189−190 °C after recrystallization). The methanol solution was evaporated on cold bath and the residue was recrystallized from hexane giving pure *p*-toluenesulfochloride (0.025 g, 3%, satisfactory IR, analysis C,H,N,S,Cl ± 0.4%, mp 66−70 °C).

Headspace Gas Mass Spectrometry Analysis. In order to analyze the gaseous phase over BTH solution, the decomposition was done in 100 mL mass spectrometry vials. A 10 mL volume of 3 mM BTH solution was prepared in deoxygenated solvent under argon. The solution was degassed by repeated freeze-pumping-thaw cycling (three times, −196 °C, 10^{-3} torr, 20 °C) and allowed to stand overnight at room temperature under vacuum. After that the vial was frozen to −100 °C, connected to mass spectrometer and gaseous phase was analyzed.

ESR Measurements. All ESR experiments were carried out at room temperature. The samples were analyzed in 100 μL Pyrex glass capillaries. The measurement conditions are shown in the text. In spin trapping experiments the spin traps were added simultaneously with initiation of the reaction under study (see Figure 1, caption). Nitric oxide production was followed by time evolution of the peak intensity of the low field component of ESR spectrum of nitronyl nitroxide (or imino nitroxide) after the addition of small aliquot of nitronyl nitroxide to deoxygenated solution of BTH. Oxygen consumption was initiated by dissolving BTH in the air-saturated solution of the spin probe (0.5 mM), and the spectra were monitored after ≤0.5 min. The calibration curve for ESR line width of oxymetry probe was made by mixing of air-saturated ($[O_2] = 2.1$ mM)[31] and deoxygenated chloroform solutions in different ratios. ESR peak to peak line width (ΔH) of 4-phenyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl used in this study show linear dependence on oxygen concentration: $\Delta H = 0.71$ G/mM × $[O_2]$ + 0.85G. The formation of *N,N*-bis(tolylsulfonyl)-aminoxyl was detected in the air-saturated solution under undissolved BTH crystals, and in some cases the aliquot of $FeCl_3$ was added. In the case of generation of disulfonated aminoxyl by oxidant (N_2O_4, Ce(IV)) the ESR spectra were obtained after oxidant addition. Hyperfine splitting constants and g-factors were determined with accuracy ±0.05 G and ±0.00008, respectively.

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