A Kinetic Study of *S*-Nitrosothiol Decomposition

Loris Grossi* and Pier Carlo Montevecchi*[a]

Abstract: Under anaerobic conditions *S*-nitrosothiols **1a**–**e** undergo thermal decomposition by homolytic cleavage of the S–N bond; the reaction leads to nitric oxide and sulfanyl radicals formed in a reversible manner. The rate constants, k_1, have been determined at different temperatures from kinetic measurements performed in refluxing alkane solvents. The tertiary nitrosothiols **1c** ($k_{1(69\,^{\circ}C)} = 13 \times 10^{-3}\,min^{-1}$) and **1d** ($k_{1(69\,^{\circ}C)} = 91 \times 10^{-3}\,min^{-1}$) decomposed faster than the primary nitrosothiols **1a** ($k_{1(69\,^{\circ}C)} = 3.0 \times 10^{-3}\,min^{-1}$) and **1b** ($k_{1(69\,^{\circ}C)} = 6.5 \times 10^{-3}\,min^{-1}$). The activation energies ($E^{\#} = 20.5$–$22.8\,Kcal\,mol^{-1}$) have been calculated from the Arrhenius equation. Under aerobic conditions the decay of *S*-nitrosothiols **1a**–**e** takes place by an autocatalytic chain-decomposition process catalyzed by N_2O_3. The latter is formed by reaction of dioxygen with endogenous and/or exogenous nitric oxide. The autocatalytic decomposition is strongly inhibited by removing the endogenous nitric oxide or by the presence of antioxidants, such as *p*-cresol, β-styrene, and BHT. The rate of the chain reaction is independent of the RSNO concentration and decreases with increasing bulkiness of the alkyl group; this shows that steric effects are crucial in the propagation step.

Keywords: chain decomposition · nitrogen oxides · radical ions · radicals · *S*-nitrosothiols

Introduction

Even though the existence of *S*-nitrosothiols (RSNOs) has been known for a long time,[1, 2] not very much was reported on their chemistry until the early nineties, mainly because their use for synthetic purposes was thought to be hindered by their instability. In the past decade the interest in these compounds has remarkably increased due to the discovery of their involvement in several biological processes.[3, 4] In particular, *S*-nitroso derivatives of essential biomolecules, like cysteine and glutathione, seem to act as in vivo biological carriers of NO,[5–8] the role of which as a neurotransmitter is now well documented.[9]

Several studies have been conducted with the aim of clarifying the mechanism of the thermal decomposition of *S*-nitrosothiols, which is crucial for the understanding of how the NO transportation in the human body takes place; but many aspects still remain unknown.

The decomposition of RSNOs has been generally assumed to take place by a unimolecular mechanism through the cleavage of the weak S–N bond,[10, 11] but a heterolytic process has been suggested as well.[12–14] In contrast to the homolytic mechanism, it has been reported that the rate of disappearance of RSNOs depends on their concentration[15] and on the presence of air.[16] Moreover, Williams et al. reported that, in aqueous solutions, RSNOs can undergo a copper(II)-catalyzed reaction as the main decomposition route.[2, 17]

RSNOs can exist in two different isomeric forms, *syn* and *anti*, due to possible partial double bond character between the sulfur and nitrogen atoms.[18] Primary and secondary RSNOs exist preferentially as *syn* conformers that are red colored, whilst tertiary RSNOs have a preference for, for steric reasons, the *anti* conformation that is green colored; these latter have been reported to be more persistent than the red ones, even though a reasonable explanation for this experimental evidence is not given.[18, 19]

Herein, we report results[20] obtained from kinetic studies on the decomposition of a number of simple *S*-nitrosothiols, synthesized from the parent thiols and peroxynitrite, as we recently reported.[21] Our aim was to throw light onto the mechanism of the S–N bond cleavage and to point out the effect of the concentration, the presence of dioxygen, nitric oxide, and antioxidants.

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Results and Discussion

For this study we have considered the two primary RSNOs **1a** and **1b**, which are red colored, the two tertiary RSNOs **1c** and **1d**, which are green colored, and the red aryl RSNO **1e**. These

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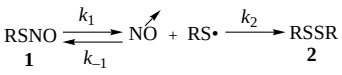
compounds were synthesized by treating at 5–10 °C a solution of the corresponding thiol in acetonitrile with hydrochloric acid and a molar equivalent of an aqueous solution (0.5 M, pH = 13.5) of peroxynitrite.[21] Under these conditions *S*-triphenylmethylnitrosothiol (**1d**) was separated as a green solid in 60% yield, whereas *S*-nitrosothiols **1a–c**,**e** were extracted from the aqueous solution with the appropriate organic solvent, and the concentration was measured by spectrophotometry. Kinetic experiments were performed on solutions of **1a–e** (12 mM) either under aerobic and anaerobic conditions. The decay of RSNOs was monitored by spectrophotometry at $\lambda = 550$ nm for **1a**[22, 23] and **1b**,[22] $\lambda = 603$ nm for **1c**[23] and **1d**, and $\lambda = 570$ nm for **1e**.[24]

Decomposition of *S*-nitrosothiols under anaerobic conditions: In preliminary experiments, solutions of **1a–c** in *n*-octane were carefully deaerated by bubbling argon at room temperature and then they were kept in a thermostatic bath. The solutions were vigorously stirred and kept under a weak stream of argon until the end of the experiment. Samples were taken at fixed time intervals up to 200 min. Several experiments were conducted at different temperatures in the range 40–70 °C. In all cases the decomposition of **1a–c** was found to follow a first-order kinetic law.

Apparently, these findings are those expected from an unimolecular homolytic scission of the S–N bond, which leads to nitric oxide and sulfanyl radicals. In agreement with this, GC-MS analyses of the reaction mixtures, which resulted after complete decomposition had been monitored by the disappearance of the color, showed the formation of the disulfide **2a–c** as the unique reaction product (Scheme 1).



a: R = $PhCH_2$; **b**: R = *n*-hexyl; **c**: R = *tert*-butyl; **d**: R = Ph_3C; **e**: R = Ph

Scheme 1. Formation of the disulfide **2**.

However, we found that the kinetic constant values depended a great deal (up to one order of magnitude) on the experimental conditions. First of all, reactions conducted under the normal laboratory lighting were faster as compared with those conducted in the dark, and this showed a significant contribution of the photochemical-induced decomposition.[25] Furthermore, the decomposition rate was found to increase considerably by increasing the speed of stirring as well as the flow of argon. We accounted for this surprising behavior by assuming that the unimolecular S–N bond scission is a reversible process (Scheme 1). In this assumption, and in the steady-state approximation for RS•, the rate of decomposition of RSNO depends on the NO concentration in Equation (1).

$$v = k_1[\mathrm{RSNO}]\{1 - k_{-1}[\mathrm{NO}]/(k_{-1}[\mathrm{NO}] + k_2[\mathrm{RS}])\} \quad (1)$$

We reasoned that an increase of the rate of stirring and/or the flow of argon caused an increase in the decomposition rate by favoring the escape of NO from the reaction vessel.

In a preliminary paper we have already discussed the possible reversibility of the S–N bond scission.[20] In the present work we found definitive evidence based on the following findings: i) a solution of **1b**, carefully deaerated at room temperature by bubbling argon and then by saturation with gaseous NO, was found to be fairly stable at 70 °C over 20 h, whereas complete decomposition occurred within 3 h in the absence of added nitric oxide; this finding clearly indicated that, in agreement with Equation (1), the rate of decomposition of RSNOs decreases with increasing nitric oxide concentration; ii) a solution of (triphenylmethyl)nitrosothiol (**1d**) decomposed in an argon atmosphere and then it was saturated with nitric oxide. A green color, due to the re-formation of **1d**, immediately developed. This finding was explained in these terms: decomposition of **1d** leads to nitric oxide, removed by the stream of argon, and (triphenylmethyl)sulfanyl radicals ($Ph_3CS^{\bullet}$), which are known to exist in equilibrium with the corresponding disulfide **2d**.[26] Subsequent addition of gaseous nitric oxide promoted the re-formation of **1d** by reversible coupling of NO with $Ph_3CS^{\bullet}$.[27]

From these overall results we argued that the first-order kinetic rate constants, k_1, of the unimolecular S–N bond scission could not be obtained under the above experimental conditions. So, we thought it prudent to run kinetic experiments in refluxing solvents, since under these conditions the concentration of nitric oxide can be considered to be almost zero, and then Equation (1) becomes $v = k_1[\mathrm{RSNO}]$. Accordingly, solutions of **1a–d** in the appropriate solvent (cyclopentane: b.p. = 50 °C; hexane: b.p. = 69 °C; cyclohexane: b.p. = 80.5 °C; and/or methylcyclohexane: b.p. = 101 °C) were at first deaerated at room temperature by bubbling with argon and then heated to a moderate reflux in the dark and in an argon atmosphere. Each kinetic experiment was repeated three times. We obtained first-order kinetic rate constants (±2% error) that we assumed to be the "true" k_1 values (see Table 1).

From the k_1 values at different temperatures we could estimate the activation energy for the S–N bond scission, $E^{\#}$, through the Arrhenius equation ($\ln k = \ln A - E^{\#}/RT$) (Table 1 and Figure 1). Our $E^{\#}$ values appear to be lower (ca. 3–4 kcal mol^{-1}) than the dissociation bond energies (DBEs) reported in the literature for related nitrosothiols.[28, 29] It is notable that the reported DBEs do not seem to be affected by the nature of the alkyl group. In sharp contrast to this report, as well as to the claim that the bulky, green nitrosothiols are

Table 1. Kinetic constants ($k_1 \pm 2\%$), activation energies ($E^{\#} \pm 0.4$ Kcal mol^{-1}), and frequency factors ($\ln A \pm 0.6$) for the decomposition of RSNOs **1a–d** under an argon atmosphere in refluxing solvents (A: cyclopentane, b.p. = 323 K; B: hexane, b.p. = 342 K; C: cyclohexane, b.p. = 353.5 K; D: methylcyclohexane, b.p. = 374 K).

RSNO	$k_1 \times 10^3$ min^{-1}				$E^{\#}$	$\ln A$
	A	B	C	D	[Kcal mol^{-1}]	
1a	0.90	6.5	18.0	105	22.3	27.7
1b	–	3.0	8.8	53	22.8	27.7
1c	–	13.0	37.0	202	21.9	27.7
1d	15.4	91	–	–	20.5	27.7

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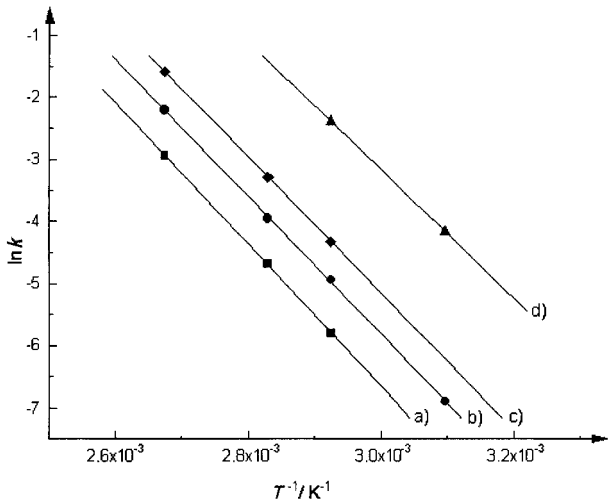

Figure 1. ln k versus $1/T$ for the thermal decomposition of **1a** (curve b), **1b** (curve a), **1c** (curve c), and **1d** (curve d) carried out under anaerobic conditions and in refluxing solvents (cyclopentane: b.p. = 50 °C; hexane: b.p. = 69 °C; cyclohexane: b.p. = 80.5 °C; methylcyclohexane: b.p. = 101 °C).

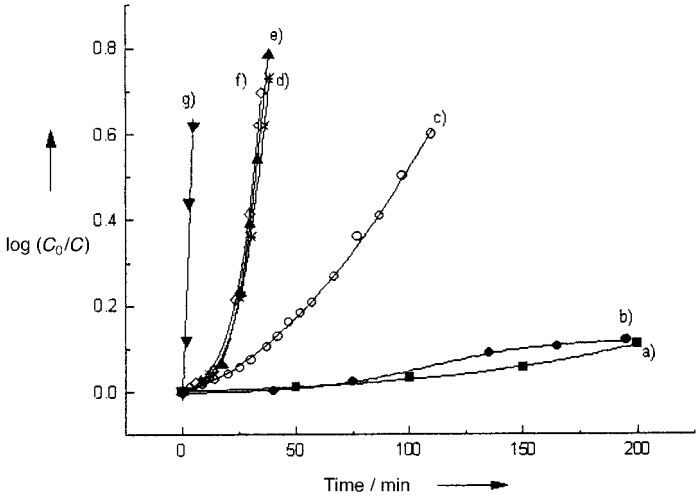

Figure 2. Kinetic curves obtained from the decomposition under aerobic conditions of solutions of *S*-nitrosothiols **1a**–**e** in *n*-pentane at 0 °C: a) 12 mM **1c**; b) 12 mM **1d**; c) 12 mM **1b**; d) 16 mM **1a**; e) 12 mM **1a**; f) 6 mM **1a**; g) 12 mM **1e**.

more stable than the nonbulky, red ones,[18, 19] our findings clearly showed that the thermal stability of RSNOs decreases with increasing bulkiness of the alkyl group.

Attempts to perform kinetic experiments in refluxing solvents with *S*-benzenenitrosothiol **1e** failed because of its thermal instability. Actually, it was possible to run experiments only at 15 °C with solutions of **1e** prepared under an argon atmosphere. Under these conditions a first-order decomposition rate was observed ($k_{exp} = 35 \times 10^{-3}$ min^{-1}). TLC and GC-MS analyses of the reaction mixture showed the disulfide **2e** to be the unique reaction product. On the assumption that $k_{exp} = k_1$ and $\ln A = 27.7$ (see Table 1), we could estimate $E^{\#} = 19.1$ kcal mol^{-1} (the DBE value reported in the literature is 19.4 kcal mol^{-1}).[28] The lower thermal stability of **1e**, as compared with that of **1a**–**d**, must be attributed to the higher stability[29] of arenesulfanyl radicals (ArS$^{\bullet}$) as compared with that alkanesulfanyl radicals (RS$^{\bullet}$).

Decomposition of *S*-nitrosothiols under aerobic conditions: Kinetic experiments under aerobic conditions were conducted at 0 °C in air-saturated solutions in *n*-pentane. Under these conditions a fast decomposition of nitrosothiols **1a**–**e** occurred; but, none of the reactions followed a simple first-order kinetic law. The rate of decomposition was found to be higher for the red, primary nitrosothiols **1a**,**b** (Figure 2, curves c,e) as compared with that for the green, tertiary nitrosothiols **1c**,**d** (Figure 2, curves a,b). Apparently, these results are consistent with previous claims that the half-life time of RSNOs depends on the presence of dioxygen[16] and the bulkiness of the alkyl group.[18, 19] The kinetic curves (Figure 2) show that the rate of disappearance of RSNOs increases over time. Such kinetic behavior is characteristic of autocatalytic chain reactions, in which a reaction product behaves as the chain carrier; the observed increase over time of the decomposition rate must be related to the progressive increase of the chain-reaction rate due to the parallel increase of the chain-carrier concentration. The higher stability exhibited by the bulky *S*-nitrosothiols **1c**,**d**, which contrasts with the lower thermal stability exhibited under anaerobic conditions (see above), should be ascribed to steric effects in the chain-propagation step. Also, it is worth noting the peculiar trend exhibited by the kinetic curve obtained from **1d** (Figure 2, curve b). After an initial period of time, in which the forecast autocatalytic behavior was observed, the increase in the chain-reaction rate tended to slow down. This trend led us to suppose that a product arising from **1d** inhibited the chain-decomposition reaction (see later for discussion).

In order to verify if the RSNO concentration can influence the rate of decomposition, as previously reported,[15] kinetic experiments were also conducted with 16 and 6 mM solutions of **1a** in *n*-pentane. But, no variation in the kinetic behavior was observed when compared with the 12 mM solution (Figure 2, curves d–f).

To determine the effect of the temperature on the decomposition rate, kinetic measurements were also conducted at 18, 33, and 50 °C with solutions of **1a** in *n*-octane. The kinetic curves (Figure 3) show that an increase of the temperature from 0 to 50 °C caused the expected increase of the decomposition rate (Figure 3, curves c,d), whereas a remarkable decrease was found on passing from 0 °C to 18 or 33 °C (Figure 3, curves a–c). This unexpected behavior of the decomposition rate led us to argue that two contrasting effects were arising by increasing the temperature (see later for discussion).

GC-MS and ^{1}H NMR analyses of the reaction mixture obtained from **1a** provided evidence for the formation of benzaldehyde (**4**) and the thiosulfonate derivative RS–SO_2R (R = $PhCH_2$), in addition to dibenzyl disulfide (**2a**) (Scheme 1). These products were formed in a 25:5:70 ratio and in 80% overall yield.

Similarly, GC-MS analyses of the reaction mixtures obtained from **1b**,**c**,**e** showed the formation of the corresponding disulfide **2b**,**c**,**e** and thiosulfonate derivative, RS–SO_2R

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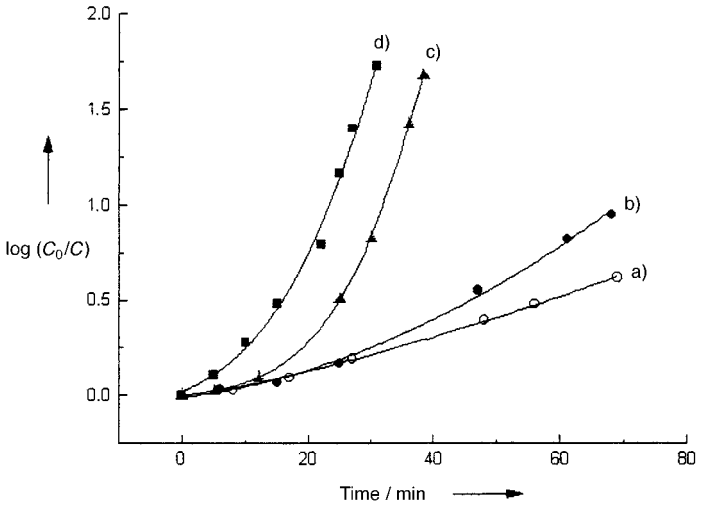
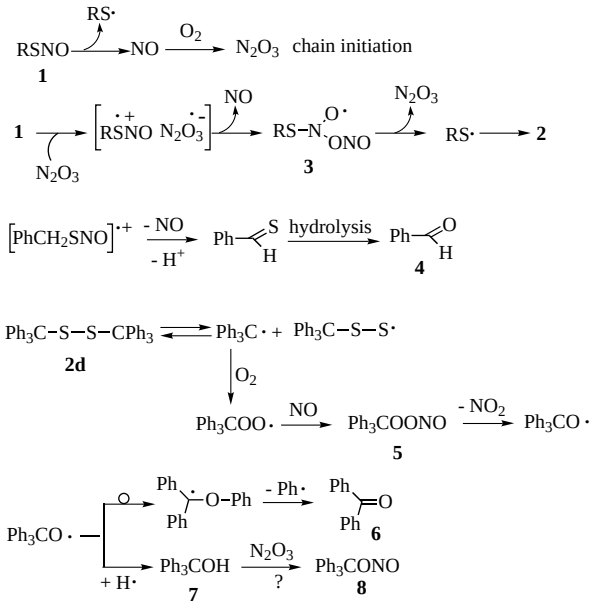


Figure 3. Kinetic curves obtained from the decomposition under aerobic conditions of 12 mM solutions of *S*-nitrosothiol **1a** at 0 °C (curve c), 18 °C (curve a), 33 °C (curve b), and 50 °C (curve d).

[R = *n*-hexyl (7%), *tert*-butyl (7%), and phenyl (13%)] (Scheme 2).

From the reaction mixture obtained from **1d** we could separate small amounts of a yellow oil (**A**), which rapidly decomposed to triphenylmethanol (**7**) on standing in air or by absorption on silica gel. TLC analysis of the reaction mixture showed the presence of triphenylmethanol (**7**) and benzophenone (**6**) as the main products (Scheme 2), besides trace amounts of two unidentified compounds and minor amounts of the yellow product **A**. Compounds **6** and **7**, but not **A**, could be separated by subsequent silica gel column chromatography in 23 and 50% yield, respectively, together with trace amounts of a mixture of the two unidentified products (Scheme 2). The oily product **A** possibly was the nitrite **8**, which could be formed by reaction of **7** with nitric oxide under aerobic conditions.[30]



Scheme 2. Radical reactions of **1** and **2d**.

From the above kinetic results we reasoned that dioxygen can induce an autocatalytic chain decomposition of RSNOs that largely predominates over the unimolecular S–N bond scission.

However, we obtained sound evidence that the presence of dioxygen alone is not sufficient to induce the autocatalytic decomposition. In fact, when a kinetic experiment was carried out on a solution of **1a** under continuous bubbling of dioxygen, a remarkable decrease in the rate of decomposition was observed (Figure 4, curve a). We hypothesized that in this case the endogenous nitric oxide, produced by homolytic cleavage of the S–N bond, was removed from the reaction medium by the stream of dioxygen. So, we assumed that the autocatalytic chain decomposition is induced by the simultaneous presence of both dioxygen and nitric oxide.

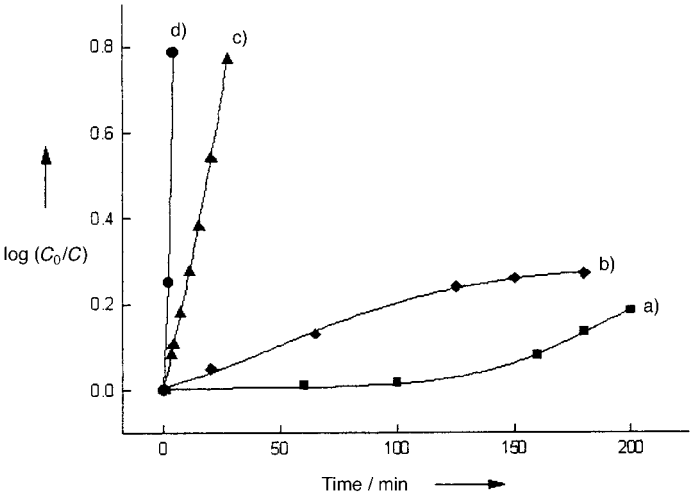


Figure 4. Kinetic curves obtained from the decomposition of *S*-nitrosothiols **1a**,**c**,**d** at 0 °C: a) 12 mM solution of **1a** in *n*-octane under bubbling of oxygen; b) 12 mM solution of **1d** in *n*-octane added with nitric oxide; c) 12 mM solution of **1c** in *n*-octane added with nitric oxide; d) 12 mM solution of **1a** in *n*-octane added with nitric oxide.

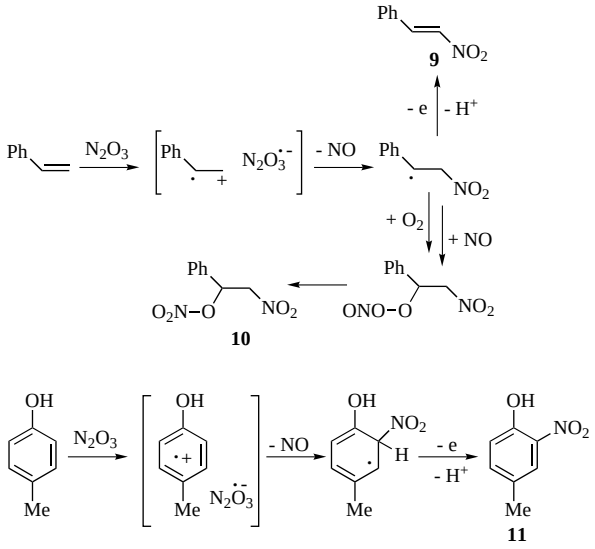
Our assumptions on the key role of nitric oxide were proved correct by the finding that a noticeable increase in the decomposition rate was observed when air-saturated solutions of **1a**,**c**,**d** were added with exogenous nitric oxide. Under these conditions a *pseudo*-first-order kinetic decay was followed by **1a**,**c** (Figure 4, curve c,d). Once more, peculiar behavior was shown by the nitrosothiol **1d**: a *pseudo*-first-order decay was initially observed, afterwards the decomposition rate markedly slowed down (Figure 4, curve b).

We also found that a fast chain decomposition occurred at room temperature when nitric dioxide was bubbled into a carefully deaerated solution of **1a** in *n*-hexane. Complete disappearance of the red color was detected within 1 min.

Since nitrous anhydride (N_2O_3) was expected to be rapidly formed under aerobic conditions from the reaction of nitric oxide and dioxygen,[5, 31] and under anaerobic conditions from nitric oxide/nitric dioxide coupling, we could infer that N_2O_3 was most likely to be the real chain carrier involved in the chain-decomposition reaction of *S*-nitrosothiols **1a**–**e**.

We have recently reported[32] that N_2O_3 is capable of oxidizing suitable organic substrates, such as styrene and

p-cresol, through an ET (electron transfer) process; the resulting radical ion pair decomposes to nitric oxide and nitro-substituted radicals, from which reaction products **9**, **10**, and **11**, respectively, are eventually formed (Scheme 3). An analogous ET process between N_2O_3 and *S*-nitrosothiols **1** might lead to a radical ion pair, which could decompose to some intermediate, possibly the radical **3**, with the release of nitric oxide (Scheme 2). The latter could be oxidized by dioxygen to N_2O_3 (chain initiation). In turn, the radical intermediate **3** could undergo homolytic S–N bond scission with formation of sulfanyl radicals (precursors of the disulfides **2**) and N_2O_3 (chain propagation).



Scheme 3. Oxidation by N_2O_3 and the formation of **9**, **10**, and **11**.

Based on this mechanism, the *pseudo*-first-order kinetic law observed when the decomposition of **1a**,**c** occurred in the presence of an excess of exogenous nitric oxide (Figure 4, curves c,d) can be explained by assuming that a nearly steady concentration of N_2O_3 was achieved. Furthermore, the unexpected dependence of the decomposition rate on the temperature (Figure 3) that suggests that two contrasting effects operate can be accounted for by assuming that both the chain-propagation reaction and the formation of the chain carrier, N_2O_3, are disfavored by increasing the temperature, since the higher the temperature the lower the concentration of gaseous NO, O_2, and N_2O_3. But, in the meantime, both reactions are favored as a consequence of the enhanced kinetic rate constants.

As for the reaction products obtained from **1a**–**c**,**e**, disulfides **2a**–**c**,**e** were derived by dimerization of the corresponding sulfanyl radicals, whereas the source of the thiosulfonate derivatives, RS–SO_2R, is still unclear. Benzaldehyde (**4**) might arise from the radical cation $[PhCH_2SNO]^{\bullet+}$ through a competitive deprotonation process followed by the release of nitric oxide and hydrolysis of the resulting unstable thioaldehyde (Scheme 2).[33]

Triphenylmethanol (**7**) and benzophenone (**6**), detected as the main products from the decomposition of **1d**, were probably derived from the disulfide **2d** initially formed. As mentioned above, the disulfide **2d** is in equilibrium with the corresponding thiyl radical, $Ph_3CS^{\bullet}$, as well as perthiyl radicals, $Ph_3CSS^{\bullet}$, and trityl radicals, $Ph_3C^{\bullet}$.[26] The latter are known to be trapped by dioxygen to give the peroxyl radical, $Ph_3COO^{\bullet}$, and then the hydroperoxide, Ph_3COOH.[26] Most likely, under our experimental conditions the peroxyl radical is rapidly scavenged by NO ($k = 10^9\,mol^{-1}\,sec^{-1}$).[34] The resulting peroxynitrite, Ph_3COONO, is expected to fragment to give the trityloxyl radical, $Ph_3CO^{\bullet}$, which can lead to trityl alcohol (**7**), by a hydrogen-abstraction reaction, and benzophenone (**6**) by an initial O-neophylic rearrangement followed by the β-elimination of a phenyl radical.[35] Subsequent reaction of alcohol **7** with N_2O_3 would lead to the nitrite **8** (product **A**; vide supra) (Scheme 2). The peculiar kinetic behavior exhibited by **1d** (Figure 2, curve b and Figure 4, curve b) can be explained by assuming that the reaction of nitric oxide with dioxygen to give the chain carrier N_2O_3 can be partially prevented by NO/$Ph_3CS^{\bullet}$ coupling to give back **1d**; moreover, the formation of the nitrite **8** would lead to N_2O_3 consumption (chain-termination reaction). So, the rate of the formation of N_2O_3 (chain carrier) should progressively decrease by progressively increasing the disulfide **2d** and the alcohol **7** concentration.

To obtain further evidence for the crucial role of N_2O_3 as the chain carrier, we performed the decomposition of **1a** in air-saturated solutions in the presence of styrene and *p*-cresol. As mentioned before, these substrates react efficiently with N_2O_3 through an initial ET process,[32] so they were expected to behave as potential inhibitors of the chain decomposition of **1a**.

Actually, we found that the presence of equimolar amounts of styrene strongly inhibited the autocatalytic reaction (Figure 5, curve a). The ^{1}H NMR quantitative analysis of the reaction mixture showed the formation of β-nitrostyrene (**9**) and 1-(1-phenyl-2-nitro)ethyl nitrate (**10**) (Scheme 3) in a 1:1 ratio and in 44% overall yield, based on starting styrene, as well as dibenzyl disulfide (**2a**) and minor amounts of

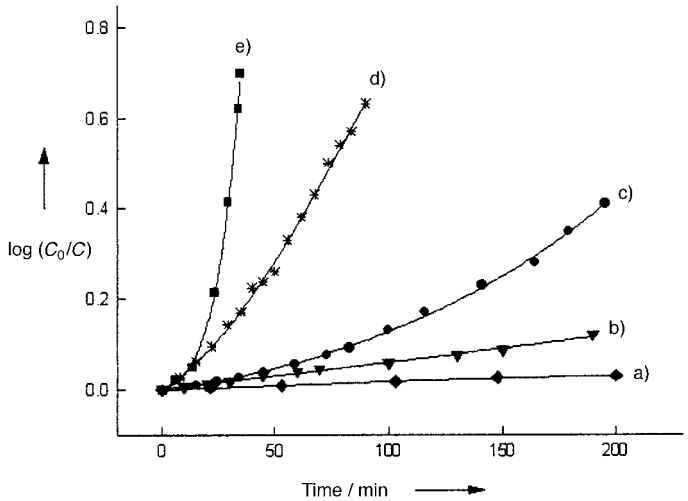


Figure 5. Kinetic curves obtained from the decomposition under aerobic conditions of 12 mM solutions of *S*-nitrosothiol **1a** at 0 °C: a) in the presence of 12 mM styrene; b) in the presence of 20 mM *p*-cresol; c) in the presence of 2 mM *p*-cresol; d) in the presence of 2 mM BHT; e) in the absence of antioxidants.

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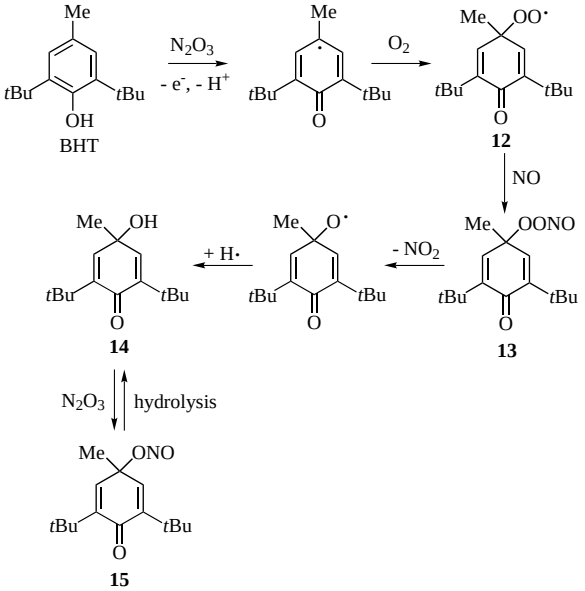
benzaldehyde (**4**), the thiosulfonate derivative, RS–SO_2R (R = $PhCH_2$), and two unidentified compounds.

Analogously, when the decomposition of a 12 mM **1a** solution was conducted in the presence of both 20.0 and 2.0 mM *p*-cresol, the autocatalytic reaction rate decreased with the increase in the *p*-cresol concentration (Figure 5, curves b,c). The ^{1}H NMR quantitative analysis of the mixture from the former reaction showed that the *p*-cresol was almost quantitatively converted (> 90%) into 2-nitro-*p*-cresol (**11**), as expected from the ET reaction between *p*-cresol and N_2O_3[32] (Scheme 3). In addition, the disulfide **2a**, benzaldehyde (**4**), and the thiosulfonate derivative, RS–SO_2R (R = $PhCH_2$), were detected in a 75:20:5 ratio.

We also found that 2,6-di-*tert*-butyl-4-methylphenol (BHT), a well-known antioxidant,[36] is capable of inhibiting the autocatalytic decomposition of **1a**, as shown by a kinetic experiment carried out in the presence of BHT (2.0 mM) (Figure 5, curve d).

When a solution of **1a** (12 mM) in *n*-pentane decomposed in the presence of equimolar amounts of BHT, the ^{1}H NMR analysis of the reaction mixture showed the formation of 2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone (**14**)[37] and an unidentified compound (**B**) in 45 and 18% yield, respectively, as well as the disulfide **2a**, unreacted BHT (30%), and minor amounts of the aldehyde **4** and the thiosulfonate derivative, RS–SO_2R. The subsequent silica gel column chromatography separated, in addition to **2a** and the thiosulfonate, RS–SO_2R, the hydroxy derivative **14** in 52% yield. No traces of the product **B** were found. In order to obtain evidence about its identity, a solution of BHT in *n*-octane was reacted for three hours with nitric oxide at 0 °C in the presence of air. ^{1}H NMR analysis of the reaction mixture showed complete disappearance of BHT and the presence of the compound **B** as the exclusive reaction product. The latter was found to give the product **14** on standing in air or by absorption on silica gel. We suggest that the product **B** might be the nitrite **15**, from which the alcohol **14** can form by subsequent hydrolysis (Scheme 4).



Scheme 4. Reaction of 2,6-di-*tert*-butyl-4-methylphenol (BHT) with N_2O_3.

The formation of the hydroxy derivative **14** can be accounted for by an initial ET process between BHT and N_2O_3 that leads to the intermediate radical cation BHT$^{\bullet+}$. The latter can give the peroxyl radical **12** through deprotonation and trapping of the resulting cyclohexadienyl radical by dioxygen. Coupling between radical **12** and nitric oxide can give the peroxynitrite **13**, from which **14** arises by loss of nitric dioxide and a subsequent hydrogen-abstraction reaction. The postulated nitrite **15** (compound **B**) might derive from the following nitrosation of **14**.[30]

Conclusion

Under anaerobic conditions *S*-nitrosothiols **1a–e** have been proved to undergo thermal decomposition by homolytic cleavage of the S–N bond that leads to nitric oxide and sulfanyl radicals in a reversible reaction. As a consequence, the decomposition rate is strongly decreased by the presence of endogenous and/or exogenous nitric oxide. Rate constants, k_1, of the unimolecular S–N bond scission have been determined at different temperatures from kinetic measurements performed in refluxing solvents. In sharp contrast to previous reports, tertiary nitrosothiols **1c** ($k_{1(69\,^\circ C)} = 13 \times 10^{-3}$ min^{-1}) and **1d** ($k_{1(69\,^\circ C)} = 91 \times 10^{-3}$ min^{-1}) decompose faster than primary nitrosothiols **1a** ($k_{1(69\,^\circ C)} = 3.0 \times 10^{-3}$ min^{-1}) and **1b** ($k_{1(69\,^\circ C)} = 6.5 \times 10^{-3}$ min^{-1}).

Activation energies ($E^{\#} = 20.5 - 22.8$ Kcal mol^{-1}), calculated through the Arrhenius equation, are significantly lower than the S–N dissociation bond energies (DBEs) reported in the literature (ca. 25 Kcal mol^{-1}).

Conversely, under aerobic conditions, the decay of *S*-nitrosothiols **1a–e** takes place by an autocatalytic chain-decomposition process catalyzed by N_2O_3. The latter is formed by reaction of dioxygen with the endogenous nitric oxide. The rate of the chain reaction is independent of the RSNO concentration and decreases with increasing bulkiness of the alkyl group; this shows that steric effects are crucial in the propagation step.

The autocatalytic decomposition is strongly inhibited by removing the endogenous nitric oxide or by the presence of antioxidants, such as *p*-cresol, β-styrene, and BHT, which are preferentially oxidized by N_2O_3. On the other hand, the addition of exogenous nitric oxide causes a noticeable increase in the chain-decomposition rate.

Experimental Section

General: NMR spectra were recorded with a Varian Gemini 200 instrument using Me_4Si as an internal standard. GC-MS analyses were performed with a Carlo Erba QMD 1000 instrument. Mass spectra were recorded with a VG 7070E instrument using electron impact ionization. Kinetic measurements were performed with Pharmacia Biotech Ultrospec 1000 UV/Vis spectrophotometer.

Materials: *S*-Nitrosothiols **1a–e** were synthesized from the corresponding thiols, commercially available, and peroxynitrite.[21] An aqueous solution of peroxynitrite (0.4 M, pH = 13.5) (2.5 mL, 1 mmol) was added at 5–10 °C under stirring to a solution (10 mL) in acetonitrile of the appropriate thiol (1 mmol) and HCl (12 M, 0.2 mL); the resulting mixture was stirred for a further 10 min. In the case of *S*-nitrosothiols **1a–c,e** the reaction mixture

was extracted with the appropriate organic solvent (30 mL), and the organic layer was washed twice with water (20 mL), twice with sodium carbonate (10%, 20 mL) to eliminate the unreacted thiol, and then dried with Na_2SO_4. Yields of **1a–c,e** were determined spectrophotometrically (**1a**: λ_{max} (ε) = 550 nm (26 $mol^{-1}dm^3cm^{-1}$);[22, 23] **1b**: λ_{max} (ε) = 550 nm (21 $mol^{-1}dm^3cm^{-1}$);[22] **1c**: λ_{max} (ε) = 603 nm (16 $mol^{-1}dm^3cm^{-1}$);[23] **1e**: λ_{max} (ε) = 570 nm (42 $mol^{-1}dm^3cm^{-1}$)[24]), and then the solutions were diluted to reach the desired concentration. In the case of **1d**, the green solid separated from the reaction mixture was filtered, washed with water, and dried under vacuum [185 mg, 60%; λ_{max} (ε) = 603 nm (17 $mol^{-1}dm^3cm^{-1}$)].

Peroxynitrite was synthesized following the previously reported procedure.[21] The concentration (usually in the range 0.45–0.50 M) was determined spectrophotometrically [λ_{max} (ε) = 302 nm (1670 $mol^{-1}dm^3cm^{-1}$)].[38] Solutions kept at −18 °C showed little decomposition over several weeks.

Kinetic measurements under anaerobic conditions: A solution of **1a–d** (12 mM) in the appropriate solvent (cyclopentane: b.p. = 50 °C; hexane: b.p. = 69 °C; cyclohexane: b.p. = 80.5 °C; and methylcyclohexane: b.p. = 101 °C) was deaerated by bubbling with argon at room temperature for 15 min, and then introduced into a thermostatic bath kept at a temperature 5 °C higher than the solvent boiling point in order to maintain a moderate reflux. The apparatus was kept in the dark under an argon atmosphere. Samples were taken at fixed periods of time up to 200 min. In all cases a first-order kinetic decay was followed. The kinetic constant values are reported in Table 1.

Kinetic measurements under aerobic conditions: Kinetic measurements were generally performed in an open flask, without stirring, with a solution of **1a–e** in *n*-pentane (12 mM) for experiments carried out at 0 °C, or solutions of **1a** in *n*-octane (12 mM) for experiments carried out at 18, 33, and 50 °C. Also, kinetic measurements at 0 °C were performed with solutions of **1a** (6 mM and 16 mM) in *n*-pentane. Experiments performed in the presence of added nitric oxide were carried out by bubbling for 10 s nitric oxide into the appropriate solution of *S*-nitrosothiol **1a,c,d** in *n*-octane. Kinetic curves are reported in Figures 2–4.

Reaction products from decomposition of 1a–c,e under anaerobic conditions: Solutions of **1a–c,e** were left to react in refluxing *n*-hexane until disappearance of the color was detected. GC-MS analysis of the reaction mixtures detected the exclusive presence of the corresponding disulfide **2a–c,e** (yield not determined).

Reaction products from decomposition of 1a–e under aerobic conditions: GC-MS analysis of the reaction mixtures obtained from the decomposition of solutions of **1b,c,e** (12 mM) showed the exclusive presence of the disulfide **2b,c,e** and the thiosulfonate derivative, RS−SO_2R (**2**/RS−SO_2R relative ratio: 93:7 (R = *n*-hexyl), 93:7 (R = *tert*-butyl), and 87:13 (R = phenyl), overall yield not determined).

GC-MS analysis of the reaction mixture obtained from **1a** (40 mL of a 12 mM solution in *n*-pentane, 0.48 mmol) showed the formation of compounds **2a** and **4**, and the thiosulfonate RS−SO_2R (R = $PhCH_2$). The *n*-pentane solvent was carefully removed under vacuum. ^{1}H NMR quantitative analysis of the resulting residue, performed by using acetophenone as the internal standard, showed the above products to be present in a 70:25:5 ratio and in 80% overall yield together with small amounts of an unidentified product showing a singlet at δ = 4.0.

From the reaction mixture obtained from **1d** (40 mL of a 12 mM solution in *n*-pentane, 0.48 mmol) small amounts of a yellow oil separated from the solution; the *n*-pentane solvent was removed, and the residue was purified by chromatography on a silica gel column; gradual elution with petroleum ether (b.p. 40–70 °C)/diethyl ether gave: i) a mixture of two unidentified products (6 mg); ii) benzophenone (**6**) (20 mg, 23%); and iii) triphenylmethanol (**7**) (62 mg, 50%). In a repeated experiment we separated the above yellow oil [MS (70 eV): *m*/*z* (%): 244 (55), 167 (70), 165 (100)] which rapidly decomposed on standing in air to give **7**.

Reaction products from the decomposition of 1a under aerobic conditions in the presence of styrene: The decomposition of **1a** (40 mL of a 12 mM solution in *n*-octane, 0.48 mmol) carried out in the presence of air and styrene (50 mg, 0.48 mmol) was complete after approximately two days. The solvent was evaporated. ^{1}H NMR analysis of the residue, by using acetophenone as the internal standard, showed the formation of the disulfide **2a** (60%), benzaldehyde **4** (yield not determined), RS−SO_2R (R = $PhCH_2$) (4%), an unknown product showing a singlet at δ = 4.0, *β*-nitrostyrene (**9**) (22%, based on starting styrene), 2-nitro-1-phenylethyl nitrate (**10**)[32] (22%, based on starting styrene), and an unknown product (7%) [^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 4.45 (dd, J_{AB} = 12.7 Hz, J_{AX} = 3.5 Hz, 1H; A part of an ABX system), 4.55 (dd, J_{AB} = 12.7 Hz, J_{BX} = 9.0 Hz, 1H; B part of an ABX system), 5.40 (dd, J_1 = 9.0 Hz, J_2 = 3.5 Hz, 1H)].

Reaction products from the decomposition of 1a under aerobic conditions in the presence of *p*-cresol: ^{1}H NMR quantitative analysis (acetophenone as the internal standard) of the reaction mixture obtained from the decomposition of **1a** (40 mL of a 12 mM solution in *n*-pentane, 0.48 mmol) carried out in the presence of air and *p*-cresol (85 mg, 0.80 mmol) showed the formation of 4-methyl-2-nitrophenol (**11**) (100% yield, 90% conversion) together with products **2a**, **4**, and RS−SO_2R (R = $PhCH_2$) in a 75:20:5 ratio (overall yield not determined), and an unknown product showing a singlet at δ = 4.0.

Reaction products from the decomposition of 1a under aerobic conditions in the presence of 2,6-di-*tert*-butyl-4-methylphenol (BHT): A solution of **1a** in *n*-octane (12 mM, 40 mL) containing BHT (0.48 mmol, 105 mg) was allowed to react at 0 °C in a open tube until the disappearance of the red color (ca. two days), and then the solvent was removed. ^{1}H NMR analysis of the residue, by using acetophenone as the internal standard, showed the presence of unreacted BHT (30%), the disulfide **2a** (60%), benzaldehyde (**4**) (yield not determined), RS−SO_2R (R = $PhCH_2$) (4%), an unknown product showing a singlet at δ = 4.0, the 4-hydroxycyclohexanedione **14**[37] (45%, based on reacted BHT), and the product **B** (see below) (18%, based on reacted BHT). Subsequent silica gel column chromatography gave, by gradual elution with petroleum ether (b.p. 40–70 °C)/diethyl ether: i) the disulfide **2a** (65 mg, 55%); ii) RS−SO_2R (R = $PhCH_2$) (5 mg, 4%); iii) the cyclohexanedione **14** (40 mg, 0.17 mmol, 52%).

Reaction of BHT with nitric oxide: A solution of BHT in *n*-octane (40 mL, 90 mg, 0.4 mol) was saturated with gaseous nitric oxide and allowed to react at 0 °C in a sealed tube. After 2 h, TLC analysis showed the complete disappearance of the starting BHT. The residue obtained after removal of the solvent was composed of the compound **B**, as evidenced by TLC and ^{1}H NMR analysis [^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 1.21 (s, 18H), 1.82 (s, 3H), 6.73 (s, 2H)]. Compound **B**, which tentatively was assigned the structure of nitrite **15**, underwent quantitative decomposition to **14** upon standing in air or by absorption on silica gel.

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