Decomposition of *S*-Nitrosothiols: Unimolecular versus Autocatalytic Mechanism

Loris Grossi,* Pier Carlo Montevecchi,* and
Samantha Strazzari

*Dipartimento di Chimica Organica "A. Mangini"
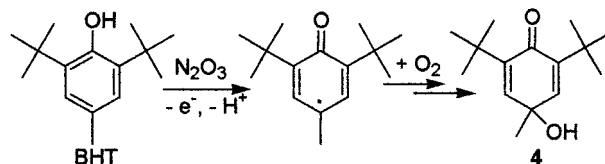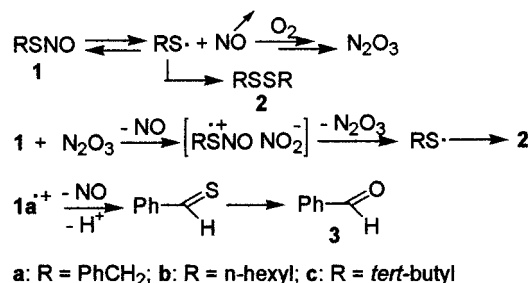Università di Bologna, Viale Risorgimento 4,
I-40136 Bologna, Italy*

We report herein that nitrosothiols (RSNOs) undergo thermal decomposition at a rate independent of the bulkiness of the alkyl group. The disappearance of RSNO is inhibited if nitric oxide, produced by reversible homolytic S−N bond scission, is not removed from the reaction mixture. When the decomposition is carried out in aerated solution, the disappearance of RSNO is faster and occurs through an autocatalytic mechanism induced by N_2O_3, which is produced by dioxygen oxidation of the endogenous nitric oxide. The chain-decomposition reaction rate is dependent on the bulkiness of the alkyl group, this suggesting that steric factors play an important role in the propagation step.

In the past decade a large number of biological roles have been ascribed to *S*-nitrosothiols;[1] in particular, it has been postulated that nitrosothiol derivatives of biological molecules, for instance cysteine and glutathione, can act as in vivo carriers of nitric oxide.[2] Despite the present great interest of chemists and biochemists toward these compounds, a concrete picture of the mechanism of the decomposition leading to nitric oxide under thermal conditions is still lacking, since the data available in the literature are often contradictory.

Since early experiments,[3] it is generally assumed that RSNOs decompose through a unimolecular mechanism by homolytic cleavage of the labile S−N bond, even if it has been reported that the half-life time ($t_{1/2}$) of RSNO is dependent on its concentration[4] and the presence of air.[5] In addition to the homolytic cleavage mechanism, the involvement of heterolytic pathways also has been hypothesized.[6] The thermal stability of *S*-nitrosothiols has been reported to be affected by steric factors.[7,8] RSNOs exist as two geometrical syn and anti isomers, due to some S−N double bond character.[7] Primary and secondary *S*-nitrosothiols seem to adopt the syn conformation, red color, whereas tertiary nitrosothiols, owing to steric factors, would prefer

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a: R = PhCH$_2$; **b**: R = n-hexyl; **c**: R = *tert*-butyl

the anti conformation, green. Bulky nitrosothiols are believed to be more stable, but a reliable explanation is still unknown.

To achieve a more confident picture of the chemical behavior of RSNOs we have undertaken a kinetic study of the decomposition of a number of simple *S*-nitrosothiols under different reaction conditions.

For this preliminary work, benzylnitrosothiol (**1a**) and *n*-hexylnitrosothiol (**1b**), both red, and *tert*-butylnitrosothiol (**1c**), green, have been considered[9,10] and the effect of the presence of nitric oxide, dioxygen, and antioxidants on their decomposition mechanism has been investigated.

The thermal decomposition was monitored spectrophotometrically at $\lambda = 550$ nm for **1a**[11] and **1b**[11a] and $\lambda = 605$ nm for **1c**[11b] in 12 mM *n*-pentane (or *n*-octane) solution under various reaction conditions.

When the experiments were conducted at 0 °C in *n*-octane deareated solutions under bubbling of argon, nitrosothiols **1a,c** decomposed within a few days at the same rate following a first-order kinetic law ($k = 2.8 \times 10^{-4}$ min^{-1}) (Figure 1, curve a). For both substrates, GC-MS analysis of the resulting reaction mixture showed the exclusive presence of the corresponding disulfide **2a,c**. But, we found that complete decomposition of nitrosothiols **1a**−**c** occurred after several weeks (more than 5−6) when a deareated *n*-pentane solution was kept in a sealed tube under argon atmosphere at both 0 and 25 °C. These results led us to infer that nitrosothiols undergo unimolecular homolytic scission of the S−N bond at a rate independent of the bulkiness of the alkyl group. In principle, the resulting sulfanyl radicals can dimerize to disulfide **2** or undergo a competing *out of cage* recombination to RSNO. The former was the exclusive reaction allowed when nitric oxide was removed through bubbling of argon. When the decomposition was carried out in a sealed tube, the reversible coupling of sulfanyl radicals with nitric oxide might efficiently compete with the dimerization, and that can account for the slower rate of disappearance of RSNOs observed (Scheme 1).

In air-saturated *n*-pentane solution, nitrosothiols **1a**−**c** decomposed at 0 °C at a rate that was found to be dependent on the nature of the alkyl group and not to follow a simple first-order

(9) As we have recently reported (see ref 10), *S*-nitrosothiols **1a,b** (80%) and **1c** (62%) were obtained by reacting the corresponding thiol (1 mmol) with aqueous (pH 13.5) 0.40 M peroxynitrite (5 mL) and 12 M hydrochloric acid (0.45 mL) in acetonitrile solution (10 mL).

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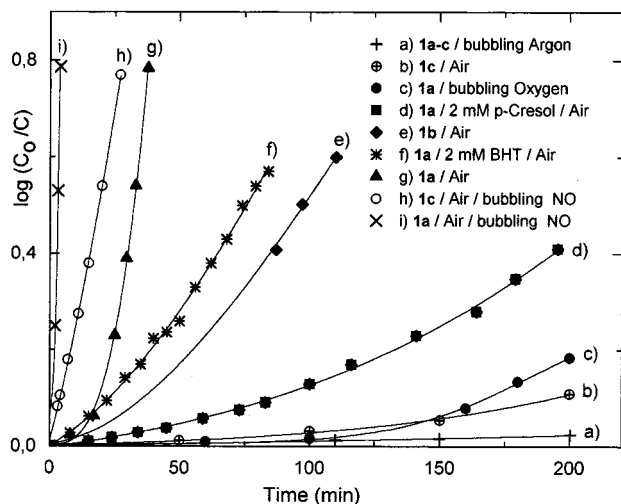
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Figure 1. Kinetic curves for the decomposition of 12 mM *S*-nitrotothiols **1a**−**c** at 0 °C in *n*-pentane (or *n*-octane) solutions.

kinetic law (Figure 1, curves b, e, and g). GC-MS and NMR analyses of the reaction mixture obtained from **1a** showed the presence of the disulfide **2a,** benzaldehyde (**3**), and the sulfone derivative (RSSO$_2$R) in 70:25:5 ratio and in 80% overall yield. Similarly, GC-MS analysis of reaction mixtures obtained from **1b,c** showed the presence of the disulfides **2b,c** besides small amounts of the corresponding sulfone derivative.[12] Kinetic measurements for the decay of **1a** were repeated in air-saturated *n*-pentane 16.7 and 6.0 mM solutions, but no effect of the concentration of **1a** on the kinetic curve (log(C_0/C) vs time) was observed. This finding contradicts the previous statement that the half-life time of RSNOs is dependent on their concentration.[4]

The kinetic curves (plots of log(C_0/C) vs time) obtained for the decomposition of **1a**−**c** in the presence of air show that the rate of disappearance increases with time. Such a kinetic behavior points to the involvement of an autocatalytic process. It can be reasoned that, in the presence of dioxygen, the unimolecular decay of **1a**−**c** takes place in competition with a chain-decomposition reaction. The increase of the rate of disappearance must be related to the progressive incursion of the chain-decomposition process due to a progressive growth of the chain carrier concentration.

It appears that the rate of the chain decay is strongly dependent on the bulkiness of the alkyl group, as shown by comparison of the kinetic curves obtained for nitrosothiols **1a,b** and **1c**. This suggests that important steric effects are involved in the chain propagation step and might account for the general claim that the stability of nitrosothiols increases with bulkiness.

The autocatalytic chain-decomposition seems to be induced by the presence of both dioxygen and nitric oxide, the latter produced from the unimolecular cleavage of nitrosothiol **1a**−**c**. In fact, when the endogenous nitric oxide was removed from the reaction mixture by bubbling of dioxygen the rate of decay of **1a** was found to be strongly diminished (Figure, curve c). In the meantime, when exogenous nitric oxide was bubbled for 10 s into an aerated *n*-octane solution at 0 °C, the rate of decomposition

of **1a,c** was found to be strongly enhanced. Under these conditions the decomposition rate was found to be first order in nitrosothiol **1a,c** (Figure 1, curves i and h).

Based on the above, and since it is well-known that nitric oxide rapidly reacts with dioxygen to give N$_2$O$_3$,[2a,13] we can infer that the latter is most likely the chain carrier responsible for the chain-decomposition of nitrosothiols.

As for the actual reaction mechanism of the N$_2$O$_3$-catalyzed decomposition of nitrosothiols, some speculative proposals can be made. We have recently reported[14] that N$_2$O$_3$ is capable of oxidizing organic substrates, viz styrene and *p*-cresol, through an initial ET process leading to nitric oxide and a radical ion pair. Similarly, an ET process between N$_2$O$_3$ and nitrosothiol would lead to nitric oxide and a radical ion pair (or a σ complex), from which sulfanyl radicals (and then disulfide **2**) arise by unimolecular release of N$_2$O$_3$ (Scheme 1). It is notable that, in this suggestion, the concentration of the endogenous nitric oxide, and then N$_2$O$_3$, increases with time: this accounts for the observed autocatalytic behavior. On the other hand, in the presence of exogenous nitric oxide the concentrations of both NO and N$_2$O$_3$ are expected to be rather constant and this accounts for the observed first-order decomposition observed under these conditions.

The formation of benzaldehyde **3**, when **1a** was investigated, can be accounted for by a competitive deprotonation of **1a**$^{•+}$ by the nitrite counterion, followed by loss of nitric oxide and eventual hydrolysis of the unstable benzothioaldehyde.

Supporting evidence to the involvement of N$_2$O$_3$ as the chain carrier in the decomposition of RSNOs came from results obtained from the decomposition of **1a** in air-saturated solution carried out in the presence of 2 mM *p*-cresol, which is known to react with N$_2$O$_3$ leading to 2-nitro-*p*-cresol.[14] Under these conditions the autocatalytic chain-decomposition of **1a** still occurred, but at a lower rate (Figure 1, curve d). Actually, the analysis of the resulting reaction mixture showed the formation of 2-nitro-*p*-cresol (>90% conversion), besides products **2a** and **3**, and the sulfone derivative. The rate of decay of **1a** was strongly diminished also by the presence of 2 mM BHT, a well-known antioxidant[15] (Figure 1, curve f). The analysis of the resulting reaction mixture showed the presence of **2a**, **3** and the sulfone derivative (80% overall yield), and the 4-hydroxycyclohexadienone **4**[16] (80% conversion). The latter was also obtained in nearly quantitative yields in a blank experiment conducted by bubbling nitric oxide in an *n*-pentane solution of BHT. Most likely compound **4** arises from an initial ET process followed by deprotonation of the BHT$^{•+}$ radical cation intermediate, and then coupling of the resulting cyclohexadienonyl radical with dioxygen (Scheme 1).

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