

ELECTRON SPIN RESONANCE SPIN TRAPPING OF THIYL RADICALS
FROM THE DECOMPOSITION OF THIONITRITES

P. David Josephy*, Detlef Rehorek‡, and Edward G. Janzen,
Guelph-Waterloo Centre for Graduate Work in Chemistry,
Department of Chemistry and Biochemistry,
University of Guelph,
Guelph, Ontario, Canada N1G 2W1.

ABSTRACT

Alkyl thiyl radicals produced by the homolytic decomposition of thionitrites are detected by ESR spin trapping using 5,5-dimethyl-1-Δ-pyrroline-N-oxide (DMPO).

Alkyl thionitrites (RSNO; thionitrous acid esters) have been prepared by nitrosation of thiols with alkyl nitrites (1), N₂O₄ (2), and other agents. Most thionitrites are both thermally (3) and photochemically (4) unstable, especially in the presence of O₂ (5). The initial step in thionitrite decomposition is believed to be homolytic cleavage of the S-N bond to give NO and an alkyl thiyl radical:



Decomposition of thionitrites yields disulfides and nitric oxide (1) and induces polymerisation of methyl methacrylate (4,6). The thiyl radical mechanism was also invoked to explain the addition of thionitrites across CC double bonds of alkenes (7). We now report the detection of the initial thiyl radicals formed by the thermal decomposition of thionitrites, using the technique of ESR spin trapping (8). Thionitrites were prepared by mixing a solution of isoamyl nitrite with a solution of the thiol and spin trap:



Typical final concentrations were thiol, 0.2M, isoamyl nitrite, 0.1M, and spin trap, 0.01M. The solutions were gassed with N₂ and transferred to the ESR flat cell immediately after mixing, without exposure to air. The strongest ESR signals were obtained using the spin trap 5,5-dimethyl-1-Δ-pyrroline-N-oxide (DMPO), (Figure). The spectra obtained with ethanethiol, 2-hydroxyethanethiol, 2-methyl-2-propanethiol, 2-propanethiol and α-toluenethiol had similar, but distinctive, hyperfine splitting constants, and are assigned to the thiyl radical adducts. Typically, the signals persisted for half an hour or more.

*author for correspondence

‡ On sabbatical leave from Karl-Marx Universität, Leipzig, DDR.

The ESR spectrum of the thiyl radical spin adduct was not observed if any component of the system was omitted. Little change was noted when experiments were carried out in the dark, suggesting that thermal decomposition of the thionitrite was sufficient for thiyl radical formation. Photolysis of solutions containing excess isoamyl nitrite generated spin adducts of alkoxy radicals.

TABLE: Hyperfine Splitting Constants of Thiyl Radicals Spin Adducts ^a

Thiol	DMPO			PBN		MNP
	a_N	a_{β}^H	a^H	a^N	a_{β}^H	a_N
CH ₃ CH ₂ SH	1.34	1.16	0.08(2H) ^b	1.38	0.20	1.79
HOCH ₂ CH ₂ SH	1.38	1.42	0.07(2H) ^b	1.39	0.20	1.82
(CH ₃) ₃ CSH	1.35	1.12		N.D.		N.D.
(CH ₃) ₂ CHSH	1.34	1.12				
PhCH ₂ SH	1.36	1.17	0.114(2H) ^b	N.D.		1.74
Ph ₃ CSH	1.295	1.38		1.41	0.19	
Cysteine ^c	1.56	1.77				
	1.52	1.67				
Cysteine ^d	1.53	1.72				
N-Acetyl cysteine ^e	1.37	1.43				
Glutathione ^f	1.49	1.54				

Footnotes:

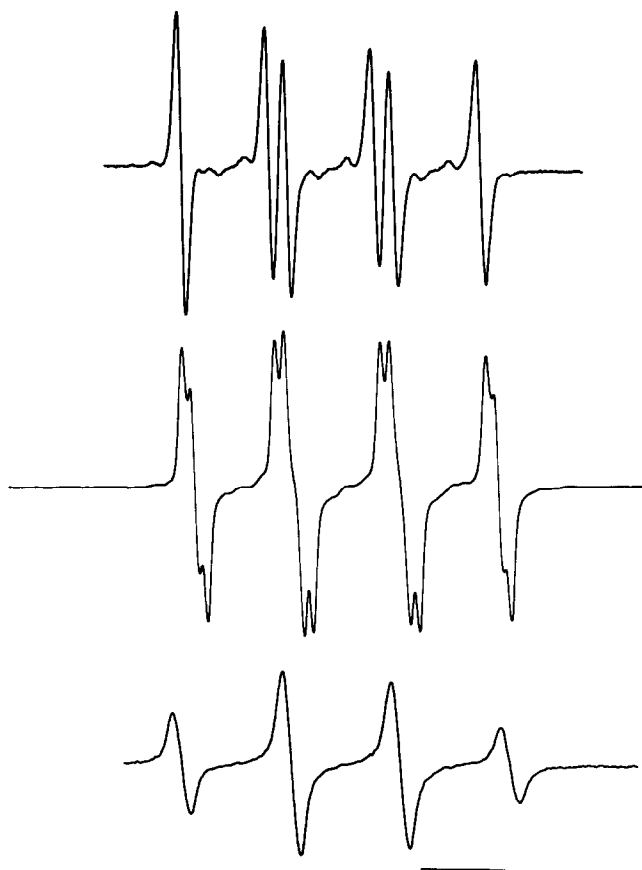
- (a) obtained by computer simulation of spectra, in mT; solvent, benzene, unless noted.
 (b) γ -H on spin adduct ring or δ -H on alkylthiyl group
 (c) in H₂O
 (d) reference 9
 (e) in CH₃CN
 (f) in H₂O/MeOH (3/1)

N.D.: not detected.

Triphenylmethyl thionitrite has been isolated as a solid (3). We prepared this compound by mixing triphenylmethyl mercaptan and isoamyl nitrite (3 equivalents) in benzene. After one hour, the solvent was removed under vacuum, and the resulting green solid was washed with large amounts of water and methanol. Triphenylmethyl thionitrite was recrystallized from CHCl₃ as dark green needles. Yield, 60%. A mixture of this thionitrite and DMPO gave a strong ESR signal with hyperfine parameters similar to those for the thiyl radical spin adducts prepared as described above.

N-Acetyl cysteine was examined in CH_3CN solution and gave a strong spectrum. To observe the cysteine thiyl radical, we used tert-butyl nitrite for better aqueous solubility, and higher DMPO concentrations (0.4M). Similarly, the thiyl radical from the tripeptide glutathione was trapped with DMPO in mixed aqueous/methanol solution. The spectra of the cysteine and glutathione thiyl radicals were short-lived, and were recorded within 5 minutes after initiation of the reaction.

A summary of the ESR hyperfine splitting constants of the thiyl radical spin adducts observed in this study is given in the Table. For cysteine, the spectrum was best computer simulated using a sum of two similar spectra with the listed splitting constants. We determined the g-values of the DMPO adducts of Ph_3CS^* (2.0067) and $\text{HOCH}_2\text{CH}_2\text{S}^*$ (2.0061; both values $\pm .0005$).



ESR Spectra of DMPO Thiyl Radical Adducts. Solutions were prepared as described in text, and ESR spectra were recorded on a Varian E-104 spectrometer. The spectra shown are those of the DMPO adducts of thiyl radicals derived from (top) 2-methyl-2-propanethiol, (middle) α -toluenethiol, (bottom) 2-hydroxyethanethiol. The scale bar indicates 1 mT.

Thiyl radical spin adducts of 2-methyl-2-nitrosopropane (MNP) are known. For example, Wargon and Williams studied the radiolysis of liquid thiols in the presence of MNP, and detected thiyl aminoxyl adducts characterized by hyperfine splitting constants of about 1.7 to 1.9 mT (10). We also trapped thiyl radical MNP adducts from some thionitrites, as shown in the table, and the observed hyperfine splitting constants were similar to those reported earlier. Recently, Graceffa reported the use of α -phenyl-N-tert-butylnitron (PBN) to trap cysteine thiyl radical formed by Ce^{4+} oxidation (11). We trapped the ethanethiyl and 2-hydroxyethanethiyl radicals with PBN as shown in the table. A mixture of triphenylmethyl thionitrite (0.25M) and PBN (0.1M) gave a weak spectrum consistent with the triphenylmethyl thiyl radical adduct (see Table). With time, a second spectrum became dominant. This 3-line spectrum ($a_N = 0.795$ mT) was probably due to the α -keto aminoxyl oxidation product of PBN (12) and was the only spectrum observed following photolysis of the solution.

We emphasize that DMPO is the spin trap of choice in this system. The DMPO spin adducts gave at least one hundred times more intense signals than the MNP or PBN spin adducts, and in many cases the latter spin traps did not give detectable signals. Probably, DMPO is more reactive towards RS^{\bullet} than are MNP or PBN. DMPO adducts show considerable dependence of hyperfine splitting constants on the structure of the thiyl radical trapped (see Table and Figure). We anticipate that DMPO will prove to be of wide applicability to studies of thiyl radicals in chemistry and biology.

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