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The Oxidative Ring Opening of the Cyclic Nitrone Spin Trap 5,5-Dimethyl-1-pyrroline-1-oxide (DMPO): Free Radical Involvment

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The preparation and reactions of the cyclic nitrone 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), were first reported by Todd and coworkers [1–3]. Recently, the free radical addition reactions of DMPO have become of interest as DMPO has found increasing use as a spin trapping agent [4]. Not the least important of the spin trapping results to emerge, is the ability to trap and detect superoxide (and alkyl peroxyl) radicals at room temperature since these radicals have been implicated in a number of biological processes [5, 6]. The electron spin resonance (ESR) spectrum of the DMPO superoxide spin adduct, 5,5-dimethyl-2-hydroperoxy-1-oxyl-pyrrolidine (DMPO–OOH), in aqueous solution, pH 7, is characterised by the hyperfine splitting constants a_N = 14.3 G, a_H = 11.7 G, a_H = 1.25 G at g = 2.0061 [7]. It decays to non-radical products, with a half-life of 90 s [8].

We investigated the reaction of μ-superoxo-bis-[pentaamminecobalt(III)] with DMPO; the properties of μ-superoxo-bis[pentaamminecobalt(III)] complex [9], are quite consistent with the presence of a bridging superoxide ligand. μ-superoxo-bis[pentaamminecobalt(III)] pentachloride and μ-peroxo-bis-[pentaamminecobalt(III)] tetranitrate were prepared by the methods previously described [10, 11]. The spin trap DMPO was prepared by the method of Bonnett *et al.* [1], and further purified by the method of Beuttner and Oberley [8]. 5,5-Dimethyl-1-hydroxy-2-pyrrolidone (DMPOXH) was prepared by the method of Elsworth and Lamchen [12]. 4-Methyl-4-nitroso-pentanoic acid (MNPA) was prepared by the method of Clark *et al.* [13].

The reaction between μ-superoxo-bis[pentaamminecobalt(III)] pentachloride (1 m*M*) and DMPO (1 m*M*) in phosphate buffer (50 m*M*) at pH 7 was followed by monitoring the A_{670} band, associated with the former species. The peroxo-complex (stable only in aqueous 7 *M* ammonia solution) showed no reaction. The final product mixture formed was extracted with chloroform (salting out with borax). The remaining aqueous phase gave the characteristic U.V. spectrum of pentaamminecobalt-(III) complexes, $[Co(NH_3)_5H_2O]^{3+}$ and $[Co(NH_3)_5OH]^{2+}$. The chloroform extract was concentrated *in vacuo* and the remaining residue was analyzed by g.l.c./mass spectrometry. The predominant product (55%) was found to be 4-methyl-4-nitro-pentanoic acid, MNOPA (^1H n.m.r. spectrum: methyl protons, = 1.50 ppm, methylene protons, = 1.3 and 1.6 ppm, and mass spectral data· molecular ion m/e = 163), with significant fractions (21 and 18%) of 4-methyl-4-nitroso-pentanoic acid (MNPA) and 5,5-dimethyl-1-hydroxy-2-pyrrolidone (DMPOXH). The rate of the reaction was measured by the rate of disappearance of the cobalt superoxo complex monitoring the decrease in the A_{670} band. The initial rate of this reaction showed the reaction to be first order with respect to DMPO, and first order with respect to the cobalt–superoxo complex with $k = 4.8\ 0.7 \times 10^{-3}$ $M^{-1}\ s^{-1}$.

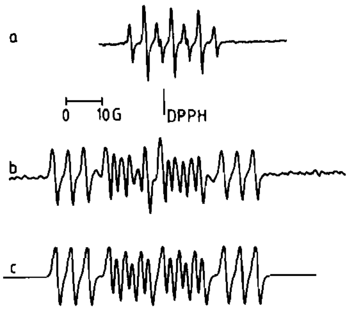

Fig. 1. ESR spectra: (a) DMPOX, (b) DMPO–PNO and (c) Computer simulation of (b). Reaction mixtures: 1 m*M* μ-superoxo complex, (a) 1 m*M* and (b) 100 m*M* DMPO in 50 m*M* phosphate buffer pH 7. ESR Instrumentation: A Varian E104, X-band spectrometer with. field set 3385 G, field scan 200 G, modulation freq. 100 kHz, modulation amplitude 1.0 G, receiver gain 4 × 10^3, time constant 0.064 s, scan time 90 s, microwave freq. 9.51 GHz, microwave power 10 mW, temperature 293 K. Signal lifetimes were followed by locking onto a peak in the ESR spectrum; signal concentrations were calculated from a calibrated double integral.

The presence of intermediates in the reaction was investigated using electron spin resonance spectroscopy (ESR). The observed ESR spectrum is given in Fig. 1a and is characterised by the hyperfine splitting constants a_N = 7.2 G and a_H = 4.1 G(2H) at g = 2.0048. The ESR spectrum is not that of DMPO–OOH. The same ESR spectrum was observed on the reaction of cumene hydroperoxide and haematin in the presence of DMPO [14] and was characterised [15] as an hydroxamyl radical. 5,5-dimethyl-1-oxyl-2-pyrrolidone (DMPOX). The lifetime of DMPOX is dependent on the presence of dioxygen; in the absence of dioxygen, the concentration of DMPOX rises to 95% of the initial concentration of DMPO and decays slowly to a wide range of products. Major

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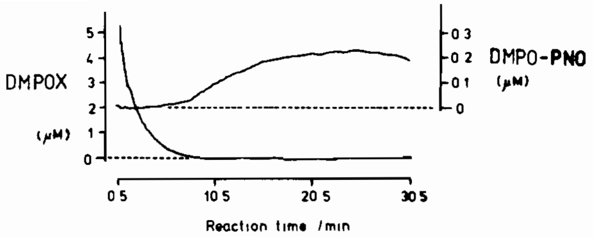
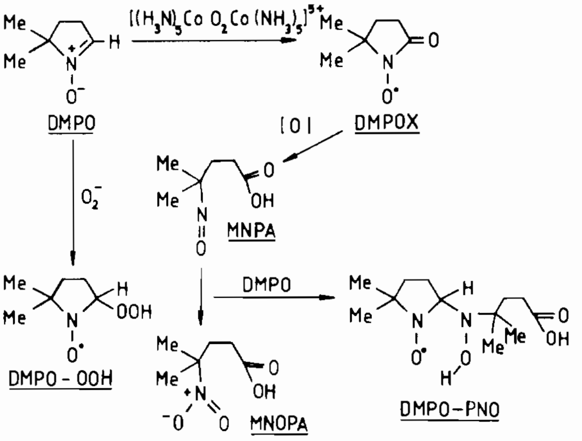


Fig. 2. Sequencial generation of DMPOX and DMPO–MPO.

fractions of the product mixture analyse as DMPOXH (14%), MNPA (29%), MNOPA (17%) and 4-methyl-4-nitroso-butane (14%). In the presence of dioxygen, however, DMPOX is short-lived – Fig. 2, decaying to 55% MNOPA. The initial rate of disappearance of DMPO and the μ-superoxo complex shows no dioxygen dependence. In the presence of excess DMPO (100:1 DMPO/cobalt superoxo complex), the ESR spectrum of a nitrogen-centred radical derived spin adduct, N-(5′,5′-dimethyl-1′-oxyl-pyrrolidine)-4-hydroxylamino-4-methyl-pentanoic acid (DMPO-PNO) is observed – Fig. 1b. DMPO–PNO is observed in the later stages of the reaction, after the decay of Fig. 2. DMPO–NPO is characterised by hyperfine splitting constants a_{N1} = 14.3 G, a_H = 16.2 G and a_{N2} = 4.2 G at g = 2.0053. A simulation of the ESR spectrum of DMPO–PNO is given in Fig. 2c. DMPO–PNO is observed during the oxidation of MNPA to MNOPA with ferricyanide, in the presence of DMPO. DMPOX is also observed during the periodate oxidation of the hydroxamate DMPOXH to MNPA.



Scheme 1. Reaction mechanisms for the addition of 'free' and coordinated superoxide to DMPO.

The sequence of events leading to the formation of MNOPA from DMPO is given in Scheme 1. We suggest that DMPOX Is formed by the spin trapping of a cobalt [III] superoxide fragment from the μ-superoxo-bis(pentaamminecobalt [III]) pentachloride, followed by rearrangement. This is similar to Rauckman and Rosen's interpretation of the cumene hydroperoxide/haematin system [15] and should be seen as a transition metal catalysed decomposition of peroxyl spin adducts of DMPO. The rapid formation of DMPOX from DMPO (formally a three electron oxidation) implicates a highly oxidising metal oxygen fragment *e.g.* cobaltyl Co(IV)=O and ferryl Fe(IV)=O states respectively. The ring opening of DMPOX is a relatively slow process but is enhanced by oxygenation, MNPA and finally, MNOPA are slowly formed. The reactivity of the spin trap DMPO towards oxidative ring opening reactions may be marked, especially when trapping peroxyl radicals in the presence of a certain redox active metal complexes. The observation of DMPOX and DMPO–PNO during spin trapping observations must be treated as artifactual. The spin trapping qualities of DMPO should be admired with respect for the other facets of its chemistry.

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References

1 R. Bonnett, R. F. C. Clark, V. M. Clark, I. O. Sutherland, and A. Todd, *J Chem Soc*, 2094 (1959).
2 R. Bonnett, V. M. Clark and A. Todd, *J Chem Soc*, 2102 (1959).
3 R. F. C. Brown, V. M. Clark, M. Lamchen and A. Todd, *J Chem Soc*, 2116 (1959).
4 E. G. Janzen and J. I. P Lui, *J Magn Res.*, *9*, 510 (1973).
5 W. Bors, M. Saran and G. Czapski, in 'Biological and Clinical Aspects of Superoxide and Superoxide Dismutase' (W. H. Bannister and J. V. Bannister, eds.), Elsevier North Holland, 1980, p 1.
6 E. G. Janzen, in 'Free Radicals in Biology', Vol. IV (W. A. Pryor ed.), Academic Press, New York, p. 116.
7 J. R Harbour, V. Chow and J. R. Bolton, *Can J Chem*, *52*, 3549 (1974).
8 G. R. Beuttner and L. W. Oberley, *Biochim. Biophys Res Commun.*, *83*, 69 (1978).
9 Crystallographic properties – R. E. Marsh and W. P. Shaefer, *Acta Cryst*, *B24*, 246 (1968); paramagnetic properties – E. A. V. Ebsworth and J. A. Weil, *J Phys Chem.*, *63*, 1890 (1959).
10 M. Ferrer, T. D. Hand and A. G. S. Sykes, *J. Chem Soc. Dalton Trans.*, 14 (1980).
11 R. G. Charles and S. Barnartt, *J Inorg Nuclear Chem.*, *22*, 69 (1961).
12 J. F. Elsworth and M. Lamchen, *J. South Afric Chem. Inst.*, *24*, 196–204 (1971).
13 V. M. Clark, B. Shlarz and A. Todd, *J Chem Soc.*, 2123 (1959).
14 R. A. Floyd and L. A. Soong, *Biochem. Biophys Res. Commun.*, *74*, 79 (1977).
15 G. M. Rosen and E. Rauckman, *Mol Pharmacol*, *17*, 233 (1980).