MOLECULAR PHARMACOLOGY, 17, 233–238

Spin Trapping of the Primary Radical Involved in the Activation of the Carcinogen *N*-Hydroxy-2-acetylaminofluorene by Cumene Hydroperoxide–Hematin

GERALD M. ROSEN AND ELMER J. RAUCKMAN

Departments of Pharmacology and Surgery, Duke University Medical Center, Durham, North Carolina 27710

Received September 24, 1979; Accepted November 1, 1979

SUMMARY

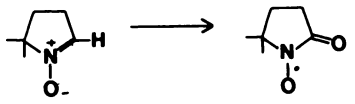
ROSEN, G. M., AND E. J. RAUCKMAN. Spin trapping of the primary radical involved in the activation of the carcinogen *N*-hydroxy-2-acetylaminofluorene by cumene hydroperoxide–hematin. *Mol. Pharmacol.* **17:** 233–238 (1980).

We have verified that the cumene hydroperoxide–hematin system reacts with 5,5-dimethyl-1-pyrroline-1-oxide to form the nitroxide 5,5-dimethyl-pyrrolidone-(2)-oxyl-(1) (DMPOX). We have investigated the mechanism of this reaction and found that it is not a simple oxidation, as has been previously reported, but that DMPOX is formed via the spin trapping of a cumene hydroperoxyl radical followed by an intramolecular carbanion displacement. The activation of the carcinogen, *N*-hydroxyl-2-acetylaminofluorene, by the cumene hydroperoxide–hematin system is most likely mediated by cumene hydroperoxyl radical.

INTRODUCTION

Spin trapping is a technique for the identification of short-lived free radicals such as hydroxyl (1) or superoxide (2). This method consists of using a nitrone or nitroso compound to "trap" a short-lived radical generating a "long-lived" nitroxide radical which can be detected with conventional electron paramagnetic resonance (epr)[1] methodology. We have recently pointed out that carefully designed control experiments are essential if one is to draw meaningful conclusions based on the spin trapping of superoxide and hydroxyl radicals in biological systems (3).

Floyd and co-workers have noted the formation of nitroxide intermediates in the activation of carcinogenic arylamines (4–6). Recently, Floyd and Soong have proposed, using spin trapping techniques, to identify the putative primary radical which attacks and activates these carcinogenic arylamines (7). They had also noted that cumene hydroperoxide–hematin oxidatively activates *N*-hydroxy-2-acetylaminofluorene, and based on this observation designed experiments using spin trapping to identify the primary free radicals produced by cumene hydroperoxide–hematin (7). They observed a nitroxide radical upon addition of 5,5 dimethyl-1-pyrroline-1-oxide (DMPO) to the cumene hydroperoxide–hematin system and concluded that this radical, 5,5-dimethylpyrrolidone-(2)-oxyl-(1) (DMPOX), did not result from spin trapping but from direct oxidation as shown below.



DMPO DMPOX

In this report, we provide compelling evidence to verify that DMPOX is formed during the reaction of DMPO with the hematin–cumene hydroperoxide system. However, we demonstrate that DMPOX is not obtained by means of a simple ionic oxidation of DMPO. Finally, we propose a mechanism to account for the formation of DMPOX from DMPO via spin trapping of cumene hydroperoxyl radical (CuOO·) followed by rearrangement to DMPOX.

MATERIALS AND METHODS

Preparation of 5,5-dimethyl-1-pyrroline-1-oxide. DMPO was prepared by a modification of the procedure of Bonnett *et al.* (8, 9) as follows and is illustrated in Fig. 1.

4-Methyl-4-nitropentan-1-al (I). 2-Nitropropane (215.4 ml, 2.4 mole) was added slowly at −10° to a mixture of absolute methanol (250 ml) and sodium (7.4 g, 0.32 mole). The stirred solution was cooled to −20° and a mixture of acrolein (18 g, 0.32 mole) and 2-nitropropane (53.8 ml, 0.6 mole) was added dropwise over 1 hr maintaining the temperature of the reaction at −20°. After the addition

This research was supported in part by Grants GM 25188 and GM 25753 from the National Institutes of Health.

[1] The abbreviations used are: epr, electron paramagnetic resonance; DMPO, 5,5-dimethyl-1-pyrroline-1-oxide; DMPOX, 5,5-dimethylpyrrolidone-(2)-oxyl-(1); CuOOH, cumene hydroperoxide; NOH-AAF, *N*-hydroxy-2-acetylaminofluorene; NOF, 2-nitrosofluorene.

0026-895X/80/020233-06$02.00/0

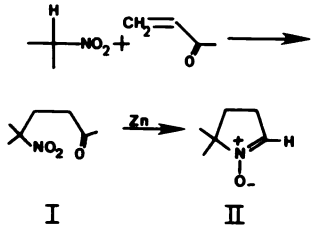


FIG. 1. *The synthesis of DMPO*

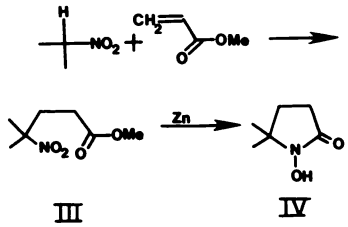


FIG. 2. *The synthesis of DMPOX*

was completed, the reaction was stirred for an additional 20 min, made acidic with hydrogen chloride and dried over anhydrous magnesium sulfate. Evaporation of solvent followed by fractional distillation gave 4-methyl-4-nitropentan-1-al (23.2 g) as a pale yellow liquid in 50% yield, bp 70–73° at 1 mm, lit. bp 88.3–89.5° at 3 mm (10). Infrared (liquid film), 1710, 1540, 1350 cm^{-1}.

5,5-Dimethyl-1-pyrroline-1-oxide (II). 4-Methyl-4-nitropentan-1-al (20 g. 0.138 mole), ethylene glycol (10 g, 0.161 mole) and *p*-toluenesulfonic acid (0.286 g, 1.5 mmole) were refluxed in 200 ml dry benzene until 2.5 ml of water was collected in a Dean–Stark trap. The benzene solution was cooled, washed with aqueous sodium carbonate, dried over anhydrous magnesium sulfate, and fractionally distilled to give 2-(3-methyl-3-nitrobutyl)-1:3-dioxolane (19 g, 75%), bp 101–105° at 0.5 mm, lit. bp 105° at 0.5 mm (9). Infrared (liquid film) 1540, 1350 cm^{-1}.

To this 1,3-dioxolane (19 g, 0.138 mole) was added a solution of ammonium chloride (5.7 g, 0.108 mole) in 114 ml of water. After this mixture was cooled to 10°, zinc dust (26.6 g, 0.407 mole) was added over a 40 min period in which the temperature of the mixture was not allowed to rise above 15°. This reaction was stirred for an additional 15 min, filtered and the filter cake washed with hot water (50°). The combined filtrate and washings were acidified with hydrochloric acid, left standing overnight and then heated to 70° for 40 min. Upon cooling, the solution was made basic and evaporated *in vacuo* to dryness. The remaining oil was extracted with chloroform, dried over anhydrous magnesium sulfate and fractionally distilled, giving 5,5-dimethyl-1-pyrroline-1-oxide (10.9 g, 70%), bp 63–66° at 0.6 mm, lit. bp 66–67° at 0.6 mm (8). Infrared (liquid film) 1573 cm^{-1}.

Preparation of 5,5-dimethylpyrrolidone-(2)-oxyl-(1) (DMPOX). DMPOX was prepared according to the methods of Kloetzel (11) and Bonnett *et al.* (9) as modified below (Fig. 2).

Methyl 4-methyl-4-nitropentanoate (III). A mixture of methyl acrylate (20 g, 0.23 mole), 2-nitropropane (62 g, 0.697 mole) and triethylamine (11.7 g, 0.116 mole) was stirred at 30° for 7 days. The unreacted reagents were removed *in vacuo*. Fractional distillation of the remaining oil gave methyl 4-methyl-4-nitropentanoate (24.2 g) as a clear liquid in 60% yield, bp 75–78° at 1 mm, lit. bp 88–89° at 2 mm (11). Infrared (liquid film) 1740, 1540, 1350 cm^{-1}.

1-Hydroxy-5,5-dimethylpyrrolid-2-one (IV). A solution of methyl 4-methyl-4-nitropentanoate (21 g, 0.12 mole) and ammonium chloride (6 g, 0.11 mole) in 50% aqueous ethanol (150 mole) was reduced with zinc dust (30 g, 0.459 mole). This mixture was allowed to stir at room temperature for 4 hr, filtered and the cake washed with hot 50% aqueous ethanol. Upon evaporation to dryness, the remaining oil was dissolved in 2 N hydrochloric acid (50 ml) and extracted with chloroform. After drying the chloroform solution with anhydrous magnesium sulfate, it was evaporated to dryness leaving a thick yellow oil. This oil was chromatographed using alumina (grade I) and a chloroform:carbon tetrachloride mixture (2:1) which removed the unreacted starting material. When the alumina column was eluted with chloroform:methanol (1:1), 1-hydroxy-5,5-dimethylpyrrolid-2-one (3.84 g) was obtained as an off-white solid which was recrystallized from petroleum ether–diethyl ether, in 25% yield, mp 78–80°, lit. mp 82–83°. Infrared (KBr pellet) 3400–3320, 1680 cm^{-1}.

5,5-Dimethylpyrrolidone-(2)-oxyl-(1). To a solution of 1-hydroxy-5,5-dimethylpyrrolid-2-one (1 mg, 7.8 mole) in water (0.5 ml) was added lead dioxide (2 mg, 8.37 mole). The solution was rapidly placed in the epr spectrometer and scanned immediately. The resulting spectrum is shown in Fig. 3. This same spectrum was obtained if other oxidizing agents were used such as peracetic acid, lead tetraacetate in acetic acid, ferrous sulfate/hydrogen peroxide, sodium tunstate/hydrogen peroxide, and sodium hydrogen carbonate/hydrogen peroxide (12).

RESULTS AND DISCUSSION

To verify the assignment of the epr spectrum observed by Floyd and Soong (7), we prepared an authentic sample of 1-hydroxy-5,5-dimethyl-pyrrolid-2-one and oxidized it to the corresponding nitroxide. A number of oxidizing agents, including one electron acceptor such as Fe^{+3} orthophenanthrene, were effective in promoting this oxidation. As shown in Fig. 3, the nitroxide obtained from

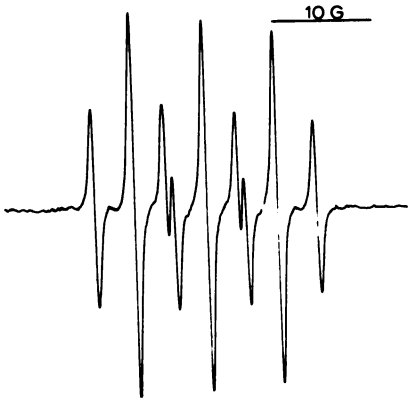


FIG. 3. *The electron spin resonance spectrum obtained when 1-hydroxyl-5,5-dimethylpyrrolid-2-one is oxidized by a variety of one electron sources*

Note Materials and Methods for details.

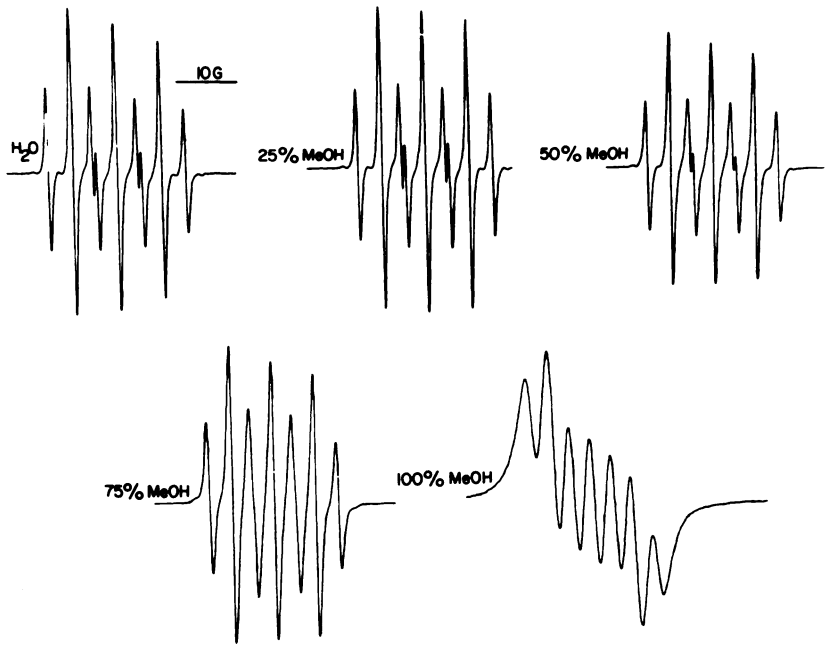


FIG. 4. *The electron spin resonance spectrum of DMPOX produced in various methanol/water ratios*

this oxidation exhibited an epr spectrum in water that is identical to that reported by Floyd and Soong (7), who assigned the DMPOX structure to their product on the basis of the spectral hyperfine splitting constants in water A_N = 7.1 G and A_H = 4.2 G and from the previous work by Aurich and Trosken (13). These authors (13) had prepared, as we have, 1-hydroxyl-5,5-dimethylpyrrolid-2-one and oxidized this hydroxylamine with lead dioxide in various organic solvents. Since these hyperfine splitting constants are highly solvent dependent (13) and since Floyd and Soong's (7) spectrum was run in water while Aurich and Trosken's (13) spectra were obtained in organic solvents, we felt it would be beneficial to determine the solvent effect of methanol/water mixtures on the hyperfine splitting constants of DMPOX. The results are shown in Fig. 4. The spectrum obtained in methanol is consistent with that reported by Aurich and Trosken (13). However, as the ratio of water to methanol increased, the hyperfine splitting characteristics of the spectrum changed considerably and finally approached that reported by Floyd and Soong (7).

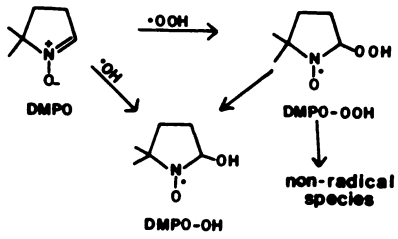
Floyd and Soong (7) found that molecular oxygen was necessary for the production of DMPOX from DMPO in the cumene hydroperoxide–hematin system and suggested that the oxygen which adds to DMPO comes from molecular oxygen. In addition, they hypothesized two other sources of this oxygen atom: water and cumene hydroperoxide. In this discussion we will examine these hypotheses and propose a more feasible mechanism for the generation of DMPOX from DMPO.

It is highly unlikely that this oxygen atom comes from molecular oxygen. To do so, molecular oxygen would have to be activated by the hematin–cumene hydroperoxide system as shown below.

$$Fe^{3+} + CuOOH \rightarrow Fe^{2+} + CuOO^{\cdot} \xrightarrow{O_2} Fe^{3+} + O_2^{-}$$

If this were correct, the generated superoxide would react with DMPO to give DMPO-OOH which we have previously shown is stable enough to be observed by epr (3).

With time, DMPO-OOH decomposes partially into the more stable DMPO-OH and partially into a nonradical species, as shown below.



Another possible fate of superoxide is its dismutation to hydrogen peroxide which could react with reduced hematin iron (Fe^{II}) to produce hydroxyl radical. Trapping of this species by DMPO would lead to the formation of DMPO-OH.

$$2\,O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + Fe^{2+} \rightarrow HO^{\cdot} + HO^- + Fe^{3+}$$

Oxidation of DMPO-OH by cumene hydroperoxide could then give DMPOX. Thus, if molecular oxygen were the source of the oxygen atom in DMPOX, one would anticipate that the characteristic epr spectrum of DMPO-OH would be observed. Finally, Floyd has confirmed this conclusion based on experiments using oxygen-17.[2] Since oxygen-17 has a spin of 5/2, its incorporation into DMPOX would lead to additional hyperfine splitting. He found that the DMPOX spectrum generated using the cumene hydroperoxide–hematin system in either an oxygen-16 or an oxygen-17 atmosphere was identical. Thus,

[2] R. A. Floyd, personal communication.

it is unlikely that molecular oxygen participates directly in the formation of DMPOX from DMPO.

In order for water to be the source of oxygen, it must add across the double bond of DMPO to give 1,2-dihydroxy-5,5-dimethylpyrroline which we have previously shown is readily oxidized by molecular oxygen (14) or superoxide (15) to DMPO-OH. Oxidation of this nitroxide by cumene hydroperoxide could give DMPOX. Although this mechanism accounts for the molecular oxygen requirement, the rate of water addition to DMPO at physiological pH is much too slow to be consistent with this reaction being important in the formation of DMPOX (3).

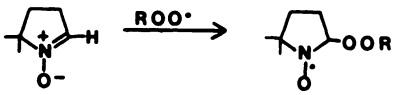
There are three possible mechanisms to account for cumene hydroperoxide participation in DMPOX formation. The first is that ferrous iron of hematin reacts with cumene hydroperoxide to produce the alkoxy radical which would be trapped by DMPO.

$$ROOH + Fe^{2+} \rightarrow Fe^{3+} + RO^{\cdot} + HO^{-}$$

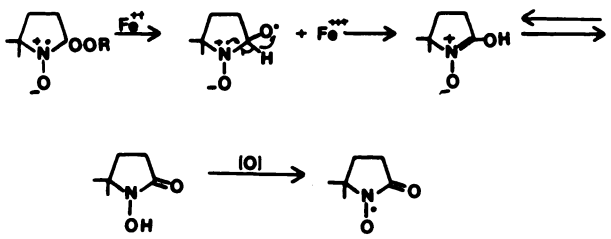
$$DMPO + RO^{\cdot} \rightarrow DMPO\text{-}OR$$

As in the case of hydroxyl radical, DMPO-OR would be stable and exhibit a characteristic epr spectrum different from DMPOX. Unlike DMPO-OH, DMPO-OR could not be oxidized by cumene hydroperoxide to give DMPOX, thus this mechanism may be elminated. A second possibility is that singlet oxygen produced during the reaction of cumene hydroperoxide and hematin reacts with DMPO to produce DMPOX. However, Hawco *et al.* (16) reported that although singlet oxygen is a product of the reaction of linoleic acid hydroperoxide and hematin, no evidence for singlet oxygen formation was found with the cumene hydroperoxide–hematin system.

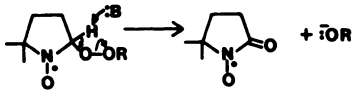
The third and most likely mechanism is that ferric hematin reacts with cumene hydroperoxide to give the cumene hydroperoxyl radical which could be trapped by DMPO leading to the unstable intermediate, DMPO-OOR.



We envision two possible mechanisms to account for the formation of DMPOX from DMPO-OOR. The first, Mechanism A, is initiated by the reduction of DMPO-OOR by ferrous hematin, while the second, Mechanism B, is a base-catalyzed intramolecular carbanion displacement reaction. We feel that the latter mechanism is best supported by the data presented by ourselves and Floyd and Soong (7).

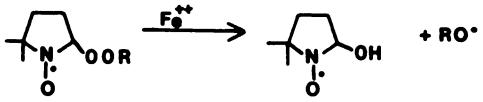


Mechanism A

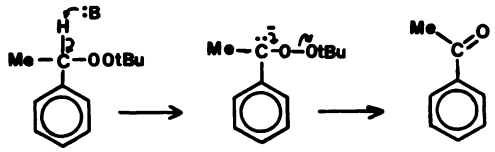


Mechanism B

First, under anaerobic conditions, Mechanism A predicts that a small steady-state concentration of DMPO-OOR should be observed. However, Floyd and Soong (7) did not detect any DMPO-OOR, which argues against Mechanism A. Second, during the homolytic cleavage of DMPO-OOR in Mechanism A, some DMPO-OH should arise as follows.



As previously mentioned, neither DMPO-OH nor DMPO-OR was observed by Floyd and Soong (7). However, Mechanism B seems reasonable in light of the work of Kornblum and De La Mare (17) who reported that at room temperature, t-butyl-α-phenethyl peroxide can be converted by base to acetophenone.



Other investigators (18) have found that nitrogen or oxygen functions gamma to the peroxide linkage readily undergo concerted cleavage reactions either thermally or with mild base.

A method for testing that latter mechanism is to react DMPO with the cumene hydroperoxide–hematin system at various pH values. At high pH, where the DMPO-OOR can easily undergo intramolecular rearrangement, only DMPOX should be observed, while at low pH, DMPO-OOR should be stable and be observed. Finally, at intermediate pH values, DMPO-OOR should be initially observed and rapidly decomposed into DMPOX. When we incubated DMPO with cumene hydroperoxide-hematin in a 0.015 M phosphate buffer with 1 mM DETAPAC at pH 7.4, we only observed the spectrum of DMPOX. When the reaction was run at pH 3.0 in 0.015 M glycine–HCl buffer with 1 mM DETAPAC, we observed a six-line spectrum with an A_H = 10.75 G, $A_H{}^{\gamma}$ = 1.75 G and A_N = 14.5 G (Fig. 5). Based on these hyperfine splitting constants and the work of Janzen (19), we have assigned this spectrum to DMPO-OOR where R = cumene. Finally, at pH 5.0, we initially observed the formation of DMPO-OOR which then rapidly decomposed into DMPOX. These observations confirm the work of Ohto *et al.* (20) who trapped cumene hydroperoxyl radical using phenyl *N*-t-butyl nitrone (PBN) in benzene.

Floyd and Soong (7) reported that the carcinogens, *N*-hydroxy-2-acetylaminofluorene (NOH-AAF) and 2-nitrosofluorene (NOF) inhibited the formation of DMPOX from DMPO in the cumene hydroperoxide–hematin system, while 2-acetylaminofluorene did not prevent the generation of DMPOX. They suggested that DMPO and

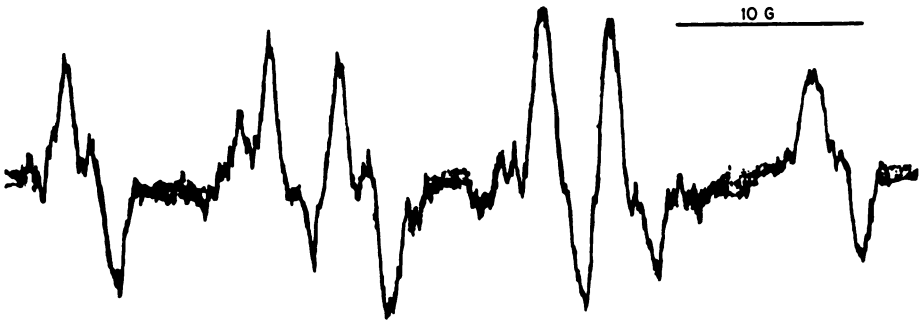
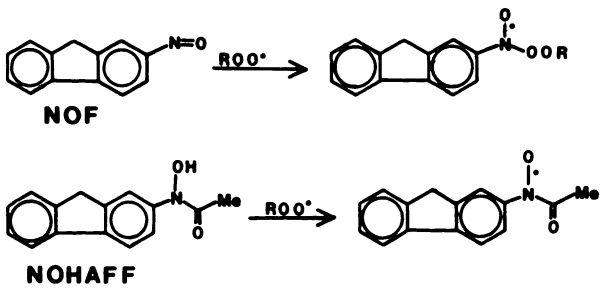


FIG. 5. *The electron spin resonance spectrum of DMPO-OOR obtained by reacting DMPO with the cumene hydroperoxide–hematin system at pH 3.00*

The microwave power was 10 mW and the modulation frequency was 100 kHz with an amplitude of 0.5 G. The sweep time was 1.67 G/min and the response time was 10 sec with A_N = 14.5 G, A_H = 10.75 G and $A_{H'}$ = 1.75 G.

either NOH-AAF or NOF compete for the same "active oxidant species." If these authors were correct in their hypothesis, and given that cumene hydroperoxyl radical is this species, then one should observe nitroxides from the reaction of NOH-AAF and NOF with the hydroperoxyl radical. However, the data they presented do not show these nitroxides when either NOH-AAF or NOF is added to the reaction mixture. The reaction of NOF with cumene hydroperoxyl radical leads to the nitroxide shown below.



We expect this nitroxide to be unstable and decompose rapidly. Support for this hypothesis comes from the work of Wargon and Williams (21) who observed that the spin trapping of methoxy radicals using 2-methyl-2-nitropropane was only possible at −78°. When the temperature of this reaction mixture was raised to 25°, the nitroxide spectrum completely disappeared. Based on these observations (21), we suggest that cumene hydroperoxyl radical initially attacks NOF forming the ensuing nitroxide which decomposes rapidly at 25° into nonradical species. Also the reaction of NOH-AAF with cumene hydroperoxyl radical leads to the corresponding NOH-AAF nitroxide radical via H· abstraction (22). Floyd and Soong (6) previously pointed out that this radical is unstable having a half-life of only 4 min. Thus, using a scan time of 1 hr as was used to observed DMPOX, one would not expect to detect the unstable NOH-AAF free radical.

In conclusion, when DMPO is reacted aerobically with cumene hydroperoxide and hematin, the only product observed is DMPOX. This nitroxide arises via the spin trapping of cumene hydroperoxyl radical followed by an intramolecular rearrangement of the unstable DMPO-OOR. Molecular oxygen is required to oxidize the ferrous hematin to ferric hematin. It should be noted that although there is an oxidation/reduction of iron in hematin, there is no evidence that the nitroxide, DMPOX, is formed via a simple ionic oxidation of DMPO, as suggested by Floyd and Soong (7).

REFERENCES

1. Harbour, J. R., V. Chow and J. R. Bolton. An electron spin resonance study of the spin adducts of HO and HO_2 radicals with nitrones in the ultraviolet photolysis of aqueous hydrogen peroxide solutions. *Can. J. Chem.* **52:** 3549–3553 (1974).
2. Harbour, J. R. and J. R. Bolton. Superoxide formation in spinach chloroplasts: Electron spin resonance detection by spin trapping. *Biochem Biophys. Res. Commun.* **64:** 803–807 (1975).
3. Finkelstein, E., G. M. Rosen and E. J. Rauckman. Spin trapping of superoxide. *Mol. Pharmacol.* **16:** 676–685 (1979).
4. Floyd, R. A., L. M. Soong and P. L. Culver. Horseradish peroxidase/hydrogen peroxide-catalyzed oxidation of the carcinogen N-hydroxy-N-acetyl-2-aminofluorence as effected by cyanide and ascorbate. *Cancer Res.* **36:** 1510–1519 (1976).
5. Floyd, R. A., L. M. Soong, R. N. Walker and M. Stuart. Lipid hydroperoxide activation of N-hydroxy-N-acetylamino-fluorene via a free radical route. *Cancer Res.* **36:** 2761–2767 (1976).
6. Floyd, R. A. and L. M. Soong. Obligatory free radical intermediate in the oxidative activation of the carcinogen N-hydroxy-2-acetylaminofluorene. *Biochim. Biophys. Acta* **498:** 244–249 (1977).
7. Floyd, R. A. and L. M. Soong. Spin trapping in biological systems. Oxidation of the spin trap 5,5-dimethyl-1-pyrroline-1-oxide by a hydroperoxide-hematin system. *Biochem. Biophys. Res. Commun.* **74:** 79–84 (1977).
8. Bonnett, R., V. M. Clark, A. Giddey and A. Todd. Experiments towards the synthesis of corrins. Part I. The preparation and reactions of some Δ′-pyrrolines. A novel proline synthesis. *J. Chem. Soc.:* 2087–2093 (1959).
9. Bonnett, R., R. F. C. Brown, V. M. Clark, I. O. Sutherland and A. Todd. Experiments towards the synthesis of corrins. Part II. The preparation and reactions of some Δ′-pyrroline-1-oxides. *J. Chem. Soc.:* 2094–2120 (1959).
10. Schechter, H., D. E. Ley and L. Zeldin. Addition reactions of nitroalkanes with acrolein and methyl vinyl ketone. Selective reduction of nitrocarbonyl compounds to nitrocarbinols. *J. Amer. Chem. Soc.* **74:** 3664–3668 (1952).
11. Kloetzel, M. C. Reactions of nitroparaffins. II. Addition of nitroparaffins to unsaturated esters. *J. Amer. Chem. Soc.* **70:** 3571–3576 (1948).
12. Rauckman, E. J., G. M. Rosen and M. B. Abou-Donia. Improved methods for the oxidation of secondary amines to nitroxides. *Syn. Commun.* **5:** 409–413 (1975).
13. Aurich, H. G. and J. Troeken. Losunysmittelabhangigkeit der ESR-spektner von alkyl-acyl-nitroxiden. *Leibigs Ann. Chem.* **745:** 159–163 (1971).
14. Rauckman, E. J., G. M. Rosen, B. B. Kitchell and J. A. Moylan. Oxidation of hydroxylamines to nitroxides by mixed function amine oxidase, in *Biological Oxidation of Nitrogen* (J. W. Gorrod, ed). Elsevier/North-Holland Biomedical Press, Amsterdam, 489–494 (1978).
15. Rauckman, E. J., G. M. Rosen and B. B. Kitchell. Superoxide radical as an intermediate in the oxidation of hydroxylamines by mixed function amine oxidase. *Mol. Pharmacol.* **15:** 131–137 (1979).
16. Hawco, F. J., C. R. O'Brien and P. J. O'Brien. Singlet oxygen formation during hemoprotein catalyzed lipid peroxide decomposition. *Biochem. Biophy. Res. Commun.* **76:** 354–361 (1977).
17. Kornblum, N. and H. E. De La Mare. The base catalyzed decomposition of a dialkyl peroxide. *J. Amer. Chem. Soc.* **73:** 880–881 (1951).
18. Grob, C. A. and P. W. Schiess. Heterolytic fragmentation. A class of organic reactions. *Angew. Chem. Engl.* **6:** 1–15 (1967).
19. Janzen, E. G., C. A. Evans and J. I. P. Liu. Radical addition reactions of 5,5-dimethyl-1-pyrroline-1-oxide. ESR spin trapping with a cyclic nitrone. *J. Magn. Res.* **9:** 510–512 (1973).

20. Ohto, N., E. Niki and Y. Kamiya. Study of autoxidation by spin trapping. Spin trapping of peroxyl radicals by phenyl N-t-butyl nitrone. *J. Chem. Soc.*: 1770–1774 (1977).
21. Wargon, J. A. and F. Williams. Electron spin resonance studies of radical trapping in the radiolysis of organic liquids. I. Evidence for the primary formation of the methoxy radical in methanol. *J. Amer. Chem. Soc.* **94**: 7917–7918 (1972).
22. Rosen, G. M. and E. J. Rauckman. Formation and reduction of a nitroxide radical by liver microsomes. *Biochem. Pharmacol.* **26**: 675–678 (1977).

Send reprint requests to: Dr. Gerald M. Rosen, Department of Pharmacology, Duke University Medical Center, 011 Research Park IV, Durham, N.C. 27710.