

2,4,6-TRIMETHOXYPHENYL *tert*-BUTYL NITRONE (MO)₃PBN, AS A DETECTOR OF HYDROXYL RADICALS IN THE PRESENCE OF PEROXIDES, SUPEROXIDE AND PEROXYL RADICALS

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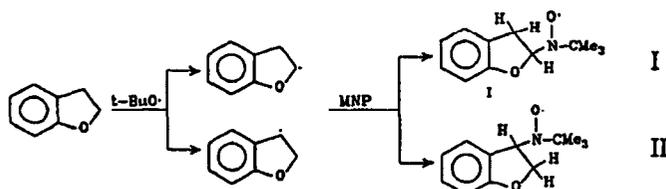
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Summary: 2,4,6-Trimethoxyphenyl *tert*-butyl nitron, (MO)₃PBN is proposed as a spin trap for hydroxyl radicals since the aminoxyl produced from internal trapping of the *ortho*-oxymethylenyl radical gives a unique spectrum not found with peroxy spin adducts. Superoxide does not produce ESR detectable spin adducts with (MO)₃PBN.

Detection of the hydroxyl radical in the presence of hydrogen peroxide or superoxide using the nitronyl spin trapping method can sometimes lead to erroneous conclusions since the spin adduct of the hydroperoxyl radical is relatively unstable (typical of a secondary hydroperoxide bonded to a free radical function in the β -position) and may itself produce hydroxyl radicals during its decomposition.¹ Thus, a need exists for spin traps which give unique signatures for various oxygen-centered radicals which are specific for each different type of radical.² Recently, the synthesis of 5,5-diethyl-3,3-dimethylpyrroline-N-oxide,³ E₂M₂PO has been reported. It was shown that this nitron does not react with superoxide to give a spin adduct, hence providing a more unambiguous detector of hydroxyl radicals. This work prompts us to communicate on the reaction of hydroxyl radicals with 2,4,6-trimethoxyphenyl *tert*-butyl nitron ((MO)₃PBN) as an example of a spin trap where reactions remote from the nitron eventually produce aminoxyls with unique spectral signatures for the radical detected.

(MO)₃PBN gives a variety of ESR spectra for spin adducts of alkyl radicals.⁴ In general, the β -H hfsc's are larger than in the equivalent structure derived from PBN. From hydroxyl radicals in water, the ESR pattern obtained with (MO)₃PBN consists of 3 sets of 3 lines (Figure 1). Because the intramolecular spin trapping of the oxymethylenyl radical derived from hydrogen atom abstraction from one of the *ortho* methoxyls is a possible source of the spectrum in Figure 1, the spin trapping reaction of radicals derived from dihydro-2,3-benzofuran by hydrogen atom abstraction was investigated with 2-methyl-2-nitrosopropane (MNP) using the photolysis of di-*tert*-butyl peroxide as the *tert*-butoxyl radical source in benzene:



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The ESR spectrum observed was very similar to that obtained from (MO)₃PBN except that the N-hfsc was smaller (due to the difference in solvent polarity), namely $a_N = 14.6$, $a_H = 1.7$ (1H), $a_H = 1.0$ (1H)G in benzene. Thus, this spectrum is assigned to II. With di-*tert*-butyl peroxalate (DBPO) in benzene at room temperature, a mixture spectrum was obtained with the following hfsc's: $a_N = 14.4$, $a_H = 1.0$ (2H)G; $a_N = 12.1$, $a_H = 1.25$ (1H), $a_H = 0.55$ (1H)G. The former component is again assigned to spin adduct II. Since the parameters for II are similar to those of the aminoxyl radical detected from the reaction of (MO)₃PBN and hydroxyl radicals, the spectrum in Figure 1 is assigned to the product of intramolecular spin trapping of the oxymethylenyl radical produced from hydrogen atom abstraction (the difference in a_N is due to the polar solvent effect):

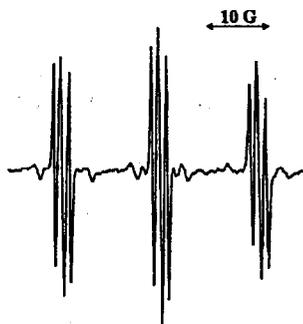
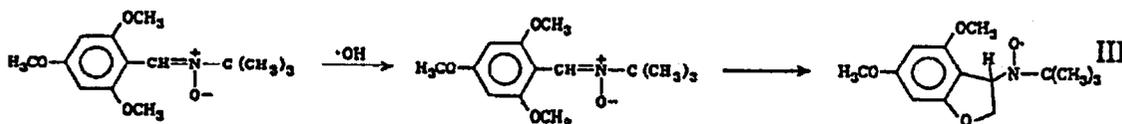
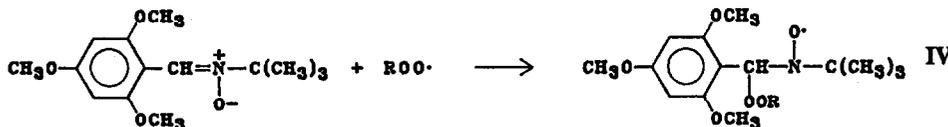


Figure 1. ESR spectrum obtained from the photolysis (Xe lamp) of 2% hydrogen peroxide in water in the presence of 0.05 M (MO)₃PBN ($a_N = 16.41$, $a_H = 1.49$ (1H), $a_H = 1.0$ (1H)G); essentially the same spectrum is obtained from the photolysis of 1% hydrogen peroxide in benzene and 0.05 M (MO)₃PBN or photolysis of a basic aqueous solution of 0.1 M sodium persulfate in the presence of 0.05 M (MO)₃PBN.



No ESR spectra were obtained from (MO)₃PBN and superoxide generated *in situ* from paraquat or from the xanthine/xanthine oxidase system.⁵ Also, the spectrum due to III is not obtained from peroxy radicals. Only "normal" adducts are found from reacting DBPO with *tert*-butyl or cumene hydroperoxide at room temperature in benzene (Figure 2):



With time, the spectra due to these adducts decay (over two hours), leaving a triplet of doublets ($a_N = 14.10$, $a_H^H = 4.64$ G) tentatively assigned to the actual hydroxyl adduct of (MO)₃PBN. Of importance is the apparent greater persistence of the peroxy adducts of (MO)₃PBN than of those obtained from PBN. The normal hydroxyl adduct is apparently also obtained in water ($a_N = 16.21$, $a_H^H = 8.85$ G) as a minor component (see Figure 1) but this aminoxyl may be the result of hydrolysis of the radical cation of (MO)₃PBN formed by electron transfer. It should be noted that in addition to III a triplet of doublets is also obtained with *tert*-butoxyl or with *iso*-butoxyl radicals (Figure 3).

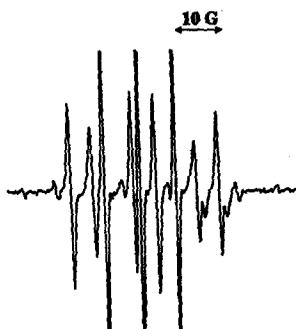
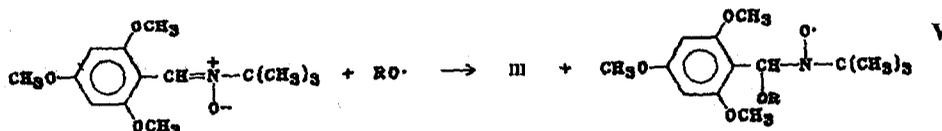


Figure 2(a). Esr spectrum obtained from the thermolysis of 0.02 M di-*tert*-butyl peroxalate in the presence of 0.1 M *tert*-butyl hydroperoxide and 0.05 M $(\text{MO})_3\text{PBN}$ in benzene; $a_N = 13.25$, $a_{\beta}^H = 4.74$ G.

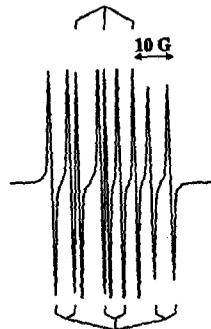


Figure 2(b). Esr spectrum obtained from the thermolysis of 0.04 M di-*tert*-butyl peroxalate in the presence of 0.2 M cumyl hydroperoxide and 0.05 M $(\text{MO})_3\text{PBN}$ in benzene; $a_N = 13.21$, $a_{\beta}^H = 5.08$ G.

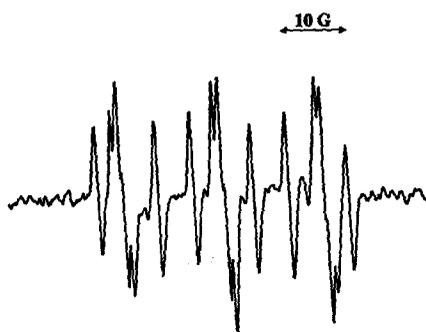


Figure 3(a). Esr spectrum obtained from the photolysis of 0.05 M isobutyl nitrite in the presence of 0.05 M $(\text{MO})_3\text{PBN}$ in benzene; $a_N = 13.97$, $a_{\beta}^H = 8.86$ G.

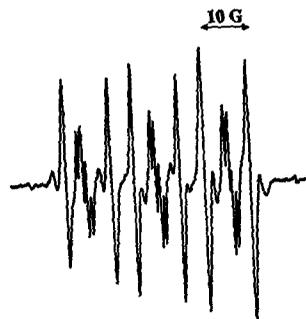


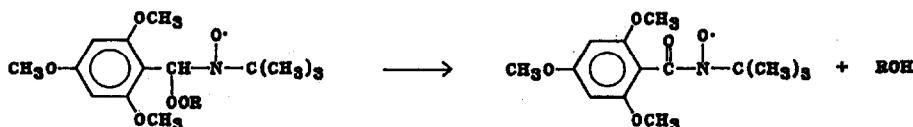
Figure 3(b). Esr spectrum obtained from the thermolysis of 0.01 M di-*tert*-butyl peroxalate in the presence of 0.05 M $(\text{MO})_3\text{PBN}$ in benzene; $a_N = 14.18$, $a_{\beta}^H = 9.27$ G.

The ratios of III to the normal spin adducts (V) are given below:

RO.	a_N	a_{β}^H	III/VI	Figure	Method/Solvent
<i>iso</i> -BuO·	13.97	8.86	~2:1	3a	photolysis of $\text{Me}_2\text{CHCH}_2\text{ONO}$ /benzene
<i>tert</i> -BuO·	14.18	9.27	~1:1	3b	thermal decomposition of DBPO/benzene

Benzoyloxy radicals give only normal spin adducts from thermal decomposition of benzoyl peroxide: $a_N = 13.36$, $a_{\beta}^H = 5.37$ G in benzene, and the acetoxy adduct appears to be formed from the reaction with lead

tetracetate: $a_N = 13.86$, $a_H^H = 7.41$ G. In benzene, photolysis of $(MO)_3PBN$ in the presence of benzophenone gives only III: $a_N = 15.04$, $a_H = 1.00$, $a_H = 0.94$, $a_H = 0.66$ (all 1H)G in benzene.



In a number of spectra, a simple triplet of three equally intense lines is also observed (see Figure 2(a) and (b)). This species is assigned to the acyl nitroxide probably produced from the decomposition of the peroxy adduct⁶ ($a_N = 7.47$ G in benzene):

In conclusion, $(MO)_3PBN$ may serve as a better spin trap than PBN for the detection and assignment of oxyl radicals. Thus, when only a triplet of doublets with appropriate hfsc's is detected, the spin adducts may be derived from peroxy or acyloxy radicals. When essentially only the pattern due to III is observed, the system must have produced hydroxyl radicals. When both III and a triplet of doublets is seen, either hydroxyl and/or alkoxy radicals were present.

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4. C.M. DuBose, Jr., Ph.D. Dissertation "Spin Trapping with α -2,4,6-Trimethoxyphenyl *N*-*tert*-Butyl Nitron," University of Georgia, 1985; work performed in the Department of Chemistry and Biochemistry at the University of Guelph.
- 5(a) Rat liver microsomes and NADPH bubbled with oxygen in a phosphate buffer solution containing $(MO)_3PBN$ and paraquat at room temperature. (b) A saturated aqueous solution of $(MO)_3PBN$ was subjected to xanthine/xanthine oxidase and the signal compared to DMPO under the same conditions in phosphate buffer. We gratefully acknowledge the assistance of Don Gibson at OMRF in performing these experiments.
6. E.G. Janzen, P.H. Krygsman, D.A. Lindsay and D. Larry Haire, *J. Am. Chem. Soc.*, in press.