

Spin-trapping of Phosphorous-containing Inorganic Radicals by a Water-soluble
Spin-trap, 3,5-Dibromo-4-nitrosobenzenesulfonate

Toshihiko OZAWA* and Akira HANAKI
National Institute of Radiological Sciences,
9-1, Anagawa-4-chome, Chiba 260

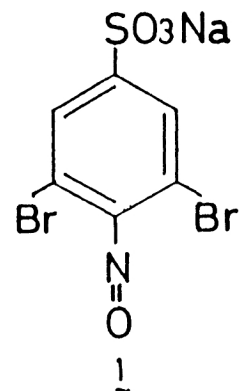
Two short-lived phosphorous-containing inorganic radicals ($\text{PO}_3^{2\cdot-}$ and $\text{HPO}_2^{\cdot-}$), which are generated by the oxidation with Ti^{3+} - H_2O_2 reaction system, are easily trapped by a water-soluble spin-trap, 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS) and their adducts are characterized by ESR spectroscopy.

In continuation of our works¹⁻⁴⁾ on the spin-trapping of short-lived free radicals, we have investigated the spin-trapping of phosphorous-containing inorganic radicals. At present there have been few reports concerning the spin-trapping of radical intermediates formed during the oxidation of the phosphorous-containing inorganic compounds, the life-time of which is too short to be directly detected by conventional ESR measurements. Norman et al.⁵⁾ and Beckwith⁶⁾ have reported that nitromethane aci-anion ($\text{CH}_2=\text{NO}_2^-$) is an efficient scavenger for the phosphorous-containing inorganic radicals generated by abstraction of hydrogen from the parent anions by hydroxyl radical ($\text{OH}\cdot$) in the continuous flow system. Lagercrantz⁷⁾ has reported that t-nitrosobutane [$\text{C}(\text{CH}_3)_3\text{NO}$] can trap some phosphorous-containing inorganic radicals produced by UV irradiation of an aqueous solution of parent ions. In order to examine the usefulness of a water-soluble spin-trap, 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS, 1), we have examined the spin-trapping of such intermediates during the oxidation with Ti^{3+} - H_2O_2 reaction system. We now report herein two phosphorous-containing inorganic radicals trapped by DBNBS.

ESR measurements were carried out on a JEOL-PE-1X ESR spectrometer (X-band) with 100 kHz field modulation. ESR spectra were recorded at room temperature in a JEOL flat quartz cell. ESR parameters were calibrated by comparison with a standard Mn^{2+} /MgO marker and 1,1-diphenyl-2-picrylhydrazyl (DPPH, $g=2.0036$).

Sodium 3,5-dibromo-4-nitrosobenzenesulfonate 1 was prepared from 3,5-dibromosulfanilic acid by the oxidation with H_2O_2 in glacial acetic acid.⁸⁾

For the generation of an oxidant, hydroxyl radical ($\text{OH}\cdot$), commercial reagents of TiCl_3 (20%, v/v) and H_2O_2 (30%, v/v) were used without further purification. The phosphorous-containing compounds, sodium phosphite ($\text{Na}_2\text{HPO}_3 \cdot 5\text{H}_2\text{O}$) and sodium hypophosphite ($\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$)



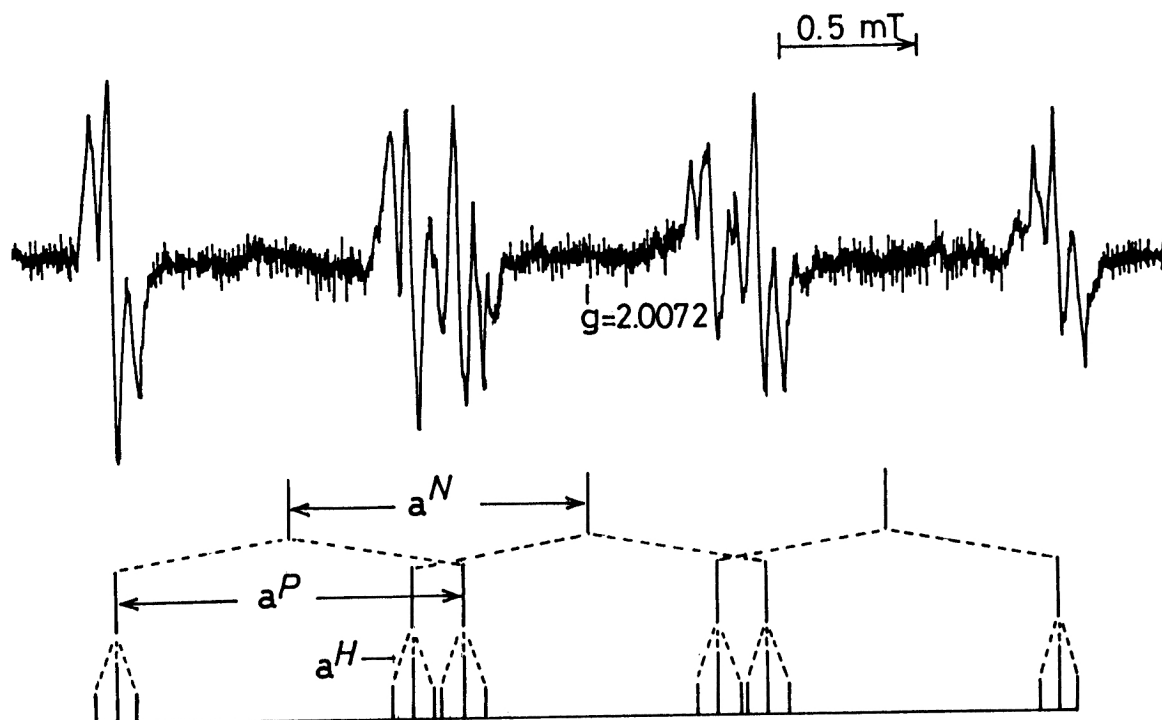
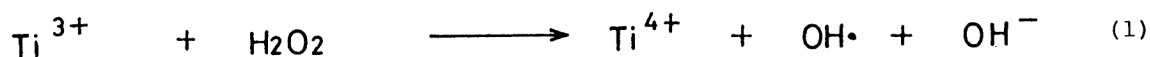


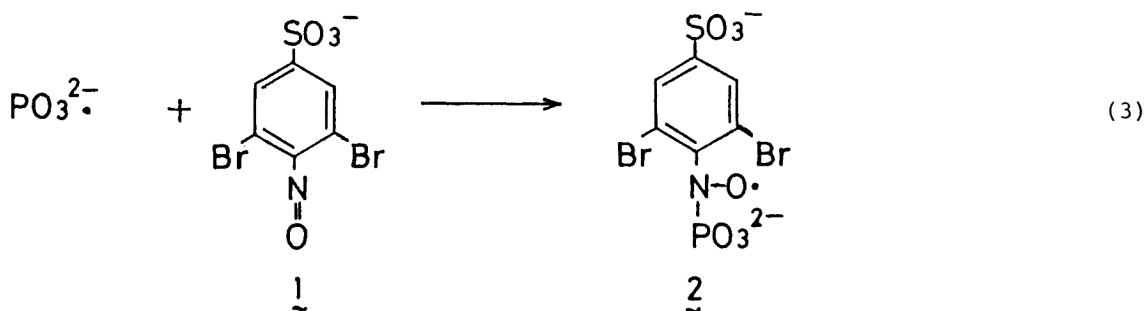
Fig. 1. ESR spectrum observed by the oxidation of phosphite ion with Ti^{3+} - H_2O_2 reaction system in the presence of DNBBS at pH 9.0 and its stick diagram. Reaction condition: Ti^{3+} , 0.01 mol dm^{-3} ; H_2O_2 , 0.05 mol dm^{-3} ; phosphite ion, 0.1 mol dm^{-3} ; DNBBS, 0.05 mol dm^{-3} .

were used as received. Other reagents were commercially available. All reaction solutions were prepared from deionized and triply distilled water and their pHs were adjusted to be 9.0 by ammonium hydroxide and sulfuric acid.^{9,10)}

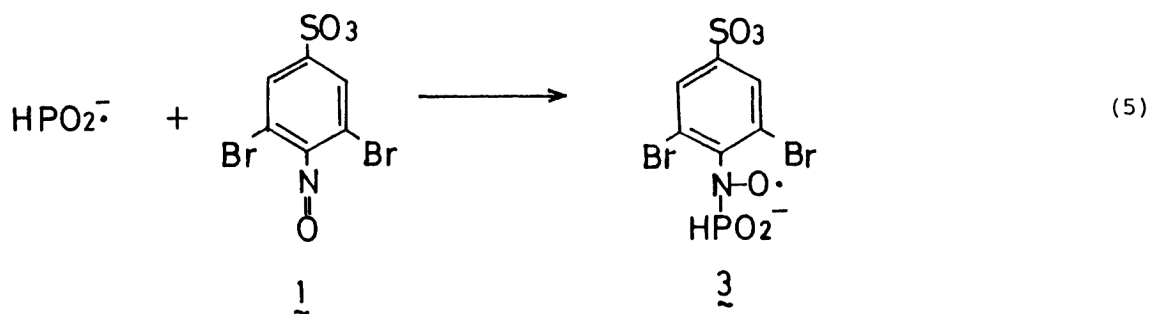
When Ti^{3+} solution (0.01 mol dm^{-3}) was mixed with the aqueous solution of H_2O_2 (0.05 mol dm^{-3}) containing DNBBS (0.05 mol dm^{-3}) and phosphite ion (0.1 mol dm^{-3}), ESR spectrum as shown in Fig. 1 was observed. The ESR spectrum could be analyzed as shown by the stick diagram in Fig. 1 and ESR parameters obtained were as follows: $a^{\text{N}}(1)=1.08 \text{ mT}$, $a^{\text{P}}(1)=1.24 \text{ mT}$, $a^{\text{H}}(2)=0.07 \text{ mT}$, and $g=2.0072$.

DNBBS does not yield observable spin-adducts with hydroxyl radical which is the immediate product from Ti^{3+} - H_2O_2 reaction system.⁴⁾ When either Ti^{3+} or H_2O_2 was omitted from the solution, no ESR spectrum was observed. Further, it has been reported that phosphite ion is oxidized by OH radical to give PO_3^{2-} radical at pH 9 (Eqs. 1 and 2).⁵⁾ The hyperfine splitting constant due to ^{31}P nucleus is almost the same as those for the radicals such as $\dot{\text{C}}\text{-O-P}^{11)}$ or $\dot{\text{C}}\text{-C-P}^{12)}$ in which phosphorous is at the β -position of the carbon atom which has the unpaired electron. Therefore, the observed radical species is assigned to the PO_3^{2-} adduct of DNBBS (2) (Eq. 3). The formation of 2 can be expressed as follows:





A similar ESR spectrum [$a^{\text{N}}(1)=1.08$ mT, $a^{\text{P}}(1)=1.24$ mT, $a^{\text{H}}(1)=0.17$ mT, and $g=2.0072$] was observed by the oxidation of hypophosphite ion with $\text{Ti}^{3+}\text{-H}_2\text{O}_2$ reaction system in the presence of DNBNS. This radical species which is assigned to the $\text{HPO}_2^{\cdot-}$ adduct of DNBNS ($\underset{\sim}{\text{3}}$) can be formed as follows:



The doublet splitting (0.17 mT) in the spectrum is assigned to the P-H proton of $\text{HPO}_2^{\cdot-}$. The hyperfine splitting due to aromatic protons was not observed probably because of a weak signal intensity.

Both the PO_3^{2-} adduct $\underset{\sim}{\text{2}}$ and $\text{HPO}_2^{\cdot-}$ adduct $\underset{\sim}{\text{3}}$ were not stable and their ESR spectra disappeared within about 10 min. Other phosphorous-containing inorganic compounds such as H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-} ions were not oxidized by OH radical.

ESR parameters obtained here are summarized in Table 1, along with other nitroxide radicals bonded to phosphorous-containing group. Studies of phosphorous-containing radicals in solutions by ESR spectroscopy have given the information about both the structures of radicals. As shown in Table 1, the ^{31}P splittings of the nitroxides of this series are much smaller in comparison with those of free untrapped phosphorous-containing radicals (about 60 mT)^{14,15} and were found to be between 1.01 and 3.16 mT. Such a large reduction of ^{31}P splittings may be caused by the spin polarization.¹²⁾

In conclusion, this study presents that a water-soluble, nitroso aromatic spin-trap, DNBNS, is very useful to trap the short-lived phosphorous-containing inorganic radicals.

Table 1. ESR parameters obtained from the nitroxide radical containing phosphorous atom

Spin-trap	Trapped radical	Hyperfine coupling const./mT	Ref.
DBNBS	PO_3^{2-}	$a^{\text{N}}(1)=1.08$, $a^{\text{H}}(2)=0.07$, $a^{\text{P}}(1)=1.24$	This work
DBNBS	HPO_2^-	$a^{\text{N}}(1)=1.08$, $a^{\text{P}}(1)=1.24$, $a_{\text{P-H}}^{\text{H}}(1)=0.17$	This work
$\text{C}(\text{CH}_3)_3\text{NO}$	PO_3^{2-}	$a^{\text{N}}(1)=1.34$, $a^{\text{P}}(1)=1.20$	7
$\text{CH}_2=\text{NO}_2^-$	PO_3^{2-}	$a^{\text{N}}(1)=2.52$, $a^{\text{H}}(2)=1.065$, $a^{\text{P}}(1)=3.16$	6
$\text{CH}_2=\text{NO}_2^-$	HPO_2^-	$a^{\text{N}}(1)=2.52$, $a^{\text{H}}(2)=1.017$, $a^{\text{P}}(1)=1.017$, $a_{\text{P-H}}^{\text{H}}(1)=0.148$	6
$\text{CH}_2=\text{NO}_2^-$	PO_3^{2-}	$a^{\text{N}}(1)=2.49$, $a^{\text{H}}(2)=1.07$, $a^{\text{P}}(1)=3.14$	5
$\text{CH}_2=\text{NO}_2^-$	HPO_2^-	$a^{\text{N}}(1)=2.51$, $a^{\text{H}}(2)=1.01$, $a^{\text{P}}(1)=2.92$, $a_{\text{P-H}}^{\text{H}}(1)=0.15$	5
$\text{CH}_3\text{CH}=\text{NO}_2^-$	PO_3^{2-}	$a^{\text{N}}(1)=2.56$, $a^{\text{H}}(1)=0.32$, $a^{\text{H}}(3)=0.03$, $a^{\text{P}}(1)=2.70$	5
$\text{CH}_3\text{CH}=\text{NO}_2^-$	HPO_2^-	$a^{\text{N}}(1)=2.55$, $a^{\text{H}}(1)=0.61$, $a^{\text{H}}(3)=0.04$, $a^{\text{P}}(1)=2.68$, $a_{\text{P-H}}^{\text{H}}(1)=0.14$	5

References

- 1) T. Ozawa and A. Hanaki, Chem. Pharm. Bull., 26, 2572 (1978).
- 2) T. Ozawa and A. Hanaki, Biochem. Biophys. Res. Commun., 136, 657 (1986).
- 3) T. Ozawa and A. Hanaki, Biochem. Biophys. Res. Commun., 142, 410 (1987).
- 4) T. Ozawa and A. Hanaki, Bull. Chem. Soc. Jpn., 60, 2304 (1987).
- 5) B. C. Gilbert, J. P. Larkin, R. O. C. Norman, and P. M. Storey, J. Chem. Soc., Perkin Trans. 2, 1972, 1508.
- 6) A. L. J. Beckwith, Aust. J. Chem., 25, 1887 (1972).
- 7) C. Lagercrantz, J. Phys. Chem., 75, 3466 (1971).
- 8) H. Kaur, K. H. W. Leung, and M. J. Perkins, J. Chem. Soc., Chem. Commun., 1981, 142.
- 9) R. O. C. Norman and P. M. Storey, J. Chem. Soc., B, 1971, 1009.
- 10) To prevent the precipitation from the solution upon addition of ammonium hydroxide, ethylenediaminetetraacetic acid (EDTA) was added to the TiCl_3 solution.
- 11) E. A. C. Lucken, J. Chem. Soc., A, 1966, 1354.
- 12) W. Damerau, G. Lassmann, and K. Lohs, J. Magn. Reson., 5, 408 (1971).
- 13) J. K. Kochi and P. J. Krusic, J. Am. Chem. Soc., 91, 3944 (1969).
- 14) A. Horsfield, J. R. Morton, and D. H. Whiffen, Mol. Phys., 4, 475 (1961).

(Received June 5, 1987)