Synthesis of 5-(Alkylphenylphosphoryl)-5-methyl-3,4-dihydro-2*H*-pyrroline *N*-Oxide as a New Spin Trapping Reagent

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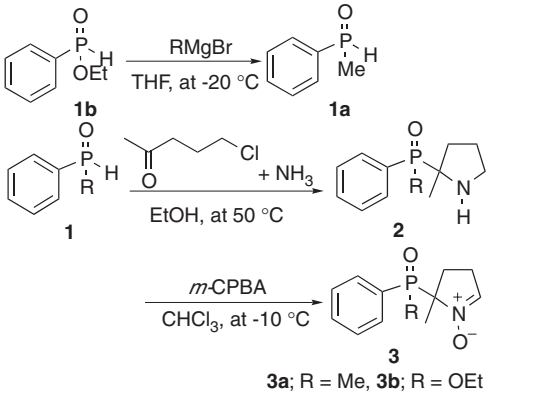
Novel DMPO analogues attached to a phenyl group at the phosphorus atom were synthesized and examined for their ability in the spin trapping of oxygen-centered radicals. The lipophilicities of these analogues were higher than those of DMPO.

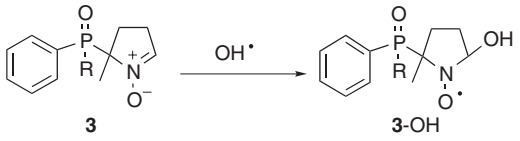
Spin trapping studies have been applied to the detection of oxygen-centered radicals in biological processes.[1] 5,5-Dimethyl-3,4-dihydro-2*H*-pyrroline *N*-oxide (DMPO) is widely used as a spin trapping reagent,[2] but has some limitations.[3] The reaction rate with superoxide is low and the spin adduct decomposes rapidly.[4] The partition coefficient of DMPO between 1-octanol and water has been found to be only 0.02–0.1.[5] Many researchers have reported the improved synthesis of DMPO for biological uses and more lipophilic derivatives.[6] One of these reports revealed that phosphoryl-DMPOs, such as 5-(diethoxy phosphoryl)-5-methyl-1-pyrroline *N*-oxide (DEPMPO), have longer life times than those of the original DMPO. Moreover, the phenyl group attached to the pyrroline ring enhanced the lipophilicity of phosphoryl-DMPO derivatives.[7] However, there is no report on the synthesis and properties of modified DMPO, which contains non-alkoxy substituents at the phosphorus atom. We report herein the synthesis of a new class of phosphoryl-DMPO containing a phenyl group at the phosphorus atom, and the substitution effect toward solubility and electronic properties.

Secondary phosphine oxide **1a** was prepared by the reaction of ethyl phenylphosphinate **1b** with methylmagnesium bromide (90% yield). Pyrroline *N*-oxide **3** was synthesized according to a method described by Tordo.[8] Pyrrolidine **2** was obtained by bubbling ammonia into ethanol solution of 5-chloropentane-2-one and phosphine oxide **1**. The oxidation of **2** with *m*-CPBA was the most sensitive step in the synthetic pathway and was carried out at $-10\,^{\circ}C$ to give **3a** and **3b** as a mixture of four diastereoisomers in 43% and 49% yields, respectively (Scheme 1). The ratio of the major **3a** to the minor product was 5:1, whereas that of **3b** was 1:1. The major isomer of the methyl derivative **3a** was able to be separated by column chromatography for use as a spin trap.[9]

The trapping of hydroxyl radicals with pyrroline *N*-oxides **3** in the Fenton reaction was carried out under the following conditions (Scheme 2): spin trap ($10\,mol\,dm^{-3}$, $200\,\mu L$), 30% H_2O_2($100\,\mu L$), $K_4[Fe(CN_6)]$ ($10\,mmol\,dm^{-3}$, $100\,\mu L$). The ESR measurements were performed with the following settings: microwave power 10 mW, field 336.4 ± 5 mT, modulation 0.1 mT, time constant 0.3 sec, sweep time 8 min. Under these conditions, the ESR signal of **3a**, **b** showed a doublet of doublets of triplets pattern which is split by a large phosphorus coupling (Figure 1). The hyperfine splitting constants calculated from the spectra are summarized in Entries 1 and 2 of Table 1. These signals were inhibited by the presence of catalase (50 U/mL).



Scheme 1.



Scheme 2.

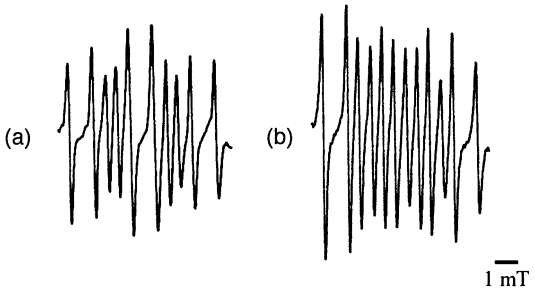
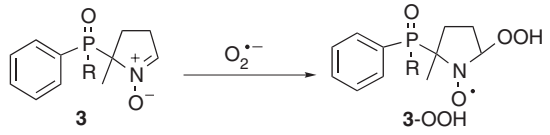


Figure 1. ESR Spectra of spin adducts obtained by the reaction with superoxide radicals (a) **3a**-OH (pH 7.4); **3b**-OH (pH 7.4).

The trapping of superoxide radicals with pyrroline *N*-oxides **3** was also examined using an aqueous reaction (phosphate buffer, pH 7.4) of xanthine ($0.4\,mmol\,dm^{-3}$, $800\,\mu L$) and xanthine oxidase (0.5 U/mL, $800\,\mu L$) in the presence of $200\,\mu L$ ($10\,mol\,dm^{-3}$) of a spin trap (Scheme 3). The ESR measurements were made with the settings: microwave power 10 mW, field 335 ± 5 mT, modulation 0.1 mT, time constant 0.1 sec, and sweep time 8 min. The ESR signal of the spin adduct obtained in the reaction of **3a** and $O_2^{\bullet -}$ is shown in Figure 2.

The signal disappeared with the addition of superoxide dismutase (320 U/mL). Hfsc's of **3**-OH and **3**-OOH are listed in Entries 3 and 4 of Table 1.



Scheme 3.

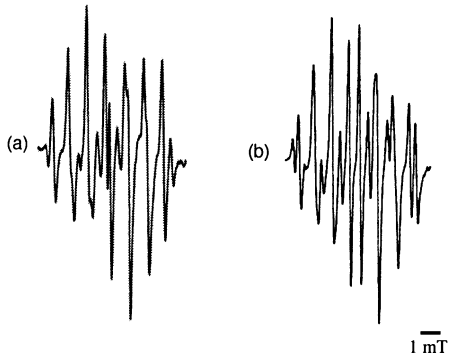


Figure 2. ESR Spectra of spin adducts obtained by the reaction with superoxide radicals (a) **3a**-OOH (pH 7.4); **3b**-OOH (pH 7.4).

Table 1. Hyperfine splitting constants of pyrroline *N*-oxide **3** adduct (mT)

Entry	Spin adduct	A_N	$A_{\beta\text{-}H}$	A_P
1	**3a**-OH	1.42	2.18	3.64
2	**3b**-OH	1.34	2.00	3.95
3	**3a**-OOH	1.24	1.21	4.17
4	**3b**-OOH	1.22	1.18	4.61

The kinetics of decay of the superoxide adducts were measured in phosphate buffer (pH 7.4). The superoxide was generated with a xanthine/xanthine oxidase system and was stopped by adding SOD. The spin adduct decay was followed by monitoring the decrease of an appropriate line of the spin adduct. The ESR signals of **3a**-OOH and **3b**-OOH were observed for 40 min and 6 min, respectively. The decay of **3a**-OOH produced under these conditions was pure first-order and had a rate constant of $1.1 \times 10^{-3}\,s^{-1}$, which corresponds to a half-life of 633 s. These half-life times are longer than that of DMPO ($t_{1/2} = 50$ s, at pH 7.0) and the same as that of DEPMPO ($t_{1/2} = 780$ s, at pH 7.0) under similar conditions.[10]

To estimate the relative lipophilicity of the pyrroline *N*-oxide **3**, we determined the partition coefficient of **3** between 1-octanol and the water system (Table 2). The presence of a phenyl group at the phosphorus enhanced the lipophilicity of **3**. It was found that the partition coefficient of the methyl derivative **3a** attained 0.7.

Table 2. Partition of pyrroline *N*-oxide **3** between 1-octanol and water[a]

	3a	**3b**	DEPMPO	DMPO
P[b]	0.70	0.44	0.06[c]	0.11 (0.10[d])

[a]Conditions: 1-octanol 5 ml, H_2O 5 ml, 0.15 mmol, at 36 °C. [b]P = [**3** in 1-octanol]/[**3** in H_2O]. [c]Ref. 8. [d]Ref. 11.

We succeeded in synthesizing lipophilic phosphoryl DMPO analogues. These analogues are suitable for spin trapping experiments that involve interfacially-generated (between the cell and the outer sphere) or intracellularly-generated free radicals.

References and Notes

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9 Satisfactory elemental analyses were obtained for all new compounds. 2-(methylphenylphosphoryl)-2-methyl-3,4-dihydro-2*H*-pyrroline *N*-oxide (**3a**); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.38–1.46 (m, 1H) 1.90 (d, J = 12 Hz, 3H) 2.085 (d, J = 18.4 Hz, 3H), 1.99–2.09 (m, 1H), 2.24-2.32 (m, 1H), 2.79–2.89 (m, 1H), 6.57 (dt, J = 1.2 Hz, 2.4 Hz, 1H), 7.27–7.912 (m, 5H). 2-(ethoxyphenylphosphoryl)-2-methyl-3,4-dihydro-2*H*-pyrroline *N*-oxide (**3b**); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.27 (t, J = 7.2 Hz, 3H), 1.31 (t, J = 7.2 Hz, 3H) 1.49 (d, J = 15.2 Hz, 3H), 1.70 (d, J = 15.2 Hz, 3H), 1.85–2.10 (m, 2H), 2.05-2.55 (m, 1H), 2.80–3.13 (m, 1H) 3.92–4.18 (m, 2H) 6.61 (db, J = 2.4 Hz, 0.5H), 6.86 (db, J = 3.2 Hz, 0.5H), 7.43–7.908 (m, 5H).

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