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Spin-Trapping Properties of 5-(Diphenylphosphinoyl)-5-methyl-4,5-dihydro-3*H*-pyrrole *N*-Oxide (DPPMDPO)

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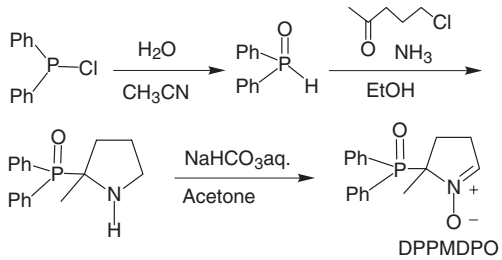
Spin-trapping properties of a novel spin-trapping reagent, 5-(diphenylphosphinoyl)-5-methyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DPPMDPO), were investigated by ESR spectroscopy. DPPMDPO had larger rate constants than DMPO and DEPMPO. DPPMDPO should be better than DEPMPO as a spin-trapping reagent for superoxide detection.

Superoxide and hydroxyl radical have been widely researched, because superoxide is the primary upstream radical of the radical reaction chain that induces oxidative stress and the hydroxyl radical is the most reactive radical species. Detection and measurement of reactive oxygen species (ROS) in vivo and in vitro have been performed by spin-trapping methods with spin-trapping reagents. Electron spin resonance (ESR) spectroscopy is a popular method for detection and identification of adducts since it is possible to detect specifically adducts with relatively stable radicals. Popular spin-trapping reagents for ROS detection are 5,5-dimethyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DMPO) and 5-(diethoxyphosphinoyl)-5-methyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DEPMPO). DMPO is most frequently used since the kinetic parameters of the trapping reactions and ESR parameters of its adducts are well documented.[1] DEPMPO is used for most effective detection and measurement of superoxide since DEPMPO has a larger rate constant for trapping and a longer adduct lifetime in comparison with DMPO.[2] However, the lifetime of DEPMPO–OOH is not sufficient in vivo. Besides, DEPMPO has poor distribution in cell membrane. Therefore, in order to improve detection, several alkoxy derivatives of DEPMPO have been developed.[3] There are a few reasons for studying DEPMPO analogues. The diethoxyphosphinoyl group increases the reactivity forward ROS,[4] and DEPMPO has sufficient amount of application data for in vivo and in vitro ROS trapping.[5,6]

In our previous study, we have described the synthesis and chemical properties of methyl- and phenyl-substituted phosphinoyl derivatives, such as 5-methyl-5-(methylphenylphosphinoyl)-4,5-dihydro-3*H*-pyrrole *N*-oxide (MPPMDPO).[7] MPPMDPO has acceptable solubility and half-life as a superoxide adduct. Unfortunately, MPPMDPO had disadvantage. MPPMDPO exists as diastereomers that are difficult to separate and that affect ESR measurements of the adducts. Therefore, for good ESR measurements, a spin-trapping reagent not have a diastereomer. In this paper, we introduce 5-(diphenylphosphinoyl)-5-methyl-4,5-dihydro-3*H*-pyrrole *N*-oxide (DPPMDPO) as a new phosphinoyl derivative.



Scheme 1.

DPPMDPO was synthesized as shown in Scheme 1. Diphenylphosphine oxide was prepared in 97% yield from chlorodiphenylphosphine. Cyclization of 5-chloropentan-2-one with ammonia and chlorodiphenylphosphine gave a pyrrolidine in 63% yield. Oxone™ oxidation of diphenylphosphine in acetone gave DPPMDPO in 47% yield. Using the Oxone™ instead of MCPBA enables large scale nitrone production in sufficient yield. The purification of DPPMDPO was performed by using recrystallization instead of column chromatography, since pure DPPMDPO easily formed colorless crystals at room temperature. DPPMDPO did not decompose in aqueous solution at room temperature for several months.

The partition coefficient is important parameter for a spin-trapping reagent since a high lipophilicity can improve its distribution in cell membrane. Therefore, we measured the partition coefficient of DPPMDPO in 1-octanol/aqueous solution by using the method described by Konorev et al.[8] The partition coefficient was calculated as the ratio between the DPPMDPO concentration in 1-octanol solution and that in aqueous solution. The concentrations were acquired from optical absorption at 227 nm for an 1-octanol solution and at 224 nm for an aqueous solutions. The observed partition coefficient value was 4.3 ± 0.2 as summarized in Table 1. As shown in Table 1, the partition coefficient of DPPMDPO was larger than that of DMPO,[9] DEPMPO,[2] and MPPMDPO.[7] This result showed that two phenyl groups on phosphinoyl group improved lipophilicity and that one of the drawbacks of DEPMPO was overcome.

Table 1. Partition Coefficients, Rate Constants, and Half-Lives of DMPO, DEPMPO, and DPPMDPO

	DMPO[8,11]	DEPMPO[2]	DPPMDPO
Partition coefficient	0.1	0.06	4.3 ± 0.2
Rate constant for			
Superoxide/M^{-1} s^{-1}	15.7	23.5	39.5 ± 0.2
Hydroxyl radical/10^9 M^{-1} s^{-1}	3.4	7.1	8.50 ± 0.01
Half-life of adduct			
Superoxide/min	1	14.8	8.3 ± 0.2
Hydroxyl radical/min	60	57	13.2 ± 1.0

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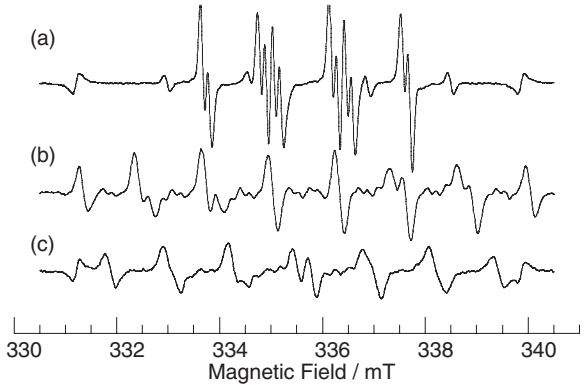

Fig. 1. ESR spectra of (a) DMPO–OOH, (b) DEPMPO–OOH, and (c) DPPMDPO–OOH. All measurements and trapping reaction were performed under the same condition.

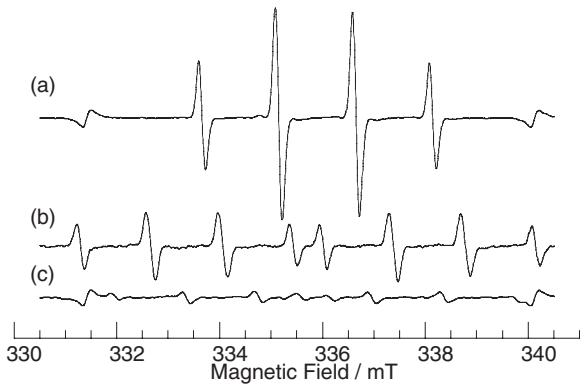

Fig. 2. ESR spectra of (a) DMPO–OH, (b) DEPMPO–OH, and (c) DPPMDPO–OH. All measurements and trapping reaction were performed under the same condition.

Figure 1 shows ESR spectra of DMPO–, DEPMPO–, and DPPMDPO–OOH. Superoxide generation was performed by using the hypoxanthine–xanthine oxidase (HPX–XOD) reaction system at pH 7.4.[10] The hyperfine splitting pattern of ESR spectrum of DPPMDPO–OOH was similar to that of DEPMPO–OOH. However, the signal of DPPMDPO–OOH did not overlap that of Mn^{2+} as an external standard. Figure 2 shows ESR spectra of DMPO–, DEPMPO–, and DPPMDPO–OH. Hydroxyl radical generation was performed by using the Fenton reaction system at pH 7.4.[11] Hyperfine splitting pattern of the ESR spectrum of DPPMDPO–OH was also similar to that of DEPMPO–OH. Figure 3 shows ESR spectra of DMPO–, DEPMPO–, and DPPMDPO–R. Carbon-centered radical generation was performed by using the Fenton reaction system containing MeOH at pH 7.4. Computer simulations of the ESR spectra of DPPMDPO–OOH, –OH, and –R gave ESR parameters and showed that DPPMDPO–OOH has a diastereomer. Calculated ESR parameters of DPPMDPO–OOH, –OH, and –R are summarized in Table 2.

In many cases, the rate constant of a ROS trapping reaction is obtained from competitive reaction by using the method described by Finkelstein et al.[12] For our study, DMPO was used in the competitive reaction of DPPMDPO. Superoxide trapping was performed in the HPX–XOD reaction system at pH 7.4, and hydroxyl radical trapping was performed in the Fenton reaction system at pH 7.4. Observed rate constants for DPPMDPO and reported values for DMPO and DEPMPO are summarized in Table 1. Unfortunately, the rate constants of several DMPO analogues were not obtained from the competitive reaction with DMPO but from inhibitory reaction of cytochrome *c* reduction, because in order to compare the rate constants the reactions must be done under the same conditions.[13] Furthermore, some of the rate constants of the other analogues have been calculated by using the inaccurate value for DEPMPO.[14] Consequently, we performed the competitive reaction with DMPO and ESR spin-trapping method the same each time. Therefore, the diphenylphosphinoyl group increased the rate constants of the ROS trapping reactions more than the diethoxyphosphinoyl group.

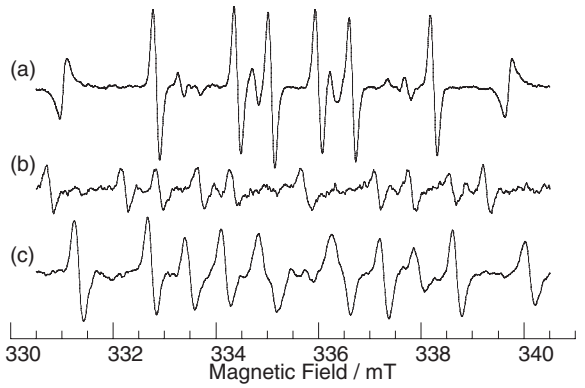

Fig. 3. ESR spectra of (a) DMPO–R, (b) DEPMPO–R, and (c) DPPMDPO–R. All trapping reactions were performed under the same condition.

Table 2. Hyperfine Coupling Constants and *g*-Values of DPPMDPO–OOH, –OH, and –R

	Hyperfine coupling constant			*g*-Value
	a_P/mT	a_N/mT	a_H/mT	
DPPMDPO–OOH				
Diastereomer A 65%	3.90	1.26	1.10	2.0069
Diastereomer B 35%	3.95	1.22	1.18	2.0072
DPPMDPO–OH	3.55	1.37	1.37	2.0068
DPPMDPO–R	3.75	1.42	2.16	2.0067

Monitoring of time dependent decay of relative ESR signal intensities gave half-lives of DPPMDPO–OOH and –OH. The half-lives were calculated from the first-order kinetic decay date. We applied the HPX–XOD superoxide generation system to produce DPPMDPO–OOH since it has been reported that other systems, such as the light-riboflavin superoxide generation system, simultaneously generate persistent carbon-centered radicals, which leads to an overestimation of the measured half-life.[15]

The signal intensity of DPPMDPO–OOH in the HPX–XOD reaction system reached a maximum around 5 min from the reaction initiation and decreased exponentially as shown in Fig. 4a. In the exponentially decaying region, time dependency completely obeyed first-order kinetic decay. In case of SOD addition to HPX–XOD reaction system to stop the superoxide trapping reaction, signal intensity decayed exponentially. The

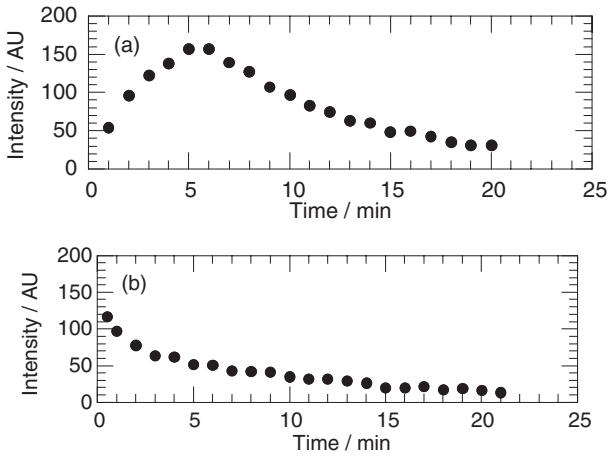

Fig. 4. Time-dependent decay of ESR signal intensities for DPPMDPO–OOH (a) and DPPMDPO–OH (b) at pH 7.4.

time dependency also obeyed first-order kinetic decay. The time dependence of the signal decay was the same regardless of the presence of the SOD. Calculated half-life of DPPMDPO–OOH was 8.3 ± 0.2 min as summarized in Table 1.

Signal intensity of DPPMDPO–OH in Fenton reaction system decayed exponentially after 3 min from reaction initiation. In the exponentially decaying region, the time dependency completely obeyed first-order kinetic decay. In addition, the signal decay of DPPMDPO–OH in the H_2O_2-UV radiation hydroxyl radical generation system showed also the same time dependency. The calculated half-life of DPPMDPO–OH was 13.2 ± 1.0 min as summarized in Table 1. Half-life of DPPMDPO–OH was shorter than those of DMPO– and DEPMPO–OH. Furthermore, the signal intensity of DPPMDPO–OH decayed rapidly immediately after initiation of the Fenton reaction as shown in Fig. 4b. These results showed that DPPMDPO–OH was not stable.

Introduction of the diphenylphosphinoyl group instead of the diethoxyphosphinoyl group enhanced stability of nitrone and increased the rate constants for superoxide and hydroxyl radical trapping reactions. Furthermore, DPPMDPO did not degrade in aqueous solution for several months. DPPMDPO appears to be a better spin-trapping reagent for superoxide detection.

References

1 G. R. Buettner, *Free Radical Biol. Med.* **1987**, *3*, 259.
2 C. Frejaville, H. Karoui, B. Tuccio, F. Moigne, M. Culcasi, S. Pietri, R. Lauricella, P. Tordo, *J. Med. Chem.* **1995**, *38*, 258.
3 F. Chalier, P. Tordo, *J. Chem. Soc., Perkin Trans. 2* **2002**, 2110.
4 J. Katzhendler, I. Ringel, R. Karaman, H. Zaher, E. Breuer, *J. Chem. Soc., Perkin Trans. 2* **1997**, 341.
5 K. J. Liu, M. Miyake, T. Panz, H. Swartz, *Free Radical Biol. Med.* **1999**, *26*, 714.
6 S. Dikalov, P. Tordo, A. Motten, R. P. Mason, *Free Radical Res.* **2003**, *37*, 705.
7 K. Shioji, S. Tsukumoto, H. Tanaka, K. Okuma, *Chem. Lett.* **2003**, *32*, 604.
8 E. A. Konorev, J. E. Baker, B. Kalyanaraman, *Free Radical Biol. Med.* **1993**, *14*, 127.
9 G. M. Rosen, E. Finkelstein, E. J. Rauckman, *Arch. Biochem. Biophys.* **1982**, *215*, 367.
10 K. Mitsuta, Y. Mizuta, M. Kohno, M. Hiramatsu, A. Mori, *Bull. Chem. Soc. Jpn.* **1990**, *63*, 187.
11 M. Kohno, M. Yamada, K. Mitsuta, Y. Mizuta, T. Yoshikawa, *Bull. Chem. Soc. Jpn.* **1991**, *64*, 1447.
12 E. Finkelstein, G. M. Rosen, E. J. Rauckman, *J. Am. Chem. Soc.* **1980**, *102*, 4994.
13 P. Tsai, K. Ichikawa, C. Mailer, S. Pou, H. J. Halpern, B. H. Robinson, R. Nielsen, G. M. Rosen, *J. Org. Chem.* **2003**, *68*, 7811.
14 F. A. Villamena, J. L. Zweier, *J. Chem. Soc., Perkin Trans. 2* **2002**, 1340.
15 G. R. Buettner, *Free Radical Res. Commun.* **1990**, *10*, 11.