Synthesis and Properties of Fluorescence Spin Traps Containing Phosphinoyl Moiety

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Abstract

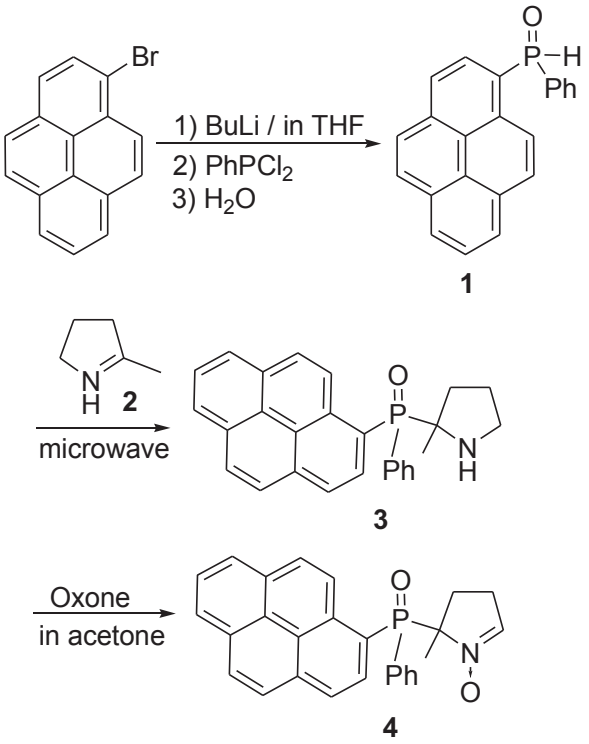
Pyrrole *N*-Oxide spin trap containing pyrenylphenylphosphoryl moiety at 5 position in pyroline ring as a fluorophore was synthesized. The trapping experiments toward several free radicals were carried out in benzene. Each ESR spectram of adducts were split by phosphorus atom and were distinguishable. The fluorescence intensity of radical adduct was lower than that of diamagnetic PyPhPMPO due to intramolecular quenching.

Introduction

Free radicals have been implicated in a number of pathological conditions.[1] These reactive species has been detected by various methods. For example, electron spin resonance (ESR) method using spin traps is most popular for detecting the free radicals.[2-5] Rosen et al. have been reported the utility of fluorophore-containing TEMPO derivative as potential probes to detect free radicals.[6] Excited singlet state of the fluorophore was quenched by diamagnetic nitoroxyl radicals.[7,8] When the radicals was attacked to the TEMPO derivatives the fluorescence emission increased compared with that of starting nitroxyl radicals. Recently, we have reported that pyrroline *N*-oxides substituted by dialkyl and/or aryl captured active free radical species to give satisfied electron spin resonance (ESR) signals of corresponding adducts. The arylphosphoryl moiety, especially pyrenylphosphoryl group, has strong fluorescence.[9,10] We report herein the synthesis and properties of novel spin traps containing pyrenylphenylphosphinoyl moiety as a fluorophore.

Results and Discussion

Synthesis of spin traps containing phenylpyrenylphosphinoyl group was carried out by addition of pyrenylphenylphosphine oxide (1) prepared from pyrenyl lithium and dichlorophenylphosphine to 2-methyl-1-pyrroline (2) under microwave irradiation. The addition was completed for 1 min to give corresponding amine 3. The oxidation of precursor amine 3 by Oxone afforded corresponding nitrone 4. The diastereomeric mixture was purified by column chromatography to give colorless crystals of nitrone 4 in 39% yield (Scheme 1). The diastereomers of nitrone 4 (PhPyPMPO), which indicated 40.1ppm and 41.8 ppm of ^{31}P NMR chemical sifts, respectively, were able to isolate with 1:1 ratio by column chromatography. An enantiomer pair (40.1 ppm) was used in ESR spin trapping experiments.



Scheme 1.

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The PhPyPMPO-OBu^t adduct was obtained by photolysis of Bu^{t}OOBu^t (0.5 M) in the presence of PhPyPMPO (10 mM) in benzene. Spin trapping of the *tert*-Butylperoxyl radical by PhPyPMPO was performed in benzene solution by photolysis of Bu^{t}OOH (0.5 M) in the presence of PhPyPMPO (10 mM). The PhPyPMPO-OOH adduct was obtained by nucleophilic addition of H_2O_2 (320 mM) to PhPyPMPO (10 mM) in pyridine followed by *in situ* oxidation of the hydroxyl amine. The obtained spectrum showed the characteristic pattern observed for DPhPMPO-OOH type adducts. The obtained ESR signal exhibited seven lines, which have small shoulder split by hydrogen atom at inside five lines. The PhPyPMPO-Me adduct was generated photolysis of methyl iodide in the presence of bis(tributyltin). The observed ESR signal was asymmetrical 10 lines because of poor stability under this condition. The PhPyPMPO-SMe adduct was obtained by photolysis of MeSSMe (0.5 M) in the presence of PhPyPMPO (10 mM). Since the hyper fine splitting constant (hfsc) of nitrogen was larger than that of PhPyPMPO-OOH, PhPyPMPO-OO-*t*-Bu, and PhPyPMPO-O-*t*-Bu, the observed ESR signal exhibited a symmetrical 7 lines. These hfsc's data calculated by computer simulation were assumed in Table 1. The ESR signals of several radical adduct were similar to that of the counterpart (41.8 ppm). Additionally, ESR spin trapping experiments of the 1:1 mixture of these enantiomer pairs were carried out. The ESR signals were similar to those of both enantiomer pair. These results suggest that the stereochemistry of phosphorus and carbon atom at 5 position of pyrroline ring was not concerned to the hfsc.

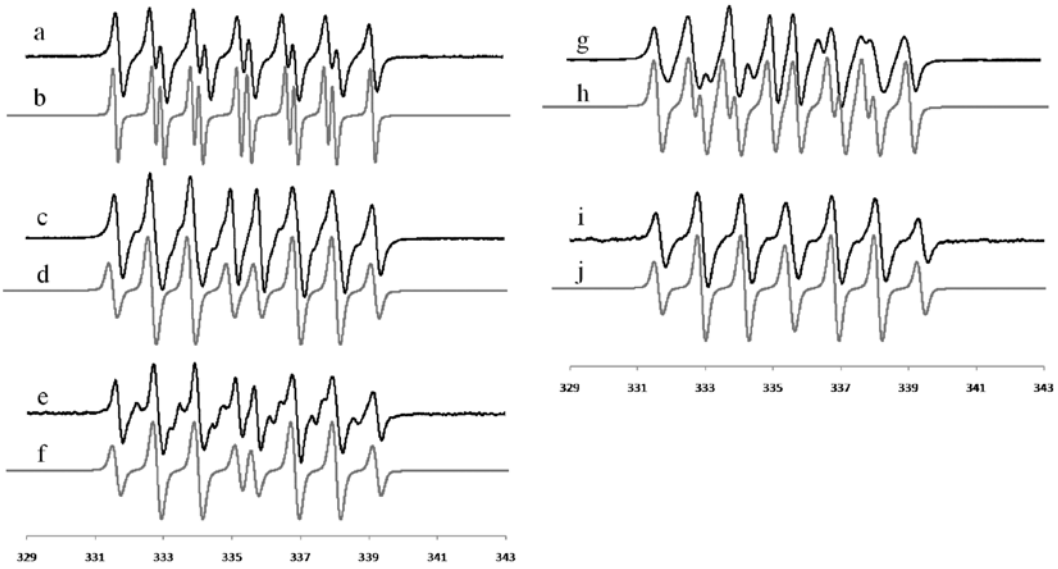

Figure 1. ESR signals of several radical adducts of ^{31}P-40.1 PhPyPMPO and their computer simulation. (a) PhPyPMPO/*t*-BuO$^{\bullet}$. (b) Computer simulation of (a). (c) PhPyPMPO/*t*-BuO$^{\bullet}$. (d) Computer simulation of (c). (e) PhPyPMPO/HOO$^{\bullet}$. (f) Computer simulation of (e). (g) PhPyPMPO/$CH_3^{\bullet}$. (h) Computer simulation of (g). (i) PhPyPMPO/$^{\bullet}SCH_3$. (j) Computer simulation of (i).

Table 1. ESR Hyperfine Splitting Constants (hfsc) of Several Radical Adducts of PhPyPMPO **4**

adduct	source	solvent	a_N (mT)	a_H (mT)	a_P (mT)
t-BuO$^{\bullet}$	*t*-BuOO-*t*-Bu, *hυ*	benzene	1.12	1.35	3.88
t-BuOO$^{\bullet}$	*t*-BuOOH, *hυ*	benzene	1.14	1.12	4.19
HO$^{\bullet}$	pyridine/H_2O_2	pyridine	1.20	1.16	4.01
$CH_3^{\bullet}$	CH_3I, bis(tributyltin), *hυ*	benzene	0.99	1.29	4.01
$CH_3S^{\bullet}$	CH_3S-SCH_3, *hυ*	benzene	1.28	1.27	3.96

The measurement of fluorescence intensity was performed in benzene in the presence of PhPyPMPO (10 μM). PhPyPMPO has a high fluorescence quantum yield due to the pyrenylphosphoryl fluorophore. Pyrenyl fluorophore, generally, has excimer fluorescence at high concentration. PhPyPMPO has also emission maximum at 381 nm. When

the photolysis of *t*-BuOOH was carried out in the presence of PhPyPMPO, the fluorescence intensity of PhPyPMPO decreased gradually. A same behavior was observed in the trapping of *t*-BuO$^{\bullet}$ and $CH_3^{\bullet}$ using PhPyPMPO. The formation of paramagnetic PhPyPMPO adduct resulted in lowering of the fluorescence intensity due to efficient intramolecular quenching of exited singlet state of fluorophore. The increasing of ESR signals of radical adducts paralleled decreasing of fluorescence intensity of PhPyPMPO. Usually, when TEMPO derivatives containing fluorophore were used for radical or redox sensor, the system required alkyl radical source such as DMSO. Similar phenomenon was observed in the direct addition of radical, involving oxygen and sulfur centered radicals, to PhPyPMPO. Additionally, since the fluorescence decreasing was also detected in diluted solution (ca.100 nM), we explored the possible use of PhPyPMPO as a radical sensor for several free radicals.

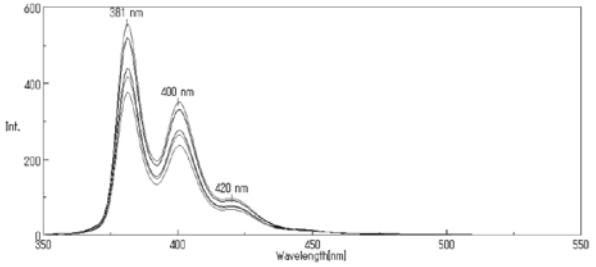

Figure 2. Fluorescence emission spectra of PhPyPMPO-OO-*t*-Bu adduct at different times.

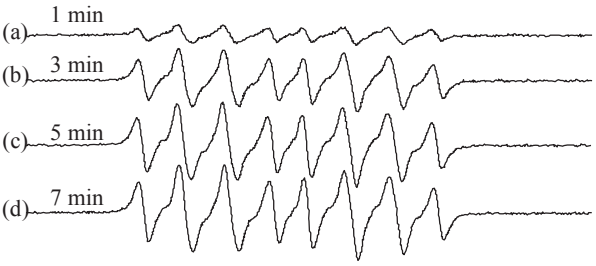

Figure 3. ESR spectra of PhPyPMPO-OO ^{t}Bu adduct at different times.

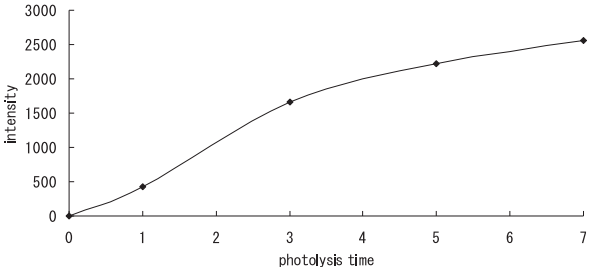

Figure 4. ESR signal intensity of PhPyPMPO-OO ^{t}Bu adduct at different times.

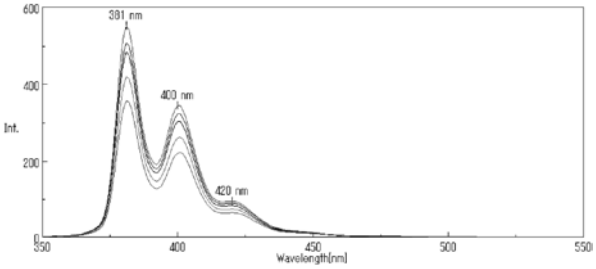

Figure 5. Fluorescence emission spectra of PhPyPMPO-O ^{t}Bu adduct at different times.

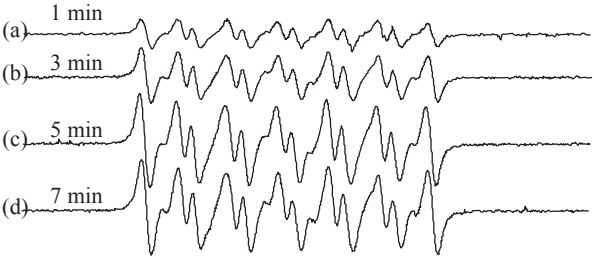

Figure 6. ESR spectra of PhPyPMPO-O ^{t}Bu adduct at different times.

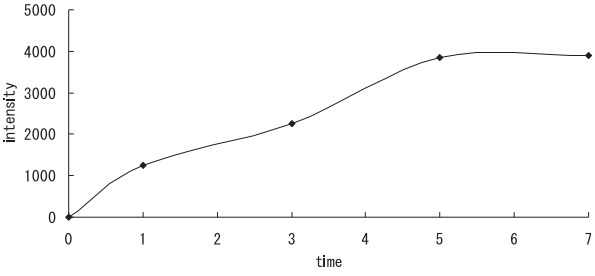

Figure 7. ESR signal intensity of PhPyPMPO-O ^{t}Bu adduct at different times.

Experiments

Materials. The solvents were distilled under nitrogen atmosphere. All chemicals were obtained from commercial supplier and used without further purification. Analytical TLC was carried out on precoated plates (Merck, silica gel 60, F254) and flush column chromatography was performed with silica (Merck, 70-230 mesh). NMR spectra (^{1}H at 400 MHz; ^{13}C at 100 MHz; ^{31}P at 161MHz) were recorded in $CDCl_3$ solvent, and the chemical shifts were expressed in ppm relative to internal TMS. ^{31}P NMR was taken in $CDCl_3$ using 85% H_3PO_4 as an internal standard with broadband ^{1}H decoupling. ESR spectra were recorded at room temperature using a spectrometer at 9.5 GHz employing 100 kHz field modulation. The melting points were uncorrected.

Synthesis of PyPhPMPO 4. Pyrenylphenylphosphine Oxide (1). To a solution of 1-Bromopyrene (17.8 mmol, 5 g) in 100 mL of THF was added 13.6 mL of t-BuLi hexane solution (21.4 mmol , 13.6 ml) at -78˚C and stirred at 2h to give pyrenyl lithium solution. The solution was added to 10 mL of THF solution containing 3.6 mL of dichlorophenylphosphine (26.7 mmol, 3.6 mL) at -78˚C and stirred at room temperature for 12h. The reaction mixture was quenched by 100 mL of water and extracted with CH_2Cl_2 (100 mL x3). The combined organic layer was dried over the Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2) to afford pyrenylphenylphosphine oxide **1** as pale yellow crystals (4.8 g, 83%): ^{31}P NMR (162.10 MHz) δ 24.41; ^{1}H NMR (400.45 MHz) δ 8.73 (1H, d, J = 452 Hz), 8.60-8.57 (1H, d, *J* =12.0 Hz), 8.40-8.34 (1H, m), 8.25-8.02 (7H, m), 7.76-7.70 (2H, m), 7,50-7.41 (1H, m); ^{13}C NMR (100.69 MHz) δ 134.93, 134.90, 133.22, 133.14, 132.88, 132.60, 132.57, 131.88, 131.22, 131.15, 131.04, 130.63, 130.26, 129.83, 129.77, 129.63, 129.19, 129.07, 127.39, 126.90, 126.87, 126.76, 125.12, 125.02, 124.54, 124.43, 124.39, 124.35, 124.12, 123,10

Anal. Calcd for $C_{22}H_{15}OP$: C, 80.97; H, 4.63. Found: C, 80.71; H, 4.90.

5-(phenylpyrenylphosphinyl)-5-methyl-1-pyrroline (3). A mixture of phosphine oxide **1** (500mg, 3.1 mmol) and 2-methyl-1-pyrroline **(2)** (0.15 mL, 3.1 mmol) was stirred and irradiated of microwave for 1 min to give crude 5-(phenylpyrenylphosphinyl)-5-methyl-1-pyrroline **3**. The residue was used next oxidation without purification.

5-(phenylpyrenylphosphinyl)-5-methyl-1-pyrroline *N*-oxide (4). A solution of Oxone (1.1g, 1.8mmol) in water (30 mL) and a solution $NaHCO_3$ (596 mg, 7.1 mmol) in 30 mL of water was added to a solution of crude **3** (600 mg, of **3**) in 30 mL of acetone at 0˚C. After stirring for 1h, 30 mL of 10% sodium thiosulfate was added to the reaction mixture and removed acetone under reduced pressure. The residue was extracted with CH_2Cl_2 (30 mL x 3) and dried over Na_2SO_4. The solvent was removed under reduced pressure and purified by column chromatography (ethyl acetate /methanol 9:1) to afford two enantiomer pairs of **4**: One enantiomer pair ^{31}P NMR 162.10 MHz) δ 40.07. Another δ 41.8.

^{31}P NMR (162.10 MHz) δ 40.07; ^{1}H NMR (400.45 MHz) δ 9.47-9.45 (1H, d, *J* = 8.0 Hz), 9.43-9.41 (1H, d, *J* = 8.0 Hz), 8.26-8.00 (9H, m), 7.54-7.44 (3H, m), 6.78 (1H, s),3.45-3.35 (1H, m), 2.45-2.40 (1H, m), 2.33-2.21 (1H, m), 2.03-2.00 (3H, d, *J* = 12.0 Hz), and 2.01-1.91 (1H, m); ^{13}C NMR (100.69 MHz) δ 136.80, 136.74, 135.55, 135.47, 134.34, 132.66, 132.57, 132.50, 131.75, 131.61, 131.17, 131.06, 130.45, 130.12, 128.99, 128.77, 128.66, 127.38, 126.51, 126.47, 126.33, 126.02, 125.98, 125.46, 125.35, 124.38, 123.99, 123.84, 122.38, 121.38, 80.89, 80.20, 31.71, 25.84, and 22.24; Anal. Calcd for $C_{27}H_{22}NO_2P\cdot H_2O$: C, 73.46; H, 5.48; N, 3.17. Found: C, 73.55; H, 5.53; N; 3.27.

^{31}P NMR (162.10 MHz) δ 41.83; ^{1}H NMR (400.45 MHz) δ 9.87-9.84 (1H, d, *J* = 12.0 Hz), 8.70-8.65 (2H, m), 8.54-8.49 (1H, m), 8.28-8.03 (7H, m), 7.56-7.52 (3H, m), 6.35 (1H, s), 3.58-3.51 (1H, m), 2.27-2.19 (1H, m), 2.17-2.06 (1H, m), 2.00-1.96 (3H, d, *J* = 16.0 Hz), 1.33-1.27 (1H, m); ^{13}C NMR (100.69 MHz) δ 136.19, 136.12, 135.38, 135.31, 134.12, 134.09, 133.48, 133.38, 132.15, 132.05, 132.02, 131.17, 131.05, 131.01, 130.31, 130.12, 129.29, 128.48, 128.35, 127.11, 126.50, 126.33, 126.25, 125.29, 125.25, 124.83, 124.73, 124.08, 123.68, 123.54, 121.25, 120.34, 80.65, 79.96, 31.48, 25.55, and 21.59; Anal. Calcd for $C_{27}H_{22}NO_2P$: C, 76.58; H, 5.24; N, 3.31. Found: C, 76.29; H, 5.59; N; 3.09.

ESR measurements. ESR Measurement. ESR spectra were recorded at room temperature using a spectrometer at 9.5 GHz employing 100 kHz field modulation. Reaction mixture was prepared in a phosphate buffer (1.0 M, pH 7.4).

(a) *tert*-Butylperoxyl Radical Trapping: *t*-BuOOH/*h*u system. The *tert*-butylperoxyl radical adduct was generated by photolysis of *t*-BuOOH (1.5 M) in the presence of a spin trap in deoxygenated benzene.

(b) *tert*-Butoxyl Radical Trapping: *t*-BuOO-*t*-Bu/*h*u system. The *tert*-butoxyl radical radical adduct was generated by photolysis of *t*-BuOO-*t*-Bu (0.5 M) in the presence of a spin

trap in deoxygenated benzene.

(e) Methyl Radical Trapping Radical Trapping: The methyl radical adduct was generated by photolysis of CH_3I (0.5 M) with hexabutyldistannane in the presence of a spin trap in deoxygenated benzene.

(d) Methyl Thiyl Trapping. Methylthiyl radical adduct was generated by photolysis of EtSSEt (1.0 M) in the presence of a spin trap in deoxygenated benzene.

References

1 Singh KK, editors. *Oxidative stress, Disease and Cancer.* London: Imperial College Press; 2006. And references therein.

2 Janzen EG, Poyer JL, Schaefer CF, Downs PE, Dubose CM. *J. Biochem. Biophys. Methods* 1995; 30: 239-247.

3 Finkelstein E, Rosen GM, Rauckman EJ. *Arch. Biochem. Biophys.* 1980; 200;1-16.

4 Janzen EG. *Free Radicals in Biology.* Pryor WA, editors. New York: Academic Press; 1980. Vol. 4, p 115-154.

5 Harbour JR, Chow V, Bolton JR. *Can. J. Chem.* 1974; 52:3549-3553.

6 S. Pou, A. Bhan, V. S. Bhadti, S. Y. Wu, R. S. Hosmane, G. M. Rosen, *FASEB J*, **1995**, *9*, 1085-1090.

7 S. A. Green, D. J. Simpson, G. Zhou, P. S. Ho, N. V. Blough, *J. Am. Chem. Soc.* **1990**, *112*, 7337-7346.

8 Z. Y. Bian, X. Q. Guo, Y. B. Zhao, J. O. Du, *Anal. Sci. Jpn.* **2005**, *21*, 553-559.

9 K. Akasaka, T. Suzuki, H. Ohrui, H. Meguro, *Anal. Lett.* **1987**, *20*, 731-745, 797-807.

10 Y. Okimoto, A. Watanabe, E. Niki, T. Yamashita, N. Noguchi, *FEBS Lett.* **2000**, *474*, 137-140.