Synthesis of a New Nitrone for Detection of Radical Species

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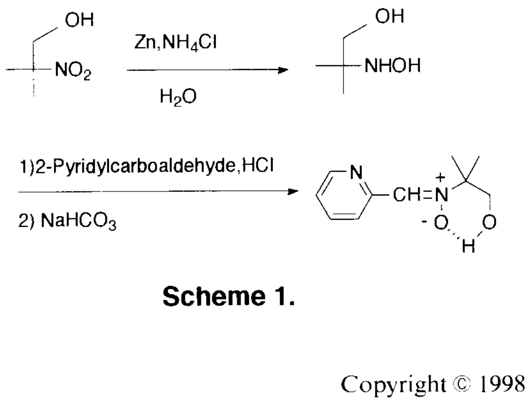
A water-soluble new nitrone, α–(2-pyridyl) *N*-(2-hydroxy-1,1-dimethylethyl) nitrone, was synthesized and found to be useful for selective detection of oxygen-centered radicals in aqueous reactions.

Spin trapping has intensively been studied to elucidate reaction mechanisms in which free radical intermediates take part.[1-4] To develop this technique many types of compounds were examined to trap radical species;[5] among them nitrones are outstanding because their spin trapping abilities are excellent and the ESR spectra of the adducts with radical species have unique characteristics for analyses of structures of the trapped radicals. For example, α-phenyl *N*-(tert-butyl) nitrone (PBN) has been preferencially used because the phenyl group attached to the carbon atom of the nitronyl function imparts stability to the parent nitrone and the tertiary alkyl group attached to the nitrogen atom lends persistence to the spin adducts for ESR measurements.[6] For biological reactions, water-soluble spin traps are necessary to trap, in many cases, hydroxyl or superoxide radicals in aqueous media. However, PBN is slightly soluble in water; therefore, its derivatives which have improved solubility in water have been required.

We have prepared a PBN derivative that has pyridyl and hydroxy groups, α-(2-pyridyl) *N*-(2-hydroxy-1,1-dimethylethyl) nitrone (PyHMEN), and found that it can be used as a spin trap to study bio-related aqueous reactions in which intermediate oxygen-centered free radical species are generated. Nitronyl alcohols such as α-phenyl *N*-(2-hydroxy-1,1-dimethylethyl) nitrone (PhHMEN) were prepared, but their detailed spin-trapping behavior was not reported.[7] We synthesized PhHMEN also, which is less soluble in water, to compare its spin-trapping reactions with those of PyHMEN.

Scheme 1 shows the synthetic route to prepare PyHMEN.[8] The ESR spectrum obtained with an aqueous solution of the nitrone showed very weak absorption probably due to a trace amount of spin adducts formed during the synthesis. The impurities could be removed almost completely by recrystallization from n-haxane-ethyl acetate; thus the small peaks in the background absorption spectrum disappeared, and consequently precise analysis on the basis of ESR spectra became feasible.



Scheme 1.

Partition of nitrones between 1-octanol and water was examined to check their solubility in water (Table 1). PBN is difficult to use in water because of its poor solubility. PyHMEN is more soluble in water than PhHMEN and can satisfactorily be used for aqueous reactions. DMPO is the most soluble of them in water; however, the spin adduct with a superoxide radical decays relatively fast. To decrease the decay rate and facilitate the ESR measurements, we prepared substituted DMPO's in a preceding work.[10]

Table 1. Partition of nitrones between 1-octanol and water[a]

	PBN	PhHMEN	PyHMEN	DMPO[b]
P[c]	21[d]	3.3[e]	0.6[e]	0.1[d]

[a] Conditions: 1-Octanol 1 ml, D_2O 1 ml, nitrone 1 mmol, at 15 ℃. [b] 5,5-Dimethyl-1-pyrroline *N*-oxide. [c] P = [conc. in 1-octanol]/[conc. in water]. [d] The value reported by Konaka et al.[9] [e] Determined by ^{1}H NMR.

Trapping of hydroxy radicals with PyHMEN,PhHMEN, and PBN in the Fenton reaction was carried out under the conditions: aqueous spin trap (0.3 mol dm^{-3}, 50 μl), H_2O_2 (15 mmol dm^{-3}, 50 μl), $FeCl_2$ (0.3 mmol dm^{-3}, 50 μl). The ESR measurements were performed with a following setting: microwave power 4 mW, field 336.4±5 mT, modulation 0.1 mT, time constant 0.3 sec, sweep time 4 min. Trapping of superoxide radicals with the nitrones was also examined using an aqueous reaction (buffered with phosphoric acid, pH 7.8) of hipoxanthine (2 mmol dm^{-3}, 50 μl) and xanthine oxidase (0.4 unit cm^{-3}, 50 μl) in the presence of a spin trap (3 mol dm^{-3}, 50 μl). The ESR measurements were made with a setting: microwave power 4 mW, field 335±5 mT, modulation 0.1 mT, time constant

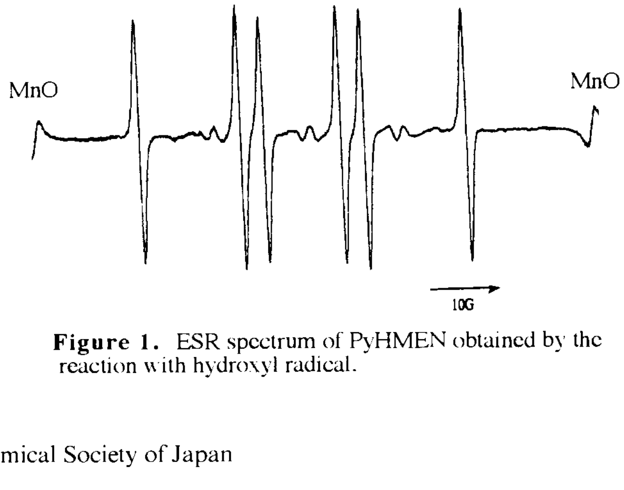


Figure 1. ESR spectrum of PyHMEN obtained by the reaction with hydroxyl radical.

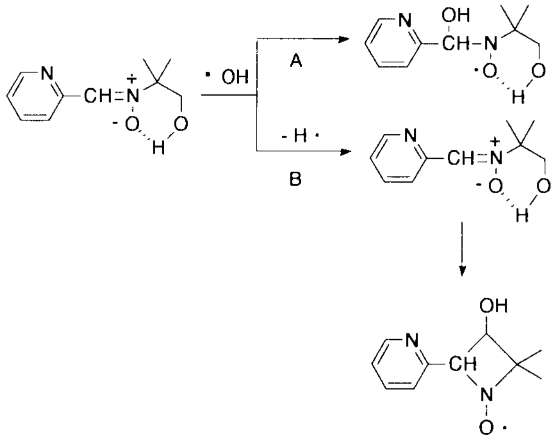
Table 2. Hyperfine splitting constants of PHDEN,PyHDEN and PBN adducts (mT)[a]

Radical	PyHMEN		PhHMEN		PBN	
	a_N	$a_{\beta\text{-}H}$	a_N	$a_{\beta\text{-}H}$	a_N	$a_{\beta\text{-}H}$
HO ·	1.56	1.96	1.44	1.44	1.48	0.28
O_2^{-} ·	—[b]	—[b]	1.44	0.28	1.53	0.28

[a] Solvent: H_2O

[b] no distinct peak was observed.

0.1 sec, sweep time 2 min. The ESR spectrum obtained in the Fenton reaction with PyHMEN is shown in Figure 1, which is typical of the spin adduct of a nitrone. The hyperfine splitting constants calculated from the spectra are presented in Table 2. The $a_{\beta\text{-}H}$ values for PyHMEN and PhHMEN are quite large, compared with that for PBN. A similar results were obtained in trapping of phenyl radicals with a cyclic nitrone, DMPO (a_N = 16.02, $a_{\beta H}$ = 23.12).[11] It therefore seems most likely that the product with a spin has a cyclic structure like a spin adduct of DMPO. As shown in Scheme 2, such a cyclic spin-labeled product would be formed through hydrogen abstraction followed by cyclization (route B), and usual spin addition (route A) seems not to occur. A very similar hydrogen abstraction to yield a cyclic product with a relatively large $a_{\beta\text{-}H}$ value was previously reported by Janzen et al.[13] The ESR signal of the product was persistent, indicating that PyHMEN is useful as a spin detecting agent for aqueous reactions. Moreover, this nitrone did not yield any product having a spin in the reaction in which superoxide radicals were generated (Table 2). Superoxide may be quenched by the pyridine ring with an electron-withdrawing nitronyl group as reported by Yamaguchi et al. for the reaction of halogenated quinolines with the superoxide radicals.[14] The selective detection of hydroxy radicals in the presence of superoxide radicals is very interesting, because discriminating detection of such oxygen-centered radicals is important to elucidate mechanisms of biological reactions.



Scheme 2.

Further investigations of the radical trapping by PyHMEN is now in progress.

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8 PyHMEN: pale yellow crystal, mp 105-106 ℃, IR (NaCl)ν 3410, 3200, 2930, 1570, 1460, 1395, 1370, 1280, 1130, 1060, 810 cm^{-1}, ^{1}H NMR($CDCl_3$)δ 1.61(s,6H), 3.82(s,sH), 4.65 (brs, 1H), 7.26 (m, 1H), 7.70 (m, 1H), 7.82 (m,1H), 8.60 (m, 1H), 9.01 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 149.4, 149.3, 136.9, 133.5, 124.3, 124.0, 74.3, 69.4, 23.6 Anal. Found: C, 61.62; H, 7.16; N, 14.36%. Calcd for $C_9H_{14}N_2O_2$: C, 61.51; H, 7.27; N, 14.42%.

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