Synthesis and Plane Selective Spin Trapping of a Novel Trap 5,5-Dimethyl-3-(2-ethoxycarbonyl-ethyl)-1-pyrroline N-oxide

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A novel cyclic nitron spin trap 5,5-dimethyl-3-(2-ethoxycarbonyl-ethyl)-1-pyrroline N-oxide has been prepared and its ability to trap a series of transient free radicals has been investigated. This nitron scavenges free radicals to give persistent nitroxides, *e.g.*, the half life-times of hydroxyl radical adducts and *tert*-butoxy radical adducts in benzene >30 min, and the life-time of acetyl adducts >60 min. The EPR spectrum of 2,2-dimethyl-4-(3-hydroxy-propyl)pyrrolidinyl-1-oxyl aminoxyl shows that the hyperfine splitting constants of two β -hydrogens are equal to 14.58 G and 23.29 G respectively, *i.e.*, the two β -hydrogens are not magnetically equivalent. Radical addition to the nitron is probably plane selective affording only one of the two possible geometric isomer pairs of the spin adduct nitroxides. The *trans* approach mechanism is proposed through configuration and conformation analysis.

Introduction

The electron spin resonance trapping technique [1–3], or called spin trapping, has been widely used to study transient free radicals in various systems [4–11]. Spin traps play a very important role in this technique (eq. (1)).

For the technique to work well, the trap should efficiently scavenge free radicals and afford persistent spin adducts of which the longer the life-times are, the better the spin trap is. Recently, 5,5-disubstituted-1-pyrroline N-oxides [12–16], 3,3,5,5-tetrasubstituted-1-pyrroline N-oxides [17–19], and 3,3,4,5,5-pentasubstituted analogues [20] have been demonstrated to be good spin traps. In addition, an asymmetric carbon on the 5-ring position can induce stereospecific radical addition [12, 13, 17]. We believe that a chiral carbon center on the C₃ ring position adjacent to the nitronyl carbon should have much greater influence on radical addition stereoselectivity or stereospecificity and on the hyperfine splitting constants (hfsc) of the spin adduct β -hydrogen. In order to search for very efficient traps and to study spin trapping stereochemistry and radical stereochemical properties, 5,5-dimethyl-3-(2-ethoxycarbonyl-ethyl)-1-pyrro-

line N-oxide (**4**), in which the 3-carbon is a chiral center, was prepared according to the route illustrated in Scheme 1. Aldehyde-enamine alkylation ability is usually weaker than ketone-enamine's. The presence of Me₂CNO₂ group substitution much more decrease the alkylation ability of enamine **2** with ethyl acrylate, and so the yield was

Scheme 1.

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very low. In this paper, we also wish to report the spin trapping results with **4** and the plane selectivity of radical addition reactions in its spin trapping processes.

Results and Discussion

The nitron **4** is soluble in water as well as in ordinary organic solvents. Therefore, nitron **4** can be used to probe short-lived free radicals not only in organic phases but also in aqueous systems. The hfs constants of the spin adducts are presented in Table I while several typical EPR spectra are shown in Fig. 1.

Fig. 1. EPR spectra of (A) hydroxyl spin adduct of **4** obtained by photolysis of zinc oxide and **4** in water; (B) hydroxyl and hydroxymethyl spin adducts of **4** obtained from photolysis of zinc oxide, methanol and **4** in water; (C) hydroxyl and CO_2^- spin adducts of **4** obtained by photolysis of zinc oxide and sodium oxalate in water; (D) SO_3^- spin adduct of **4** obtained from photolysis of zinc oxide, sodium sulfite and **4** in water; (E) acetyl (high), and carboxymethyl and/or methyl (low) spin adducts of **4** obtained when photolysis of zinc oxide, acetic acid and **4** in water. Splitting parameters, see Table I.

Spin trapping ability test

The photolysis of zinc oxide in water can generate hydroxyl radicals [21, 22] (eq. (2, 3)).

In the presence of **4**, the observed EPR spectrum showed a 1:2:2:1 pattern of four peaks which can be interpreted in terms of equivalent hyperfine splitting from both the β -hydrogen and the aminoxyl nitrogen atom ($a_N = a_H^\beta = 15.53$ G). Such a 1:2:2:1 quartet of EPR signals was given when hydroxyl radicals were trapped respectively with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) [16, 17, 21–26], 3,3,5,5-tetramethyl-4-ethoxycarbonyl-1-pyrroline N-oxide (TMEPO) and its bispiro-analogue [20]. At the same condition, the 1:2:2:1 pattern of EPR spectrum was also resulted when DMPO was used as spin trap to replace nitron **4**. All these results support the assignment that the observed 1:2:2:1 quartet EPR spectrum is due to the trapping of hydroxyl radicals by nitron **4**. Photolysis of zinc oxide dispersion in aqueous alcohols, such as methanol, ethanol, *n*-propanol, *n*-butanol, and in aqueous solution of $\text{Na}_2\text{C}_2\text{O}_4$, $\text{CH}_3\text{CO}_2\text{Na}$, $\text{CF}_3\text{CO}_2\text{Na}$, and acetonitrile, respectively, in the presence of **4** afforded the 1:2:2:1 pattern EPR signals of hydroxyl adducts besides other signals. In benzene, spin trapping with **4** when photolysis of *tert*-dibutyl peroxide (DBPO), or the mixture of bromoethane with DBPO also gave the hydroxyl adduct four peaks besides the *tert*-butoxy adduct signals (eqs. (4, 5)).

As listed in Table I, hfs constants of hydroxyl adducts are varied from 15.01 G to 15.77 G in different aqueous solutions, and equal to *ca.* 13.50 G in benzene solutions. The phenomenon that hfs constants of a same spin adduct are differing with different systems and different solvents is very common. For example, the hydroxyl adducts of DMPO in water employed various hfs constants from 14.77 G to 15.3 G referring to the systems in which radicals were trapped [23–26]. The solvent polarity can change the distribution of the unpaired spin density on nitroxyl group:

A solvent with larger polarity such as water introduces the polarization of the nitroxyl group to increase the spin density on the nitrogen atom resulting the increase of hfs constants of spin adducts. Therefore, it is reasonable that the hydroxyl adduct hfs constants are larger in water than in benzene.

In zinc oxide dispersion in water, the hydroxyl adduct hfs constants of nitrone **4** and DMPO are respectively 15.53 G and 14.90 G. The difference is due to their structural variation. For examples, the hfs constants of DMPO hydroxyl adducts in aqueous hydrogen peroxide were 14.8 G for a_{N} and a_{H}^{β} while these of 3,3,5,5-tetramethyl-1-pyrroline N-oxide (TMPO) hydroxyl adducts were 15.5 G for a_{N} and 16.6 G for a_{H}^{β} [17]; photolysis of 1% H₂O₂ in phosphate buffer, the hfs con-

stants of TMEPO hydroxyl adducts were 15.5 G for a_{N} and a_{H}^{β} while these of TMEPO analogue hydroxyl adducts were 14.7 G at the same condition [20]; the hfs constants were 15.8 G for 5-methyl-5-*n*-butyl-1-pyrroline N-oxide hydroxyl adducts and 14.9 G for 5,5-di-*n*-propyl-1-pyrroline N-oxide hydroxyl adducts [16]. The reason is that different substitutions on the five-membered ring can change the preferential conformation of spin adduct aminoxyl ring resulting the changing of the C-H _{β} orientation with respect to the semi-occupied *p*-orbital of aminoxyl nitrogen and the changing in distribution of unpaired spin density on nitroxyl group and nearby atoms.

The hydroxyl adducts of nitrone **4** were very stable and their intensities of the EPR signals were almost same within 30 min in benzene solution.

The photolysis of zinc oxide dispersed in the mixture of water and alcohol is the source of hydroxyalkyl radicals [22] (eq. (6, 7)) which can be

Source ^b	Radical addend	Solvent	a_{N}	a_{H}^{β}	a_{H}^{α}
ZnO + H ₂ O	$\cdot\text{OH}$	H ₂ O	15.53	15.53	
ZnO + CH ₃ OH + H ₂ O	$\cdot\text{CH}_2\text{OH}$	H ₂ O	15.86	25.58	
	$\cdot\text{OH}$		15.77	15.77	
ZnO + EtOH + H ₂ O	$\cdot\text{CH}(\text{OH})\text{CH}_3$	H ₂ O	15.67	24.22	
	$\cdot\text{OH}$		15.44	15.44	
ZnO + EtCH ₂ OH + H ₂ O	$\cdot\text{CH}(\text{OH})\text{Et}$	H ₂ O	15.72	24.24	
	$\cdot\text{OH}$		15.51	15.51	
ZnO + BuOH + H ₂ O	$\cdot\text{CH}(\text{OH})\text{CH}_2\text{Et}$	H ₂ O	15.68	24.32	
	$\cdot\text{OH}$		15.45	15.45	
ZnO + Na ₂ SO ₃ + H ₂ O	$\cdot\text{SO}_3^-$	H ₂ O	14.41	19.11	
ZnO + Na ₂ C ₂ O ₄ + H ₂ O	$\cdot\text{CO}_2^-$	H ₂ O	15.69	24.60	
	$\cdot\text{OH}$		15.61	15.61	
ZnO + CH ₃ CO ₂ Na + H ₂ O	$\cdot\text{CH}_2\text{CO}_2^-$ ($\cdot\text{CH}_3$)	H ₂ O	15.43	26.16	
	$\cdot\text{OH}$		15.46	15.46	
ZnO + CF ₃ CO ₂ Na + H ₂ O	$\cdot\text{CO}_2^-$	H ₂ O	15.74	24.40	
	$\cdot\text{OH}$		15.71	15.71	
ZnO + CH ₃ CO ₂ H + H ₂ O	$\cdot\text{CH}_2\text{CO}_2\text{H}$ ($\cdot\text{CH}_3$)	H ₂ O	16.08	26.75	
	$\cdot\text{COCH}_3$		14.73	16.23	
	$\cdot\text{COCH}_3$	C ₆ H ₆	12.94	14.37	
DBPO ^c	$\cdot\text{OBu}^t$	C ₆ H ₆	13.04	4.08	1.34 (1 H)
	$\cdot\text{OH}$		13.50	13.50	
DBPO + EtBr	$\cdot\text{OBu}^t$	C ₆ H ₆	13.12	4.09	1.25 (1 H)
	$\cdot\text{OH}$		13.56	13.56	
PhNHNH ₂ ^d	$\cdot\text{C}_6\text{H}_5$	C ₆ H ₆	14.10	23.06	
ZnO + THF + H ₂ O	$\cdot\text{CH}(\text{CH}_2)_3\text{O}$	H ₂ O	15.48	22.99	
	$\cdot\text{OH}$		15.01	15.01	
ZnO + CH ₃ CN + H ₂ O	$\cdot\text{CH}_2\text{CN}$	H ₂ O	14.57	7.39	
	$\cdot\text{OH}$		15.18	15.18	
BP ^e + CH ₃ OH	$\cdot\text{CH}_2\text{OH}$	C ₆ H ₆	13.65	5.90	
CH ₃ MgI	$\cdot\text{CH}_3$	C ₆ H ₆	12.74	4.35	

Table I. ESR hyperfine splitting constants of nitrone **4** spin adducts^a.

^a The splitting constants are given in gauss; ^b photolysis with a 200 W high pressure mercury vapor lamp; ^c di-*tert*-butylperoxide; ^d complexed with di-benzo-C-24-O-8; ^e benzo-phenone.

scavenged by **4** to offer a doublet of triplet of EPR spectrum.

The larger a_N and a_H^β values for the hydroxyalkyl spin adducts are probably due to an increase in spin density on the nitroxyl nitrogen atom and a decrease in the β -CH dihedral angle because of the intramolecular hydrogen bonding [14, 27] as illustrated (**6–9**). In addition, the two kinds of generated radicals, *i.e.*, hydroxyl and hydroxyalkyl radicals, were trapped within the same solution by **4** and distinct EPR spectra were observed while DMPO can scavenge hydroxyl and hydroxyalkyl radicals only in aqueous methanol and *n*-propanol cases at the same conditions (hfsc, given in Table II). These prove the nitron **4** is suitable for the multi-radical system.

With the nitron **4**, the inorganic radicals $\dot{\text{SO}}_3^-$ produced by photolysis of $\text{ZnO} + \text{Na}_2\text{SO}_3$ in water (eq. (8)), and $\dot{\text{CO}}_2^-$ generated from $\text{ZnO} + \text{Na}_2\text{C}_2\text{O}_4$ in water (eq. (9)) and from $\text{ZnO} + \text{CF}_3\text{CO}_2\text{Na}$ in water (eq. (10)) have been trapped.

According to EPR spectrum, photolysis of zinc oxide in aqueous acetic acid in the presence of **4** generates at least two kinds of spin adducts, one is acetyl radical adduct ($a_N = 14.73$ G, $a_H^\beta = 16.23$ G) and the other is $\dot{\text{CH}}_2\text{CO}_2\text{H}$ and/or $\dot{\text{CH}}_3$ radical adduct ($a_N = 16.08$ G, $a_H^\beta = 26.75$ G). There are two possible approaches by which the acetyl adduct is resulted. The first is direct addition of acetyl radical (eq. (11, 12)) to nitron **4**. The second is the rapid decomposition of the acetoxyl spin adduct of **4** as the decomposition of the acetoxyl-DMPO adduct [28].

The acetyl adduct of nitron **4** is so stable, lifetime > 60 min, that the aminoxyl radical can be extracted from the aqueous solution to benzene and the a_N and a_H^β values are sensitive to the solvent polarity.

The nitron **4** also can trap phenyl and 2-tetrahydrofuranyl radicals, and their hfs constants are usually larger than these of the corresponding DMPO adducts [14]. But the a_H^β values of cyanomethyl and methyl radical adducts of nitron **4** are abnormally small. Such phenomenon was also found when 3,3,5,5-tetrasubstituted DMPO analogues trapped 2-cyano-2-propyl radicals in organic solvent [17]. As given in Table I, the hfs constants of hydroxymethyl adducts of **4** are

Source ^b	Radical addend	a_N	a_H^β
ZnO + H ₂ O	$\dot{\text{O}}\text{H}$	14.90	14.90
ZnO + CH ₃ OH + H ₂ O	$\dot{\text{C}}\text{H}_2\text{OH}$	15.90	22.58
	$\dot{\text{O}}\text{H}^\cdot$	14.95	14.95
ZnO + EtOH + H ₂ O	$\dot{\text{C}}\text{H}(\text{OH})\text{CH}_3$	15.85	22.96
ZnO + EtCH ₂ OH + H ₂ O	$\dot{\text{C}}\text{H}(\text{OH})\text{Et}$	15.90	23.19
	$\dot{\text{O}}\text{H}$	14.78	14.78
ZnO + BuOH + H ₂ O	$\dot{\text{C}}\text{H}(\text{OH})\text{CH}_2\text{Et}$	15.93	23.38
ZnO + HOCH ₂ CH ₂ OH + H ₂ O	$\dot{\text{C}}\text{H}(\text{OH})\text{CH}_2\text{OH}$	15.84	23.22
ZnO + Na ₂ C ₂ O ₄ + H ₂ O	$\dot{\text{C}}\text{O}_2^-$	15.71	18.76
ZnO + Na ₂ SO ₃ + H ₂ O	$\dot{\text{S}}\text{O}_3^-$	14.44	16.22
ZnO + CH ₃ CO ₂ Na + H ₂ O	$\dot{\text{C}}\text{H}_2\text{CO}_2^-(\dot{\text{C}}\text{H}_3)$	15.93	22.58
ZnO + CF ₃ CO ₂ Na + H ₂ O	$\dot{\text{C}}\text{O}_2^-$	15.65	18.76
ZnO + THF + H ₂ O	$\dot{\text{C}}\text{H}(\text{CH}_2)_3\text{O}$	15.71	19.59
	$\dot{\text{O}}\text{H}$	14.80	14.80

Table II. EPR hyperfine splitting constants of DMPO spin adducts in water^a.

^a The splitting constants are given in gauss; ^b photolysis with a 200 W high pressure mercury vapor lamp.

15.86 G for nitrogen splitting and 25.58 G for β -hydrogen splitting in water, but 13.65 and 5.90 G in benzene. It has been reported that *t*-BuO-DMP $\dot{\text{O}}$ nitroxides showed a_{H}^{β} value 8.16 G in benzene and 16.0 G in water [29], and HO-TMP $\dot{\text{O}}$ adducts showed a_{H}^{β} value 7.2 G in acetonitrile and 16.6 G in water [17]. In order to scavenge ethyl radical, the benzene solution of DBPO mixed with bromoethane in the presence of **4** was irradiated and the ethyl spin adduct was not detected, but the *tert*-butoxy spin adduct and hydroxyl radical adduct were observed. The reason is probably that the *tert*-butoxy-trapping rate is much faster than the rate of bromine atom transfer reaction between *tert*-butoxy and bromoethane.

As DMPO radical adducts, hfs constants of nitron **4** radical adducts are sensitive to radical addenda. For example, the a_{H}^{β} values for α -hydroxycarbon and $\dot{\text{C}}\text{O}_2^-$ spin adducts are between 25.58 G and 24.22 G, these for α -carboxycarbon radical adducts are 26.16–26.75 G, and that for acetyl adduct is 16.23 G in aqueous solution. For O-centered radicals, the a_{H}^{β} values are much smaller, 4.08–4.09 G for *tert*-butoxy adduct, and 13.50–13.56 G for hydroxyl adduct in benzene solution.

In order to clarify the relative spin trapping abilities of nitron **4** and DMPO further, several radical systems were investigated when the two traps were present in a same solution. Photolysis of zinc

oxide dispersion in water in the presence of 7.3×10^{-3} M DMPO and 7.1×10^{-3} M nitron **4** affords only one spin adduct of which hfs constants are 15.60 G for a_{N} and 15.60 G for a_{H}^{β} . By comparison with the hfs constants obtained when nitron **4** and DMPO were used as traps separately, the data are assigned to the hfs constants of the hydroxyl radical adduct of nitron **4** instead of DMPO because of the closeness of 15.60 G to 15.53 G for nitron **4** hydroxyl adduct. When zinc oxide dispersion in the mixture of water and methanol containing 6.8×10^{-3} M DMPO and 6.6×10^{-3} M nitron **4** was irradiated, three radical adducts were detected, 15.84 G of a_{N} and 22.58 G of a_{H}^{β} for HOCH₂-DMP $\dot{\text{O}}$ adduct, 15.71 G of a_{N} and 25.57 G of a_{H}^{β} for HOCH₂-**4** adduct, and 15.80 G of a_{N} and a_{H}^{β} for HO-**4** adduct. The intensities of HOCH₂-DMP $\dot{\text{O}}$ adduct EPR signals are much stronger than these of HOCH₂-**4** EPR signals (height:height, *ca.* 5.3:1). For zinc oxide dispersion in water and ethanol, two radical adducts were observed, 15.90 G of a_{N} and 23.03 G of a_{H}^{β} for CH₃CH(OH)-DMP $\dot{\text{O}}$ adduct, and 15.48 G of a_{N} and a_{H}^{β} for HO-**4** adduct. Other radical systems such as ZnO + *n*-PrOH + H₂O, ZnO + *n*-BuOH + H₂O, ZnO + THF + H₂O, ZnO + Na₂C₂O₄ + H₂O and ZnO + Na₂SO₃ + H₂O in the presence of nitron **4** and DMPO were also examined and the competing spin trapping results are collected in Table III. These results indicate that the ability

Source ^b	a_{N}	a_{H}^{β}	Radical trapped by nitron 4 ^c	Radical trapped by DMPO ^c
ZnO + H ₂ O	15.60	15.60	$\dot{\text{O}}\text{H}$	
ZnO + CH ₃ OH + H ₂ O	15.80	15.80	$\dot{\text{O}}\text{H}$	
	15.71	25.57 ^d	$\dot{\text{C}}\text{H}_2\text{OH}$	
	15.84	22.58 ^d		$\dot{\text{C}}\text{H}_2\text{OH}$
ZnO + EtOH + H ₂ O	15.48	15.48	$\dot{\text{O}}\text{H}$	
	15.90	23.03		$\dot{\text{C}}\text{H}(\text{OH})\text{CH}_3$
ZnO + EtCH ₂ OH + H ₂ O	15.49	15.49	$\dot{\text{O}}\text{H}$	
	15.90	23.47		$\dot{\text{C}}\text{H}(\text{OH})\text{Et}$
ZnO + BuOH + H ₂ O	15.50	15.50	$\dot{\text{O}}\text{H}$	
	15.90	23.54		$\dot{\text{C}}\text{H}(\text{OH})\text{CH}_2\text{Et}$
ZnO + THF + H ₂ O	15.05	15.05	$\dot{\text{O}}\text{H}$	
	15.90	23.09 ^e	$\dot{\text{C}}\text{H}(\text{CH}_2)_3\text{O}$	
				$\dot{\text{C}}\text{H}(\text{CH}_2)_3\text{O}$
ZnO + Na ₂ C ₂ O ₄ + H ₂ O	15.68	19.21 ^e		
	15.52	15.52 ^f	$\dot{\text{O}}\text{H}$	
	15.65	24.61 ^f	$\dot{\text{C}}\text{O}_2^-$	
	15.71	18.76 ^f		$\dot{\text{C}}\text{O}_2^-$
ZnO + Na ₂ SO ₃ + H ₂ O	14.44	19.08 ^g	$\dot{\text{S}}\text{O}_3^-$	
	14.47	16.22 ^g		$\dot{\text{S}}\text{O}_3^-$

Table III. The competing spin trapping results of nitron **4** and DMPO in water^a.

^a The splitting constants are given in gauss; ^b photolysis with a 200 W high pressure mercury vapor lamp; ^c concentrations of nitron **4** in various solutions are from 6.6×10^{-3} M to 7.1×10^{-3} M while these of DMPO are from 6.8×10^{-3} M to 7.3×10^{-3} M; ^d the height ratio of hydroxymethyl adduct signals of DMPO to that of nitron **4** is *ca.* 5.3:1; ^e the height ratio of 2-tetrahydrofuran adduct signals of DMPO to that of nitron **4** is *ca.* 1.5:1; ^f see Fig. 2; ^g the height ratio of $\dot{\text{S}}\text{O}_3^-$ adduct signals of DMPO to that of nitron **4** is *ca.* 4.8:1.

Fig. 2. EPR spectrum recorded when photolysis of 35 mg zinc oxide, 5 mg sodium oxalate, 7.1×10^{-3} M nitron **4** and 7.3×10^{-3} M DMPO in 0.5 ml of water, (A) hydroxyl spin adduct of nitron **4**, (B) $\dot{\text{C}}\text{O}_2^-$ spin adduct of nitron **4**, (C) $\dot{\text{C}}\text{O}_2^-$ spin adduct of DMPO.

of DMPO to trap hydroxyalkyl radicals, 2-tetrahydrofuranyl radical, $\dot{\text{C}}\text{O}_2^-$ and $\dot{\text{S}}\text{O}_3^-$ radicals is better than that of nitron **4**, but the activity of nitron **4** to scavenge hydroxyl radical is greater than that of DMPO. Thus, mono-substitution on C_3 ring position probably increase the spin trapping selectivity of substituted 1-pyrroline N-oxide for hydroxyl radical. Fig. 2 is an EPR spectrum acquired from photolysis of zinc oxide dispersed in aqueous solution of $\text{Na}_2\text{C}_2\text{O}_4$ in the presence of 7.3×10^{-3} M DMPO and 7.1×10^{-3} M nitron **4**. The half life-times of $^-\text{O}_2\text{C-DMP}\dot{\text{O}}$ adduct, $^-\text{O}_2\text{C-4}$ adduct and HO-4 adduct are respectively *ca.* 50 min, 59 min and 85 min upon EPR study results. So nitron **4** spin adducts are relatively persistent.

Spin trapping stereochemistry

The significantly different ^1H NMR values of nitron **4** 5,5-dimethyl protons indicate the substitution group on the 3-carbon has different influences on the two methyl groups and the nitron should exist in a preferential conformation in a solution. Likewise, the EPR spectrum of 2,2-dimethyl-4-(3-hydroxypropyl)pyrrolidinyl-1-oxyl aminoxyl radical (**5**), which was obtained by reduction of **4** with sodium borohydride similar to the methods reported [13, 30–32] and following auto-oxidation in the presence of air (eq. (13)),

showed that the two a_{H}^{β} values are equal to 14.58 G and 23.29 G respectively ($\Delta a_{\text{H}}^{\beta} = 8.71$ G), and so the two β -hydrogens are dramatically magnetically non-equivalent. This is due to the orientation of one β -hydrogen at the pseudoequatorial position of a preferential conformation with respect to the semi-occupied *p*-orbital of the aminoxyl nitrogen is different from that of the other β -hydrogen at the pseudoaxial position as presented in the following two possible conformations (**5'**, **5''**) of

which the EPR spectra should be same with each other (Fig. 3 A). It is noticeable that the reduction of nitron **4** with excess sodium borohydride at room temperature gives 2,2-dimethyl-4-(3-hydroxypropyl)pyrrolidinyl-1-hydroxy instead of 2,2-dimethyl-4-(2-ethoxycarbonylethyl)pyrrolidinyl-1-hydroxy according to the IR spectrum of reduced product. We have previously reported that 5-alkyl-5-methylpyrrolidinyl-1-oxyl nitroxides employ two non-equivalent β -hydrogens [12, 13]. In comparison, DMPO was also reduced and auto-oxidized to give 2,2-dimethylpyrrolidinyl-1-oxyl

Fig. 3. EPR spectra of (A) aminoxyl radical **5** in benzene solution indicating non-equivalence of two β -hydrogens, $a_{\text{N}} = 14.58$ G, $a_{\text{H}_1}^{\beta} = 14.58$ G, $a_{\text{H}_2}^{\beta} = 23.29$ G; (B) aminoxyl radical DTPO in benzene solution indicating equivalence of two β -hydrogens, $a_{\text{N}} = 14.31$ G, $a_{\text{H}}^{\beta} = 19.04$ G (2H).

(DTPO) nitroxide radical of which EPR spectrum showed hfs constants of two β -hydrogens are 19.04 G respectively, indicating the equivalence of the two β -hydrogens (Fig. 3 B).

Owing to the presence of the chiral 3-carbon, there are two possible spin trapping approaches, *i.e.*, the radical *trans*-addition and *cis*-addition. If the nitron cannot stereoselectively or stereospecifically scavenge transient free radical, trapping one radical should give at least four possible spin adduct isomers as illustrated in Scheme 2. The *syn*-isomers of spin adducts should have the same a_{H}^{β} values, the reason of which is that the chemical structural environment for aminoxyl and β -proton is same with each other between the two isomers, and so were the two *anti*-isomers. However, the a_{H}^{β} value of the *syn*-isomers must be different from that of the *anti*-isomers because the *syn*-isomers is chemically different from the *anti*-isomers and the a_{H}^{β} value is very sensitive to the structural difference as reflected in the nitroxide **5** and 5-alkyl-5-methylpyrrolidinyl-1-oxyls [12, 13]. Therefore, if the spin trapping reaction affords *syn*-isomers and *anti*-isomers at the same time, the observed EPR spectrum should show at least two different a_{H}^{β} values. Kotake and co-workers have discovered some diastereomers of α -phenyl N-*tert*-butyl nitron (PBN) and DMPO spin adducts show different a_{H}^{β} values in some solvents ($\Delta a_{\text{H}}^{\beta} < 1.0$ G) [33]. Diastereomers of nitron **4** spin adducts must have much larger $\Delta a_{\text{H}}^{\beta}$ values because of the presence of two chiral carbon centers within the five-membered ring.

However, when nitron **4** trapped hydroxyl radical, *tert*-butoxy radical, phenyl radical, hydroxy-

Scheme 2.

methyl radical, 1-hydroxyethyl radical, 1-hydroxypropyl radical, 1-hydroxybutyl radical, $\dot{\text{S}}\text{O}_3^-$ radical and $\dot{\text{C}}\text{O}_2^-$ radical, the EPR spectrum of the corresponding spin adduct showed only one a_{H}^{β} value as DMPO. The results indicate that the spin trapping reactions are most probably plane selective, and the selectivity is so high that the EPR spectrometer can detect only one of the two spin adduct pairs. The nitron **4** should prefer such a structural model that the nitronyl group, 3-carbon, and 5-carbon are on a same plane, and the 4-methylene is puckered from the 3-(2-ethoxycarbonyl-ethyl) substitution. Because of the hindrance of the 3-substitution, the transient free radical mentioned above should add to the nitronyl function in the direction *trans* to the 3-substitution group to afford *anti*-isomers as illustrated in Scheme 3.

Scheme 3.

In conclusion, nitron **4** can trap a series of short-lived free radicals, particularly hydroxyl radical. The spin adducts are very stable, and a_{H}^{β} values are sensitive to individual radical addendum. The spin trapping process is probably plane selective to give *trans*-adducts.

Experimental Section

Instrumentation

Microanalyses were obtained using a Heraeus-CHN-Rapid elemental analyser. IR spectra were recorded on a Carl Zeiss Jena Specord-75 spectrometer. Mass spectra were obtained using an AEI MS-50 spectrometer. ^1H NMR spectra were determined on a Varian EM-360 (60 MHz) or JEOL

JNM-FX 100 spectrometers using tetrachloride carbon or deuteriochloroform as a solvent and tetramethylsilane as an internal standard. EPR spectra were recorded on a Bruker ESP-300 EPR spectrometer at room temperature. UV spectra were acquired with a HITACHI-340 ultraviolet spectrometer.

Spin trapping procedure

Zinc oxide powder suspended in distilled water in a 10-ml beaker was dispersed by ultrasound for 10 min. The aqueous dispersion (0.5 ml) containing about 35 mg zinc oxide, 60 μ l of 0.071 M aqueous solution of **4** or 40 μ l of 0.11 M DMPO aqueous solution were placed in an EPR tube and deoxygenated. The mixture was then irradiated with a 200 W high pressure mercury vapor lamp. When trapping a hydroxyalkyl radical, 50 μ l of the corresponding alcohol was added before deoxygenation. When salts were used for generation of radicals, 5 mg of each salt was added to a tube before deoxygenation and irradiation. DMPO was prepared by the literature procedure [34]. In 100 ml of double-distilled water, 1.7 g (15 mmol) of DMPO was dissolved. The 0.15 M solution was twice purified with 2 $\times$ 0.08 g active charcoal refluxing for 20 min, filtered. The concentration was 0.11 M determined on an UV spectrometer. This DMPO solution all showed no EPR signals without photolysis and after photolysis for 30 min. The 0.071 M nitron **4** aqueous solution was obtained as the method for preparation of DMPO solution. Any EPR signals were not detected without and after photolysis of nitron **4** solution.

Preparation of ethyl 4-(2-methyl-2-nitropropyl)-5-oxopentanoate (3)

Ester-nitro-aldehyde **3** was first prepared according to the procedure used for the preparation of ethyl 4-ethyl-5-oxo-pentanoate in the literature [35]. To a mixture of piperidine (29.3 g, 0.345 mol) and anhydrous K_2CO_3 (10 g, 0.072 mol) was added dropwise nitro-aldehyde **1** (20 g, 0.138 mol) for 25 min at 2 °C, and stirred overnight. Ether (300 ml) was added, filtered, and evaporated under a reduced pressure to remove excessive piperidine. To a solution of the obtained crude enamine **2** in 100 ml of freshly distilled acetonitrile was added dropwise a solution of ethyl acrylate (18 g, 0.18 mol) in 60 ml of dry acetonitrile for 60 min at 2 °C, stirred for additional 5 h at room temperature. Then, the reaction mixture was refluxed for 40 h, cooled, and 10 ml of acetic acid in 60 ml of water was added, refluxed for 8 h. After cooling,

15 g of sodium chloride was added, and the organic layer was dried over anhydrous sodium sulfate, filtered, evaporated. The residue dissolved in 250 ml of benzene was passed through a short silica gel column to remove black impurities, evaporated and followed by distillation to give 4.75 g (14% overall yield) of **3**, b.p.: 162–165 °C/0.5 mm Hg; IR (Neat): 2717, 1717, 1527, 1176 cm^{-1} ; 1H NMR ($CDCl_3$): δ 1.24 (t, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.82–2.02 (m, 2H, CH_2), 2.21–2.68 (m, 5H, CH_2 , $CHCO$, CH_2CO_2), 4.06 (q, 2H, CH_2O), 9.57 (s, 1H, CHO).

Preparation of 5,5-dimethyl-3-(2-ethoxycarbonyl-ethyl)-1-pyrroline N-oxide (4)

The procedure used for the preparation of DMPO and its analogues [34] was adapted for the preparation of nitron **4** as following. Zinc dust (2.76 g, 0.038 mol) was added to a solution of **3** (4.65 g, 0.019 mol) in 80 ml of 95% ethanol that had been precooled to 3 °C. Under brisk mechanical stirring, glacial acetic acid (5.06 g, 0.084 mol) in 20 ml of 95% ethanol was added dropwise for 20 min while maintaining the reaction temperature below 8 °C. The mixture was stirred vigorously for additional 2 h, stored in a refrigerator for 20 h, filtered, washed with 20 ml of ethanol, evaporated. The residue was dissolved in 20 ml of chloroform, stored again in a refrigerator for one day, filtered, evaporated and followed by chromatography on a silica gel column with chloroform as the eluent to give 2.71 g (67%) of a slightly yellowish oil which was further purified by distillation to prepare analytical sample, of which the structure was assigned as 5,5-dimethyl-3-(2-ethoxycarbonyl-ethyl)-1-pyrroline N-oxide (**4**) on the basis of the following data. B.p.: 164–166 °C/1 mm Hg; UV (H_2O): λ_{max} = 229 nm; IR (Neat): 3069, 1717, 1567, 1227, 1173 cm^{-1} ; 1H NMR (CCl_4): δ 1.24 (t, 3H, CH_3), 1.28 (s, 3H, ring 5- CH_3), 1.35 (s, 3H, ring 5- CH_3), 1.60–1.93 (m, 2H, CH_2), 2.17 (d, 2H, ring CH_2), 2.31 (t, 2H, CH_2CO_2), 2.97 (m, 1H, ring 3- CH), 4.00 (q, 2H, CH_2O), 6.59 (d, 1H, $CH=N$); MS (EI, rel. int. %): m/z 213 (M^+ , 50), 198 ($M-CH_3$, 21), 168 ($M-OEt$, 43), 126 (100), 125 (26), 112 (23), 110 (21).

$C_{11}H_{19}NO_3$ (213.28)

Calcd	C 61.95	H 8.98	N 6.57
Found	C 61.66	H 8.41	N 6.81

HRMS (m/z) for $C_{11}H_{19}NO_3$

Calcd	213.1365
Found	213.1363

Reduction of 5,5-dimethyl-3-(2-ethoxycarbonyl-ethyl)-1-pyrroline N-oxide (4)

Nitrone **4** (0.06 g, 0.28 mmol) and sodium borohydride (0.20 g, 5.3 mmol) in 10 ml of distilled water was standing for two days. The mixture was saturated with sodium chloride, extracted with 2×20 ml of diethyl ether, dried over anhydrous sodium sulfate, filtered, evaporated to give 0.04 g (82%) of clear liquid. IR spectrum indicated that nitronyl group and ester group were all reduced. IR (Neat): 3300 (br., OH), 2927, 2853, 1444, 1356, 1047 (vs., C–O), 1007 cm⁻¹. A small amount of this product was dissolved in 0.5 ml of benzene, measured directly with an EPR spectrometer, and the aminoxyl radical **5** was detected. The hfs con-

stants are $a_N = 14.58$ G, $a_{H_1}^{\beta} = 14.58$ G, $a_{H_2}^{\beta} = 23.29$ G.

Reduction of 5,5-dimethyl-1-pyrroline N-oxide (DMPO)

The procedure is as same as that used for reduction of nitrone **4**. Yield: 80%; IR (Neat): 3200 (OH), 2957, 2866, 1445, 1356 cm⁻¹. DTPO aminoxyl radical was detected within the reduced product because of the auto-oxidization of 2,2-dimethylpyrrolidinyl-1-hydroxy. The hfs constants are $a_N = 14.31$ G, $a_{H_1}^{\beta} = 19.04$ G (2H).

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- [1] E. G. Janzen and B. J. Blackburn, *J. Am. Chem. Soc.* **90**, 5909 (1968).
[2] E. G. Janzen and B. J. Blackburn, *ibid.* **91**, 4481 (1969).
[3] E. G. Janzen, *Acc. Chem. Res.* **4**, 31 (1971).
[4] T. Ozawa and A. Hanaki, *Chem. Lett.* **1987**, 1885.
[5] K. P. Madden, H. Taniguchi, and R. W. Fessenden, *J. Am. Chem. Soc.* **110**, 2753 (1988).
[6] T. Ozawa and A. Hanaki, *Bull. Chem. Soc. Jpn.* **60**, 2304 (1987).
[7] G. M. Rosen, B. E. Britigan, M. S. Cohen, S. P. Ellington, and M. J. Barker, *Biochim. Biophys. Acta* **969**, 236 (1988).
[8] P. Graceffa, *Biochim. Biophys. Acta* **954**, 227 (1988).
[9] K. Stolze, D. R. Duling, and R. P. Mason, *J. Chem. Soc. Chem. Commun.* **1988**, 268.
[10] G. Gronchi, P. Courbis, P. Tordo, G. Mousset, and J. Simonet, *J. Phys. Chem.* **87**, 1343 (1983).
[11] E. G. Janzen and G. A. Coulter, *J. Am. Chem. Soc.* **106**, 1962 (1984).
[12] Y.-K. Zhang and G.-Z. Xu, *J. Chem. Soc. Chem. Commun.* **1988**, 1629.
[13] Y.-K. Zhang and G.-Z. Xu, *Magn. Reson. Chem.* **27**, 846 (1989).
[14] E. G. Janzen and J. I.-P. Liu, *J. Magn. Reson.* **9**, 510 (1973).
[15] D. L. Haire and E. G. Janzen, *Can. J. Chem.* **60**, 1514 (1982).
[16] M. J. Turner, III and G. M. Rosen, *J. Med. Chem.* **29**, 2439 (1986).
[17] P. Barker, A. L. J. Beckwith, W. R. Cherry, and R. Huie, *J. Chem. Soc. Perkin Trans. 2* **1985**, 1147.
[18] G. M. Rosen and M. J. Turner, III, *J. Med. Chem.* **31**, 428 (1988).
[19] E. G. Janzen, R. V. Shetty, and S. M. Kunanec, *Can. J. Chem.* **59**, 756 (1981).
[20] A. Dehnel, D. Griller, and J. M. Kanabus-Kaminska, *J. Org. Chem.* **53**, 1566 (1988).
[21] J. R. Harbour and M. L. Hair, *J. Phys. Chem.* **83**, 652 (1979).
[22] C.-P. Chen, X.-M. Ren, D.-H. Lu, and Y.-K. Zhang, *GanGuangKeXue Yu GuangHuaXue*, in press.
[23] J. R. Harbour, V. Chow, and J. R. Bolton, *Can. J. Chem.* **52**, 3549 (1974).
[24] P. R. Marriott, M. J. Perkins, and D. Griller, *Can. J. Chem.* **58**, 803 (1980).
[25] E. G. Janzen, D. E. Nutter (Jr.), E. R. Davis, B. J. Blackburn, J. L. Poyer, and P. B. McCay, *Can. J. Chem.* **56**, 2237 (1978).
[26] F. P. Sargent and E. M. Gardy, *Can. J. Chem.* **54**, 275 (1976).
[27] E. G. Janzen and I. G. Lopp, *J. Magn. Reson.* **7**, 107 (1972).
[28] W. A. Pryor, C. K. Govindan, and D. F. Church, *J. Am. Chem. Soc.* **104**, 7563 (1982).
[29] D. L. Haire, U. M. Oehler, P. H. Krygsman, and E. G. Janzen, *J. Org. Chem.* **53**, 4535 (1988).
[30] R. F. C. Brown, V. M. Clark, and S. A. Todd, *J. Chem. Soc.* **1959**, 2105.
[31] A. L. Castelhana, D. Griller, and K. U. Ingold, *Can. J. Chem.* **60**, 1501 (1982).
[32] P. H. Morgan and A. H. Beckett, *Tetrahedron* **31**, 2595 (1975).
[33] Y. Kotake and K. Kuwata, *Can. J. Chem.* **60**, 1610 (1982); Y. Kotake, K. Kuwata, and E. G. Janzen, *J. Phys. Chem.* **83**, 3024 (1979); Y. Kotake and K. Kuwata, *Bull. Chem. Soc. Jpn.* **54**, 394 (1981).
[34] D. L. Haire, J. W. Hiborn, and E. G. Janzen, *J. Org. Chem.* **51**, 4298 (1986).
[35] G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkowicz, and R. Terrell, *J. Am. Chem. Soc.* **85**, 207 (1963).