Syntheses and Spin Trappings of 3-Hydroxymethyl-5,5-dimethyl-1-pyrroline *N*-oxide and 3-(3-Hydroxypropyl)-5,5-dimethyl-1-pyrroline *N*-Oxide

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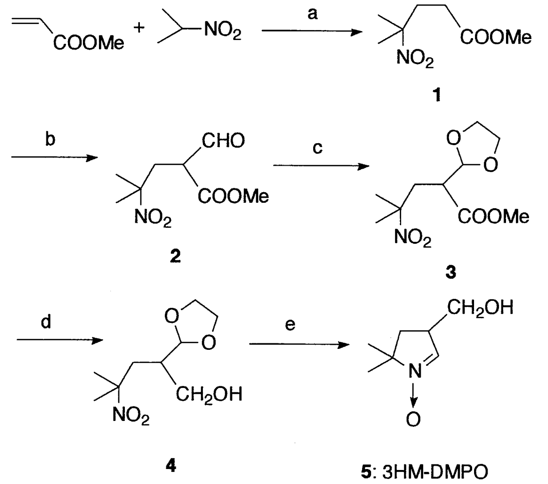
New nitrones of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO)-type , 3-hydroxymethyl-5,5-dimethyl-1-pyrroline *N*-oxide (3HM-DMPO) and 3-(3-hydroxypropyl)-5,5-dimethyl-1-pyrroline *N*-oxide (3HP-DMPO), were synthesized and tested for the ability in radical trapping of oxygen-centered radicals. 3HM-DMPO trapped superoxide but did not hydroxyl radicals, whereas 3HP-DMPO served as a trap for both radicals.

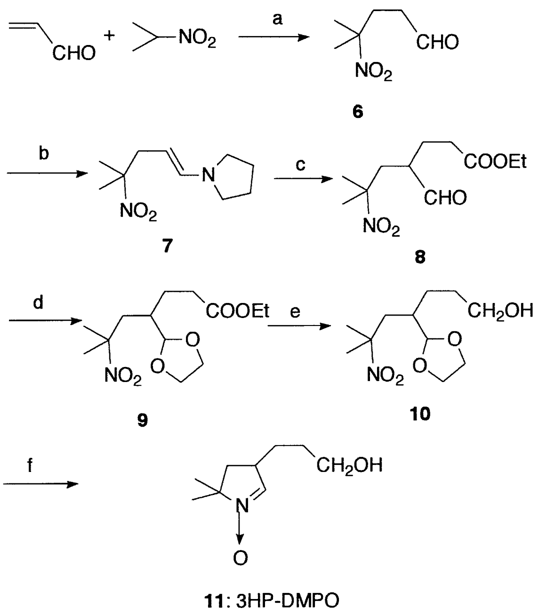
Spin trapping is a useful technique to study transient radical species, and has been intensively investigated in various systems.[1-4] Among many spin traps a cyclic nitron, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) ,has been shown to be an effective scavenger of alkyl,[5] hydroxyalkyl,[6] as well as alkoxyl[7] radicals. In particular,the marked abilities of DMPO to intercept hydroxyl and superoxide radicals have attracted much attention in biological and medical regions.[8] A problem in the use of DMPO to trap superoxide is stability of its spin adduct during ESR measurements. Many attempts to improve the stability of the adduct with superoxide were made[9] ; however, no satisfactory result has been obtained as yet. In this study we focused on hydrogen bond formation in the adduct to improve the stability. In order to enable hydrogen bonding we prepared two novel DMPO-type nitrones, 3-hydroxymethyl-5,5-dimethyl-1-pyrroline *N*-oxide (3HM-DMPO) and 3-(3-hydroxypropyl)-5,5-dimethyl-1-pyrroline *N*-oxide (3HP-DMPO), and examined their ability in trapping hydroxyl and superoxide radicals and the stabilities.

Scheme 1 shows the synthetic route of 3HM-DMPO from 2-nitropropane and methylacrylate according to the Bonnett's method:[10] After the Micheal addition of nitropropane to methylacrylate the product was formylated with ethylformate and sodium ethoxide in ether, the formyl group of the resultant **2** was protected with ethylene glycol, and then yielded **3** was reduced with lithium aluminium hydride (LAH) to give **4**, which was cyclized using zinc to produce 3HM-DMPO (**5**); the total yield was about 1%.



a) Triton B, Dioxane, 85℃ (89%); b) HCOOEt, EtONa, Ether, 25℃ (12%); c) Ethylene glycol, TsOH, Benzene,100℃ (66%); d) $LiAlH_4$, Ether (50%); e) 1. Zn, NH_4Cl, H_2O 2. HCl, 70℃ (33%).

Scheme 1.



a) CH_3ONa, 15℃ (78%); b)Pyrrolidine, K_2CO_3, RT (100%); c) 1. Acetonitrile, 80℃ 2. HCl, H_2O, 100℃ (33%); d) Ethylene glycol, TsOH, Benzene, 100℃ (99%); e) $LiAlH_4$, Ether, 50 ℃ (49%); f) 1. Zinc, NH_4Cl, H_2O, 15℃ 2. HCl, 70℃ (66%).

Scheme 2.

3HP-DMPO was synthesized from 2-nitropropane and acrolein as shown in Scheme 2: The compound **8** was prepared according to the method described in a literature.[9-a)] After the protection of formyl group of **8** with ethylene glycol, the resultant **9** was reduced with LAH to produce **10**, the cyclization of which under acidic condition in the presence of zinc powder yielded 3HP-DMPO(**11**); the total yield was about 8%.

Figure 1 shows the ESR spectrum of the spin adduct of 3HM-DMPO(1.1mol dm^{-3}) with the superoxide radical anion generated by the reaction of hipoxanthine (HPX, 2mmol dm^{-3}) and xanthine oxidaze (XOD, 0.4unit/ml) in water (buffered with phosphoric acid, pH 7.8). The ESR measurements were carried out on a JES-FR 30 spectrometer (JEOL, Tokyo) in a following setting: microwave power 4 mW, field 335±5 mT, modulation 0.1 mT, time constant 0.1 sec, sweep time 2

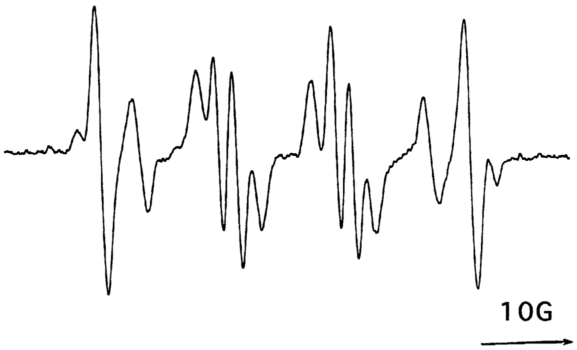


Figure 1. ESR spectrum obtained by the hipoxanthine-xanthine oxidase reaction in the presence of 3HM-DMPO.

min. The ESR absorption profile of 3HM-DMPO adduct with O_2^{-} • was almost the same as that of DMPO-O_2^{-} •. The hyperfine splitting constants (hfsc) obtained were a(N) = 1.41, a(βH) = 0.97, a(γH) = 0.21 mT. The reported values of DMPO-O_2^{-} • are a(N) = 1.26, a(βH) = 1.04, and a(γH) = 0.13 mT. The ESR spectrum obtained here disappeared on addition of superoxide dismutase. This result means that the spectrum comes from the reaction of 3HM-DMPO and O_2^{-} •. The adduct of 3HM-DMPO-O_2^{-} • was relatively stable so that the ESR signal could be observed for 8 min, while the signal with DMPO disappeared after 4 min. The half-life of the ESR spectrum of the adduct of 3HM-DMPO-O_2^{-} • was about 4 min, whereas that for DMPO-O_2^{-} • was about 2 min under the same conditions. The stabilization of the spin adduct of the modified DMPO may be ascribed to hydrogen bonding formed between the introduced hydroxy group and the trapped anionic peroxy group.

When we used 3HP-DMPO as a spin trapping reagent, remarkable stabilization by the hydroxy substituent was observed. The ESR spectrum of its spin adduct with superoxide did not change for 10 min (Figure 2). The major components displays a set of lines consistent with a large doublet split into 1:1:1 triplet : a(N) = 1.56 mT and a(βH) = 2.56 mT. This stabilization can not simply be explained in terms of such formation of hydrogen bonding, because the nine-membered hydrogen bonding seems to be less effective for the stabilization.

In trapping of hydroxy radical, more characteristic features were observed with these traps. The trapping of hydroxy radical was carried out under the conditions: a spin trap 740 μl (38 mmol dm^{-3}), H_2O_2 10 μl (9.8 mol dm^{-3}), $FeCl_2$ 250 μl (6.8 mol dm^{-3}) in water. The ESR measurements were carried out in following setting: microwave power 4 mW, field 336.4 ± 5 mT, modulation 0.1 mT, time constant 0.3 sec, sweep time 4 min. The ESR absorption profile of 3HP-DMPO adduct with hydroxy radical was almost the same as that with superoxide adduct (hfsc: a(N) = 1.6, a(βH) = 2.52 mT). 3HM-DMPO, however, did not trap hydroxyl radical under the conditions examined. The selective trapping of superoxide by 3HM-DMPO in the presence of hydroxyl radical is very interesting, because the discrimination between such radicals is important to elucidate the mechanism of biological reactions in which some kinds of oxygen-centered radical species take part.

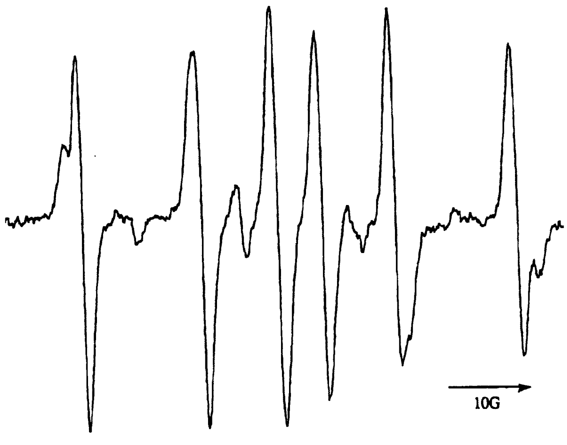


Figure 2. ESR spectrum obtained by the hipoxanthin-xanthine oxidase reaction in the presence of 3HP-DMPO.

Further investigations of the radical trapping by 3HM-DMPO and 3HP-DMPO are now in progress.

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11 3HM-DMPO: a slightly yellow viscous liquid, IR(NaCl)ν 3300, 1587cm^{-1}; ^{1}H-NMR(CDCl$_3$)δ 1.40(s,3H), 1.45 (s,3H), 1.95 (dd, J=5.9, 12.7Hz,1H), 2.22 (dd, J=8.4,12.7Hz, 1H), 3.50 (m,1H), 3.78 (m,1H), 6.88ppm (d,J=1.7Hz, 1H); ^{13}C NMR (CDCl$_3$)δ 25.8, 26.1, 36.5, 40.7, 62.5, 74.1, 137.1ppm.

12 3HP-DMPO: a slightly yellow viscous liquid, IR (NaCl)ν 3350, 1580cm^{-1} : ^{1}HNMR(CDCl$_3$) δ 1.40(s,3H), 1.45(s,3H), 1.48-1.67 (m,4H), 1.72 (dd,J=7.7, 12.9Hz, 1H), 2.35 (dd, J=9.4, 12.9Hz, 1H), 2.93 (m, 1H), 3.56-3.72 (m, 3H), 6.86 ppm (d. J=1.7Hz, 1H) ; ^{13}C NMR (CDCl3) δ 138.1, 74.2, 63.8, 61.9, 41.1, 37.4, 30.1, 26.6, 25.5 ppm.