CHEMISTRY LETTERS, pp. 497-500, 1993.

Proposed Mechanism for Production of Stable Aminoxyl Radical Impurities in the Synthesis of Substituted 5,5-Dimethylpyrroline-N-oxide (DMPO) Spin Traps

Edward G. JANZEN,*† Yong-Kang ZHANG, and Masana ARIMURA††
National Biomedical Center for Spin Trapping and Free Radicals, Molecular Toxicology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, Oklahoma 73104, U.S.A.

Paramagnetic impurities in nitrone spin traps are bothering researchers in the practice of spin trapping. By use of mass spectroscopy, a hydroxylamine dimer of 2,5,5-trimethylpyrroline-N-oxide (M_3PO) has been found in samples of M_3PO. Air oxidation of the hydroxylamine may produce the aminoxyl radical impurity detected. Mechanisms for the generation of impurities in DMPO and derivatives are proposed.

5,5-Dimethylpyrroline-N-oxide (DMPO) and C-phenyl-N-*tert*-butyl nitrone (PBN) are the two most widely used spin traps for detecting free radicals in biological systems. We have recently begun a program of synthesis in order to modify these spin traps with the expectation that better spin traps can be produced.[1,2] A major problem in this kind of project is to find methods which produce "ESR-grade" spin trapping materials. This means that at some suitable concentration of spin trap in aqueous solution, e.g. 0.1 M, no signal is obtained by EPR spectroscopy at maximum gain of the instrument. Although such standards can be achieved with PBN or substituted PBN's with careful recrystallizations and repeated sublimations, this cannot be said for DMPO or its simple derivatives. With DMPO for example, even low temperature vacuum distillation and/or treatment with carbon black after benzene extraction[3] produces a weak aminoxyl EPR signal at 1 x 10^6 gain with a Bruker ESP–300 spectrometer from buffer solutions at concentrations of 0.1 M DMPO. In practice a small "control signal" can be ignored if the spin adduct of interest gives a strong signal-to-noise spectrum. In principle, however, it would be better to understand the mechanism of production of the impurity signal and perhaps by proper choice of conditions or substituents completely eliminate this problem in spin trapping.

We have been recently studying the mechanism of decay of spin adducts in aqueous solutions[4-6] so that spin traps can be selected which give the most long-lived spin adducts. In this connection the spin trapping chemistry of 2,5,5-trimethylpyrroline-N-oxide (M_3PO) is being re-evaluated.[2,7] The problem with this spin trap which otherwise has some excellent characteristics is the strong impurity triplet EPR signal which is produced during the synthesis and/or purification by vacuum distillation.[2] Others have encountered the same problem.[8,9] Chromatography has not been successful in improving M_3PO samples. A typical EPR spectrum obtained in aqueous buffer solution is shown in Fig. 1.

---

†Alternate address: Departments of Clinical and Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G2W1 Canada.
††On leave from Department of General Education, Osaka University, Osaka.

Fig. 1. EPR spectrum of 0.1 M M3PO solution in water using the best purified sample of M3PO available; instrument settings for ESP–300E Bruker EPR spectrometer: modulation amplitude 0.975 G, microwave power 20 mW, scan time 83.9 s, sweep width 100 G; receiver gain: 1 x 10^6.

In a separate study of the fragmentation patterns obtained from low-energy collision-induced decompositions of PBN and DMPO spin adducts, the mass spectra of the alkyl adducts of M_3PO have been obtained (10). In the case of the impurity signal of M_3PO we searched for a possible dimer precursor to the triplet EPR signal. In fact the presence of a m/z = 254 component could be established in a sample of M_3PO [m/z = 127 (M^+)]. Moreover when the MS/MS capability of the instrument was used, the m/z = 254 component was found to fragment in the same manner as hydroxylamine derivatives of alkyl adducts of M_3PO. At lower pressures, only the m/z = 254 component could be found. At higher pressures of M_3PO, $[dimer + 1]^+$ (m/z = 255) and $[trimer + 1]^+$ (m/z = 382) peaks could also be detected. Formation of these species is believed to be due to ion-molecule reactions in the ionization chamber. Examples of this type of reaction have been seen before in thermospray MS studies of PBN spin adducts.[11)]

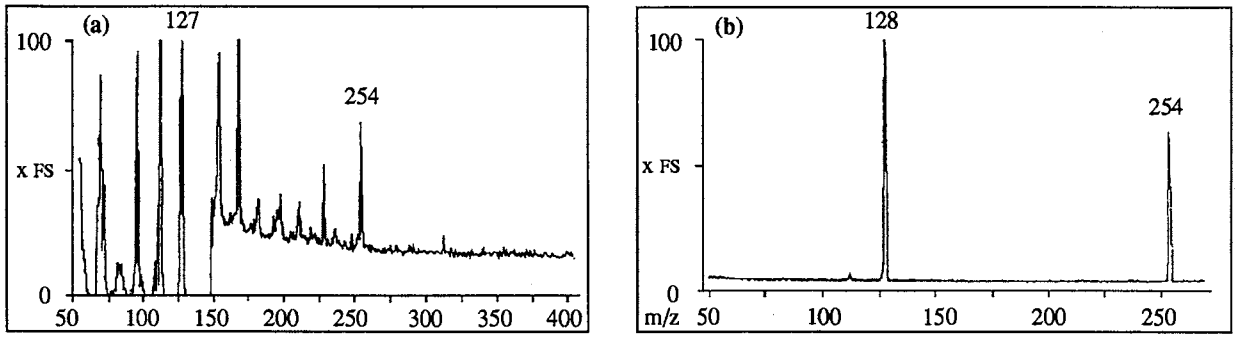
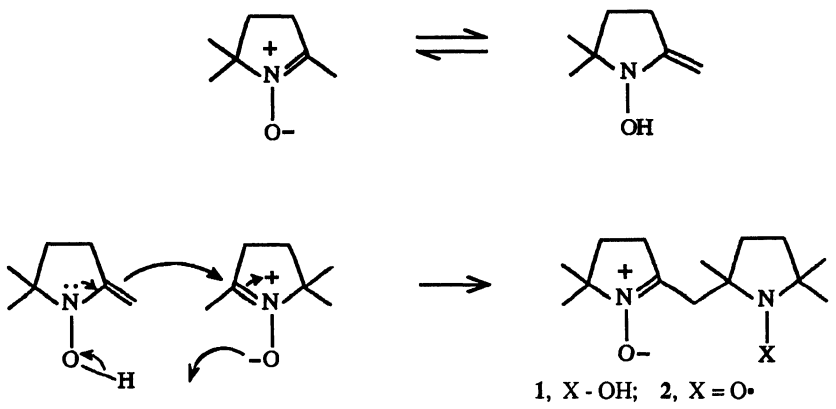


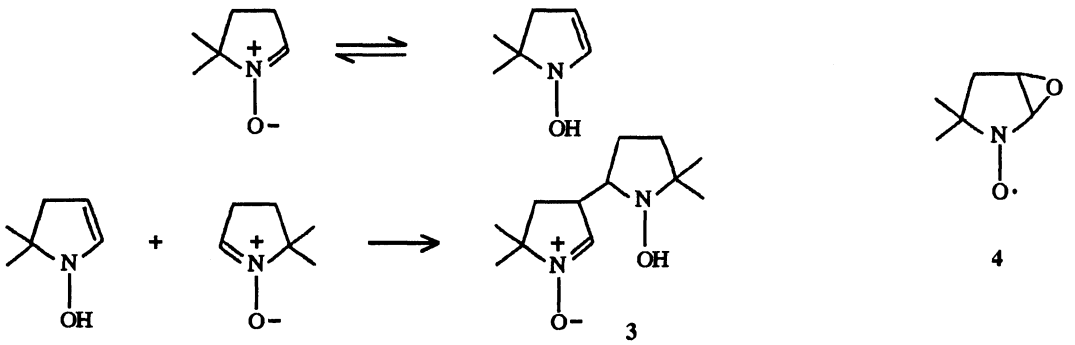
Fig. 2. a) Mass spectrum of M3PO: 15 eV electron impact ionization, probe temperature 28 °C, ion chamber temperature 130 °C; b) Collision induced dissociation mass spectrum of m/z = 254, collision gas: Ar.

A dimer hydroxylamine of M_3PO (1) could be produced by an enamine type of addition to M_3PO. If it is assumed that a small amount of the enamine of M_3PO is formed in equilibrium with M_3PO, perhaps catalyzed by surfaces such as the glass distillation apparatus or chromatography stationary phase, the following coupling reaction can occur:



1, X - OH; 2, X = O•

This reaction is quite consistent with the mechanism typically given for addition of electron-rich enamines to electron-attracting double bonds.[12] Once the dimer hydroxylamine of M_3PO is formed, air oxidation would produce the dimer aminoxyl (**2**). Since all β-positions are carbon groups, the EPR spectrum would be a triplet and the aminoxyl would be as stable as a spin label. That this kind of unconjugated "nitronyl nitroxide" is very stable has been known for almost 70 years[13] and a nitronyl nitroxide of similar structure, known as "the Banfield and Kenyon radical," is produced from C,C-dimethyl-N-phenyl nitrone.[14]



3 4

A similar possibility is also present in DMPO derivatives when the 3-position remains unsubstituted. If DMPO itself undergoes enolization, the same coupling could occur. In fact, a carbon-centered adduct of DMPO is often detected as an impurity by EPR. The dimer hydroxylamine of DMPO (**3**) may be the precursor. In addition another structure (**4**) which could also come from oxygenation of the N-hydroxyl enamine of DMPO has been identified by MS and NMR analysis having similar EPR hyperfine splitting constants.[15]

In summary, dimerization of DMPO to produce the hydroxylamine precursor of a dimer aminoxyl appears to be enhanced by methyl substitution in the 2-position. Coupling in the 3-position by a similar mechanism for DMPO or derivatives with non-enolizable hydrogens in the group attached in the 2-position, e.g. 2-phenyl-DMPO[2] seems somewhat less likely. Further work is underway to produce stable nitronyl spin traps which can be purified to EPR-grade levels taking these factors into account.

This work is being supported by NIH Grant No. RR05517 awarded by the National Center for Research Resources and the Oklahoma Medical Research Foundation (through purchase of the Mass Spectrometer). Grateful acknowledgement is hereby made. This is publication No. 8 from the National Biomedical Center for

Spin Trapping and Free Radicals. One of the authors (Y.K.Z.) would like to express his thanks to the Institute of Chemistry of the Chinese Academy of Sciences for his postdoctoral leave to the U.S.A.

References

1) R.D. Hinton and E.G. Janzen, *J. Org. Chem.*, **57**, 2646 (1992).
2) E.G. Janzen and Y-K. Zhang, *J. Magn. Res.*, in press.
3) E.G. Janzen, L.T. Jandrisits, R.V. Shetty, D.L. Haire, and J.W. Hilborn, *Chemico-Biological Interactions*, **70**, 167 (1989) and personal communication, Dr. Yashige Kotake
4) E.G. Janzen, R.D. Hinton, and Y. Kotake, *Tetrahedron Lett.*, **33**, 1257 (1992).
5) E.G. Janzen, Y. Kotake, and R.D. Hinton, *Free Rad. Biol. Med.*, **12**, 169 (1992).
6) Y. Kotake and E.G. Janzen, *J. Am. Chem. Soc.*, **113**, 9503 (1991).
7) E.G. Janzen, C.A. Evans, and J.I-P. Liu, *J. Mag. Res.*, **9**, 513 (1973).
8) Personal communication, Dr. Keisuke Makino.
9) Personal communication, Dr. Kalman Hideg.
10) Dr. Masana Arimura, manuscript in preparation.
11) E.G. Janzen, P.H. Krygsman, D.A. Lindsay, and D. Larry Haire, *J. Am. Chem. Soc.*, **112**, 8279 (1990).
12) F.A. Carey and R.J. Sundberg, "Advanced Organic Chemistry, Part B: Reactions and Synthesis," Plenum Publishing, New York, New York (1977), pp. 220-221.
13) F.H. Banfield and J. Kenyon, *J. Chem. Soc.*, **1926** , 1612.
14) A.R. Forrester, J.M. Hay, and R.H. Thomson, "Organic Chemistry of Stable Free Radicals," Academic Press, London (1968), pp. 180, 189, 208.
15) K. Makino, H. Imaishi, S. Morinishi, T. Hagiwara, T. Takeuchi, A. Murakami, and M. Nishi, *Free Rad. Res. Commun.*, **6**, 19 (1989).

(Received September 24, 1992)