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SPIN TRAPPING AND ASSOCIATED VOCABULARY*

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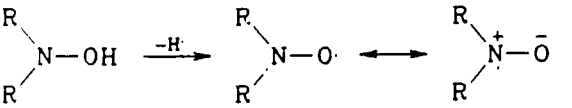
Spin trapping as a technique for detecting free radicals in biological systems is developing rapidly. Two conferences have focused on this method and this paper introduces the contributions resulting from the second meeting held in the Ontario Veterinary College of the University of Guelph on July 2-7, 1989. On the preceding pages can be found a poem on free radicals in biology and medicine, and the names of the sponsors and financial supporters. I am very grateful to Dr. Jim Bolton for his helpful efforts with the manuscripts.

KEY WORDS: Spin trapping, EPR, ESR, free radicals, nitroxide, aminoxyl, spin adducts, double adducts, mass spectroscopy, MS.

INTRODUCTION

Spin trapping is a term coined 20 years ago[1] to describe a method of detecting short-lived reactive free radicals[2] by providing a nitrone or nitrose compound for an addition reaction to occur which produces an ESR detectable aminoxyl radical.[3] The first conference on this topic was held in 1981 at the University of Guelph and a special issue devoted to 35 contributions from speakers at this meeting was published in the Canadian Journal of Chemistry.[4] Eight years later the "2nd International Symposium on Spin Trapping and Aminoxyl Radical Chemistry" was held at the same place and this special issue is dedicated to 43 papers submitted by participants of this meeting.

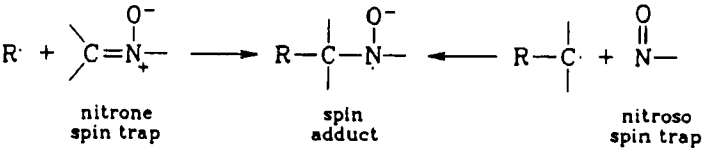
Nomenclature describing the radical produced from the hydrogen atom abstraction of a hydroxylamine has evolved through the terms: nitroxyl, nitroxide and aminoxyl (IUPAC).



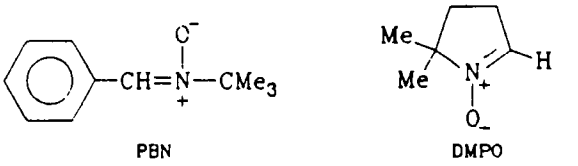
Today, all three names are still in use. The last of these would seem to provide the most systematic method for naming new radicals of this type since only the correct name for the secondary amine precursor is needed.

In spin trapping, the following vocabulary has developed. The compound trapping the radical is called the spin trap and the addition product of the radical is identified as the spin adduct.

*Nomenclature, like the Sabbath, was made for man; not *vice versa* (*J. Am. Chem. Soc.*, **84**, 2596 (1962)).



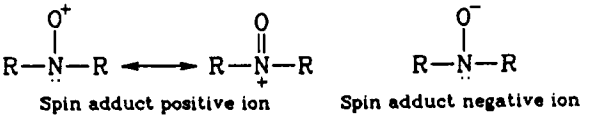
The high resolution ESR spectra of spin adducts provide hyperfine splitting constants which, in principle, are unique for each radical trapped. Tables[3,5] have been published and databases are being created[6,7] which allow searches for specific adducts in certain solvents since a considerable solvent effect on the magnitude of the splitting constants is found in some cases.[8] Spin adduct spectra usually fall into easily recognized patterns previously organized into various types.[9] Nitrone spectra are the simplest to analyze since the adding radical (addend) is one bond away from the radical function. The most common nitrones used are PBN and DMPO:



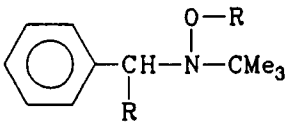
The ESR spectra can be generally described at the PBN-type (a triplet of doublets) or the DMPO-type (a doublet of triplets) although exceptions exist. The difference in spectral types comes mainly from the fact that the former is produced from an open chain aminoxyl while the latter is due to a cyclic nitroxide.

Because of the high reactivity of certain spin traps (e.g. DMPO) it is quite possible that spin adducts can be formed by routes not involving the actual addition of the addend group to the nitrone as a free radical. The best approach in order to test this question is to provide an alternate reaction route for the free radical by setting up a competitive reaction scheme. This method has been quite successful for DMPO, for example in studies of hydroxyl radical trapping (where either dimethyl sulfoxide, ethanol or sodium formate is added to the system).

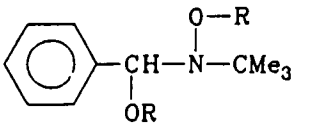
Since the detection of a spin adduct spectrum by itself is not enough proof of assignment for the structure of the aminoxyl, other methods are evolving to confirm or even establish the structure of spin adducts. The earliest was the use of isotopic enrichment in the radical addend (^{13}C, ^{15}N or ^{17}O) but recently the incorporation of ^{13}C into PBN itself has been shown to be useful for radical structure assigment.[10] High pressure liquid chromatography[11] was also shown to be suitable for certain cases of PBN spin adducts. However, more general methods are presently coming out of the development of various mass spectrometric techniques.[12-16] If the parent ion can be detected, the molecular weight of the spin adduct is the mass over charge number obtained from the spectrum. Both positive as well as negative [17] ion spectra have been detected:



Quantitating spin trapping events should take into account all reactions of free radicals with nitrones. Little is known about alternate reactions of free radicals with nitrones other than addition to the nitronyl carbon but undoubtedly such exist.[18] However, some information is appearing on further reactions of free radicals with spin adducts. Not only are double spin adducts formed, but these may come from either the same[12] or a different pair of radicals,[19] e.g.:

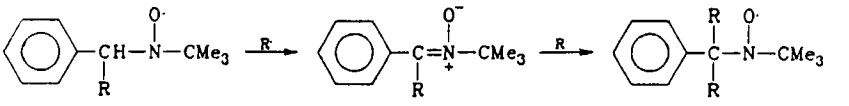


PBN double adduct

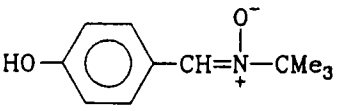


PBN mixed double adduct

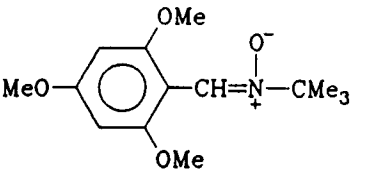
If the β-hydrogen of the spin adduct is abstracted by a radical encountering the aminoxyl a new nitrone would be formed and it is likely that evidence for such events will also be eventually found. Spin trapping with this new nitrone would give a more persistant nitroxide with no β-hydrogen, e.g.:



Nomenclature associated with short-hand forms for the names of spin traps and their spin adducts is still evolving. PBN comes from α-phenyl N-*tert*-butyl nitrone and derivatives with substituents attached to the phenyl ring have been identified in the form of a hyphenated prefix[20], e.g.:

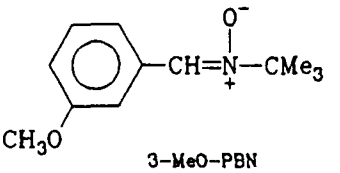


4-HO-PBN

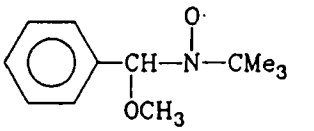


2,4,6-$(MeO)_3$PBN or "$(MO)_3$PBN"

However, a confusing usage is developing where the spin adduct is also designated in the same way. It is strongly suggested that if this is the method selected that a dot be used to distinguish this molecule as a spin adduct aminoxyl radical from the substituted nitrone, e.g.:

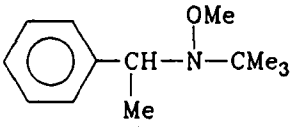


3-MeO-PBN

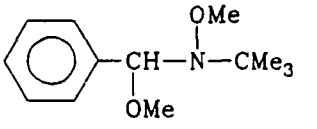


MeO-PBN·

The following double adducts can thus be designated:

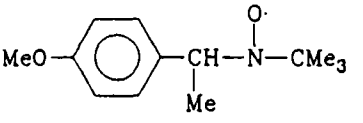


Me-PBN-Me

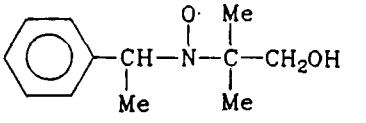


MeO-PBN-Me

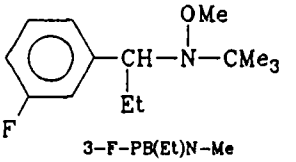
A complicated situation develops if substituents exist on either the phenyl or *tert*-butyl group, but a designation which could accommodate such possibilities is shown below:



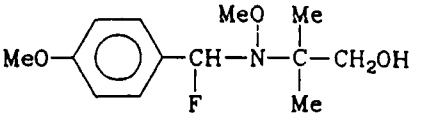
4-MeO-PB(Me)N·



P(HO)B(Me)N·

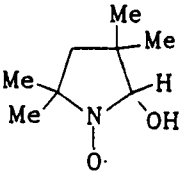


3-F-PB(Et)N-Me

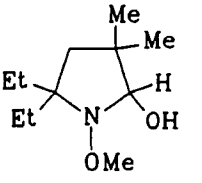


4-MeO-P(HO)B(F)-N-Me

DMPO has become entrenched[21] as the acronym for 5,5-dimethylpyrroline-N-oxide, but with more methyl groups and other alkyl substituents being used, the designation should better have been M_2PO for two methyl groups, M_4PO for four methyl groups[22] etc. For 3,3-dimethyl-5,5-diethylpyrroline-N-oxide[23] for example, the acronym becomes M_2E_2PO. For spin adducts, the following examples illustrate the method:



HO-M_4PO·



HO-M_2E_2PO-Me

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