Short Communication

Detection of a New Nitroxide Radical Derived from the Spin Trap 2-Methyl-2-nitrosopropane

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Since it was first independently developed by Janzen,[1] Lagercrantz[2] and Perkins[3] in 1968, the spin trapping technique has been widely used as a method of stabilizing short-lived free radicals. The radical under study was made to react with a diamagnetic scavenger molecule resulting in a nitroxide (aminoxyl*) spin adduct. In many cases this spin adduct is stable enough to be detected by ESR spectroscopy.

The most popular spin traps are compounds of either the nitroso R–NO or the nitrone R′–CH=N$^+$(O$^-$)–R″ type. Both have advantages and drawbacks. Nitrones are usually more effective as spin traps, especially against oxygen-centred radicals,[4] which are of great interest in biological systems. This has been thoroughly reviewed by Janzen.[5] On the other hand, the nitroso traps give spin adducts of which the ESR spectra are more easily interpreted. Therefore they have been the spin traps of choice for carbon-centred radicals . However nitrogen-centred,[6] oxygen-centred[7] and phosphorus-centred[8] radicals and others[9] have also been trapped by nitroso scavengers.

From the very beginning 2-methyl-2-nitrosopropane, (MNP; also called *t*-nitrosobutane) has been the most popular of the nitroso spin traps. It has been used in numerous studies of different kinds.[9] A field of great interest is the study of radiation damage to biological molecules in both the solid and the aqueous state.[10] This has also included studies of model systems, where hydroxyl radicals of various origins have been made to react with amino acids or nucleic acid bases.[11] In these cases it is very important that the spin trap itself does not take any part in the reactions under study. Ideally it should only trap the radicals produced and make them detectable by ESR spectroscopy. However, this is not always the case, a fact which has been shown by Riesz[12] and Makino.[13] Both diamagnetic and paramagnetic species may occur in aqueous solutions of MNP. Some of these even appear before irradiation of any kind has been performed, whereas others seem to be produced by photolysis or radiolysis.[14] In this study a formerly unknown radical is presented. It is *t*-butyl(nitroso)nitroxide which is derived solely from the spin trap itself after oxidation by hydroxyl radicals.

In the solid state MNP is dimeric. The dimer is slightly soluble in water (ca. 0.05 mol l^{-1}). When it is dissolved, the monomer is slowly formed according to eqn. (1). At ambient temperature it may take several hours for equilibrium to be established. The solution then shows a faint blue colour since the monomer absorbs light at 662 nm.

$$(CH_3)_3C-N(O)=N(O)-C(CH_3)_3 \rightleftharpoons 2(CH_3)_3C-NO \quad (1)$$

It is only the monomeric form of MNP that is active as a spin trap [eqn. (2)]. Therefore it is necessary to transform as much of the dimer into monomer as possible before any trapping attempts are made. This may be done with heat or with UV light as the dimer absorbs at 287 nm. Thus several experimenters have prepared their spin trap solutions by stirring a mixture of MNP and water in the dark for several hours at a slightly raised temperature, 30–45 °C. There is, however, a risk that part of the MNP may evaporate during this process since it has a fairly high vapour pressure. In photolysis experiments the monomerization can, in many cases, be brought about by the same UV source that initiates the reaction to be studied. This is especially useful when experiments have to be carried out at very low temperature in non-aqueous media.

$$(CH_3)_3C-NO + R\cdot \rightarrow (CH_3)_3-N(O\cdot)-R \quad (2)$$

In this laboratory another method has been used. This is simply to heat a small amount of a freshly prepared solution of MNP to its boiling point for a few seconds and then immediately to chill it in an ice bath. The MNP readily dissolves during the heating process and the monomer–dimer equilibrium is quickly established. It is possible that a small amount of the MNP will boil away in this case too. However, this seems to be more than balanced by the

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enhanced solubility and the favourable equilibrium position at the elevated temperature. The MNP solution shows a bright blue colour and it is very effective as a spin trap. An additional advantage is that most of the dissolved oxygen will evaporate from the solution during the heating process.

Experimental

All chemicals used were of the highest commercial quality and were used without further purification. MNP was bought from Sigma Chemicals Co, sodium hydroxide from EKA Nobel and hydrogen peroxide from Merck AG. The water was doubly distilled in Pyrex glass.

The aqueous MNP solutions were prepared by mixing ca. 10 mg of MNP with 1 ml of distilled water in a small test tube. The mixture was heated almost to its boiling point until all the MNP had dissolved, i.e. 5–10 s. The solution was then immediately chilled in an ice bath to slightly below room temperature. Then 0.2 ml of 30 % H_2O_2 were added. Finally a few drops of 0.01 M NaOH were added in order to raise the pH of the solution from 4–5 to the desired level. In most of the experiments this was just above 7. Usually the test solution was transferred directly to a flat quartz cell, which was put into the cavity of the ESR spectrometer. However, the same results were achieved with MNP solutions which had been stored in the ice bath for at least an hour. The test solution was irradiated *in situ* in the ESR cavity with the unfiltered light from a 200 W high-pressure mercury arc from Heraeus.

The ESR spectra were recorded with a Bruker ESP 200 MRD spectrometer, which was operated in the X-band at 2 mW microwave power. First-derivative spectra were recorded using 100 kHz field modulation with an amplitude of 0.02 mT.

Results

During experiments with MNP as the spin trap, a radical was detected that must be derived solely from the scavenger itself. It was produced during UV photolysis of a solution of MNP and hydrogen peroxide in water at pH 6.9–7.5. The radical is rather short lived and it disappears within a few seconds when the light is switched off. It shows a very well resolved ESR spectrum of nine narrow lines of almost equal intensity (Fig. 1). This indicates a radical with two nitrogens close to the free spin centre. The spectrum is strong enough to make the additional lines from the minor nitrogen isotope ^{15}N visible (Fig. 2). The intensities of these lines are not much greater than the noise level, which makes their identification slightly uncertain. However, they do not contradict our interpretation. The radical is therefore assigned the structure $(CH_3)_3C–N(O\cdot)–NO$.

In addition to the main spectrum there is also a spectrum of di-*t*-butylnitroxide and a number of weaker lines of uncertain origin. The strongest of these apparently form a spectrum consisting of six (or nine) lines with a_N = 1.475 mT and a_H = 0.181 mT (d) [or 0.09 mT (t)]. This indicates the presence of a trapped radical with the possible structure $CH_3\dot{C}H(OH)$ or $(CH_3)_2\dot{C}–CH_2OH$.

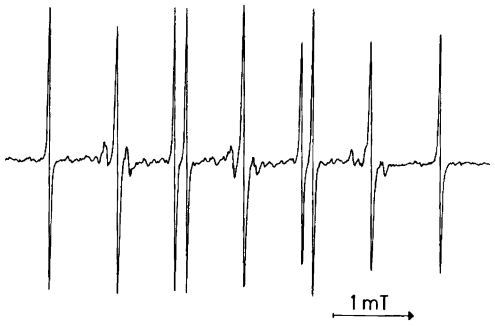


Fig. 1. ESR spectrum of the radical $(CH_3)_3C–N(O\cdot)–NO$. g = 2.0055, a_{N1} = 1.568 mT, a_{N2} = 0.854 mT for the isotope ^{14}N. The line width is ca. 0.05 mT.

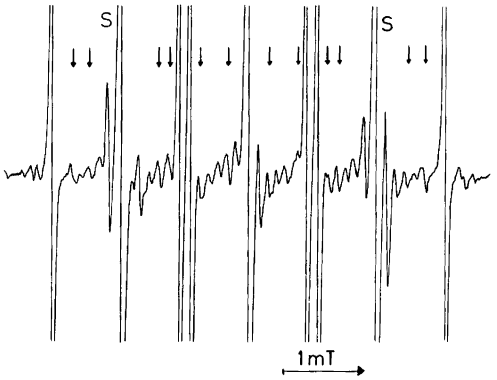
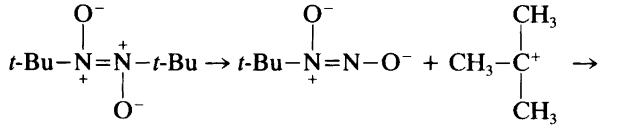


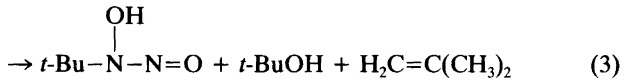
Fig. 2. ESR spectrum of the same radical as in Fig. 1 but amplified to show the additional ^{15}N lines. a_{N1} = 2.188 mT, a_{N2} = 1.194 mT for the isotope ^{15}N. The lines marked S form the spectrum of di-*t*-butylnitroxide. The third line is hidden by the central line of the main spectrum. g = 2.0055, a_N = 1.707 mT.

Discussion

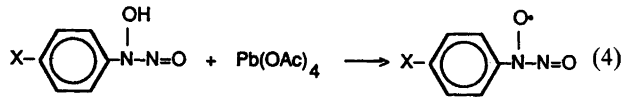
According to Makino,[13] the compound *t*-butyl(nitroso)hydroxylamine is spontaneously produced in aqueous solutions of MNP [eqn. (3)]. After γ-irradiation of such a solution, *t*-butyl(nitroso)hydroxylamine and MNP may go through several different reactions. Some of these will result in free radicals, which can be trapped by MNP and the products which can be separated by liquid chromatography (HPLC). In this way Makino[14] has identified a number of spin adducts originating from MNP only. However, none of these are identical with that found in this study. This radical appears only in neutral solution and it is not stable enough to survive chromatographic separation.

In a study by Balaban *et al.*[15] it is shown that aromatic nitrosonitroxides may be produced by oxidation of aromatic nitrosohydroxylamines in organic solvents according to eqn. (4). The ESR spectra of these radicals exhibit a





(3)

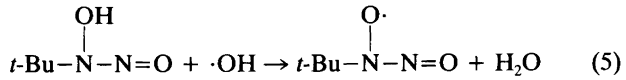


(4)

somewhat different hyperfine structure as compared with that shown in this study; i.e. the nitrogen hyperfine coupling constants are smaller than for the radical presented here. This is in accordance with the more delocalized electron density of aromatic structures. Part of the difference may also depend on the various solvents. The g values, however, are very close, which points to a similar structure.

In this case it was not possible to record an ESR spectrum of *t*-butyl(nitroso)nitroxide formed by oxidation of *t*-butyl(nitroso)hydroxylamine of MNP origin with lead tetraacetate. This might be explained by the very short lifetime of the aliphatic nitrosonitroxide found in this study as compared with the aromatic compounds used by Balaban.

Thus it is argued that MNP is partly converted into *t*-butyl(nitroso)hydroxylamine by heating the MNP solution, according to eqn. (3). This is also confirmed by the UV spectrum of the MNP solution. It shows the characteristic absorption band at ca. 246 nm due to the basic form of the hydroxylamine. Then the hydroxylamine is oxidized by hydroxyl radicals to *t*-butyl(nitroso)nitroxide, the ESR spectrum of which is recorded. The hydroxyl radicals are photolytically generated from added hydrogen peroxide [eqn. (5)].



(5)

The pK_a of *t*-butyl(nitroso)hydroxylamine is 6.0.[13] Thus at acidic pH, the amino group is protonated, which will prevent any reaction with hydroxyl radicals. This is in accordance with this study, where no radicals were produced at pH lower than ca. 6.5. At still higher pH, hydroxide-ion attack on the hydroxylamine will hamper the production of *t*-butyl(nitroso)nitroxide.

Conclusions

It is possible to use MNP as a spin trap with the technique used here, i.e. simple heating of the aqueous MNP mixture. The concentration of monomer is high and the spin trapping efficiency is quite good. However, problems may occur due to a high concentration of *t*-butyl(nitroso)hydroxylamine. This is particularly the case, when the heating time exceeds a few seconds. In the presence of hydroxyl radicals the unstable radical *t*-butyl(nitroso)nitroxide is formed. Its ESR spectrum has been recorded and is presented here. Usually its presence does not interfere severely, but one should be aware of the possibility of overlapping spectra.

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References

1. Janzen, E. G. and Blackburn, B. J. *J. Am. Chem. Soc. 90* (1968) 5909.
2. Lagercrantz, C. and Forshult, S. *Nature (London) 218* (1968) 1247.
3. Chalfont, G. R., Perkins, M. J. and Horsfield, A. *J. Am. Chem. Soc. 90* (1968) 7141.
4. Janzen, E. G. *J. Am. Chem. Soc. 100* (1978) 2824.
5. Janzen, E. G. In: Pryor, W. A., Ed., *Free Radicals in Biology*, Academic Press, New York 1980, Vol. 4, p. 115.
6. Lagercrantz, C. and Forshult, S. *Acta Chem. Scand. 23* (1969) 708.
7. Forshult, S., Lagercrantz, C. and Torssell, K. *Acta Chem. Scand. 23* (1969) 522.
8. Adevik, G. and Lagercrantz, C. *Acta Chem. Scand. 23* (1970) 2253.
9. Büttner, G. R. *Free Radical Biol. Med. 3* (1987) 259.
10. Makino, K., Moriya, F. and Hatano, H. *J. Chromatogr. 332* (1985) 71.
11. Riesz, P. and Rosenthal, I. *Can. J. Chem. 60* (1982) 1474; *Radiat. Phys. Chem., 30* (1987) 381.
12. Riesz, P. and Rustgi, S. *Radiat. Phys. Chem. 13* (1979) 21.
13. Makino, K., Suzuki, N., Moriya, F., Rokushika, S. and Hatano, H. *Radiat. Res. 86* (1981) 294.
14. Makino, K. *J. Phys. Chem. 84* (1980) 1012.
15. Balaban, A. T., Negoita, N. and Baican, R. *J. Magn. Reson. 9* (1973) 1.

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