Conversion of Nitric Oxide into a Nitroxide Radical using 2,3-Dimethylbutadiene and 2,5-Dimethylhexadiene

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Reaction of nitric oxide with 2,3-dimethyl-1,3-butadiene gave an unstable nitroxide radical characterised by hyperfine coupling to four equivalent β-protons in addition to the normal ^{14}N triplet: however reaction with the tetramethyl derivative (2,5-dimethyl-2,4-hexadiene) gave an extremely stable nitroxide, which may be of use in pollution studies and especially in studies of nitric oxide in mammalian cells.

One of the most exciting recent developments in inorganic biochemistry has been the discovery of the important roles played by the small molecule nitric oxide (·NO), which had previously been viewed simply as a dangerous toxic pollutant. It is now clear that mammalian cells synthesize ·NO specifically from L-arginine by nitric oxide synthase to serve a variety of important biological roles. For example, it plays an essential part in the process of vasodilation, it is one of the radicals involved in the action of macrophages, and it acts as a messenger in the central nervous system.[1]

Its physical properties are very like those of dioxygen, with which it reacts efficiently, so that except in hypoxic systems it has only a short time to fulfil its various roles. Unfortunately, despite the fact that it is a simple radical, it cannot be detected by EPR spectroscopy under normal circumstances, though solid-state spectra have been reported at 77 K.[2]

It reacts efficiently with deoxyhaemoglobin ($HbFe^{II}$) to form the nitrosyl derivative $HbFe^{II}$-NO. This is also a radical-like centre, with the spin largely on the NO group. It gives characteristic EPR spectra, which are best studied at low temperatures, but are detectable at room temperature.[3] This

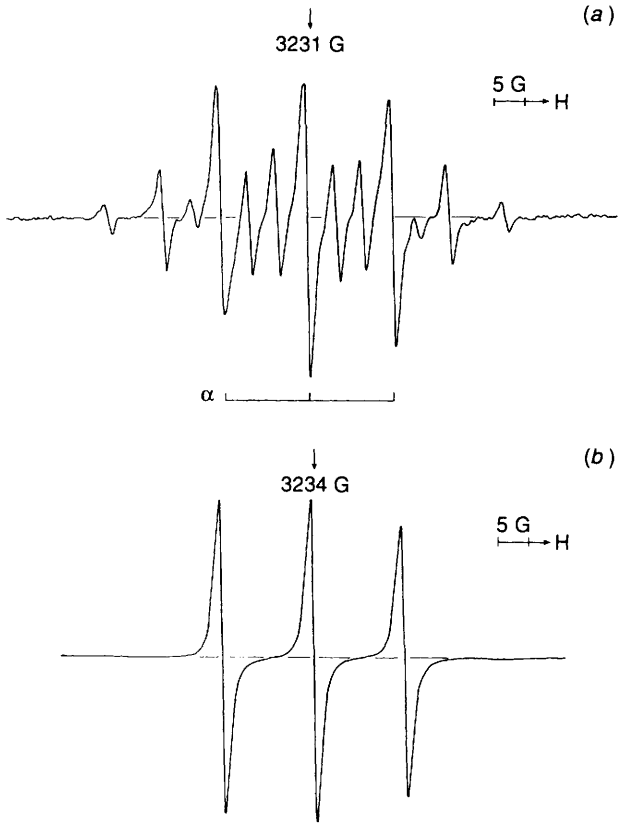
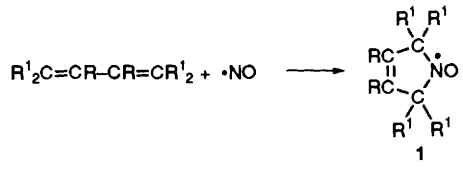


Fig. 1 First derivative X-band EPR spectrum assigned to (*a*) the nitroxide radical **1** (R = Me, R^1 = H), showing a quintet splitting from four equivalent protons in addition to the ^{14}N triplet; [there is an additional triplet feature (*a*) which was always obtained together with the triplet of quintets: the source of this species is unknown] and (*b*) the nitroxide radical **1** (R = H, R^1 = Me), showing the stable ^{14}N triplet

is a most important species for studying ·NO, but it does require the absence of oxygen since oxyhaemoglobin, $HbFe^{II}O_2$, reacts with ·NO to form methaemoglobin rather than $HbFe^{II}NO$. It is also possible to detect ·NO by trapping it with various transition-metal complexes, and it has recently been suggested that the spin-trap Me_3CNO reacts with ·NO to give the novel radical $Me_3C(ON)N^{\cdot}O$.[4]

Our interest in converting ·NO into a stable radical having well-defined EPR features arose because of our development of a whole-body radio-frequency EPR imaging spectrometer.[5,6] If this could be achieved it would be a very powerful method for studying the generation of ·NO *in vivo*. We and others have, therefore, sought new methods of trapping ·NO for EPR detection, the most obvious method being to use it to form a nitroxide radical, $R_2\dot{N}O$, which would both prove the presence of ·NO uniquely, and also be stable for some time in biological samples. Reactions of type (1) have been our aim. Butadiene derivatives, like .NO, are lipid soluble and hence would trap ·NO in such environments. The magnitude of $A(^{14}N)$ would act as an indicator for this, being *ca.* 2 G less in lipid solvents than in water. Normally, one would select R^1 = Me in order to give high stability to the nitroxide. However, by using R^1 = H a clear quintet from four equivalent protons would be expected, which would serve to prove the reaction uniquely. We have tested both.



(1)

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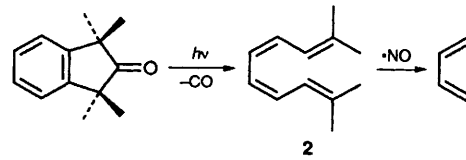
Using R^1 = H and R = Me, we have prepared radical **1**, with four equal ^1H couplings of *ca.* 10 G, and a ^{14}N splitting of 14.2 G (Fig. 1). The frozen system gave anisotropic parameters equally characteristic of a nitroxide radical, with $A_{//}(^{14}N)$ = 32.0 G. Reactions were carried out using pure gaseous nitric oxide and tetrachloromethane or cyclohexane solvents. Solutions of the butadiene (*ca.* 0.1 mol dm^{-3}) were degassed prior to use and care was taken to avoid any oxygen uptake.

We stress that this system was chosen to establish unambiguously the structure of the adduct. As expected, it has a relatively short halflife of a few minutes at room temp., and hence would not be suitable in practice. However, the derivative with R^1 = Me and R = H proved to be suitable in every respect. It reacts rapidly with very dilute solutions to give a well-defined nitroxide triplet of very long lifetime. Not only is this a unique and highly sensitive method for trapping ·NO and identifying and quantifying it in biological systems, but it has potential, by suitably substituting polar groups, for detecting ·NO either in lipid phases or in aqueous solutions.

The most important work related to our study is that of Korth *et al.*[7] They have used reaction (2), in which UV



(2)

2

photolysis is used to generate the unstable *ortho*-quinodimethane derivative **2**, which then adds ·NO to give a stable ntiroxide. The major disadvantage of this method is the need to photolyse, which would make it unsatisfactory for imaging. Otherwise, the gain is that the active centres are permanently *cis*, whereas for the open-chain compounds the *trans*-conformer is expected to be favoured. It is thought probable that reaction (1) occurs in two steps. In the event, however, the requried species is formed very rapidly from the open chain compound, and photolysis is eliminated.

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