

■ SPIN TRAPPING:

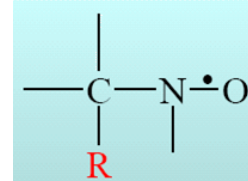
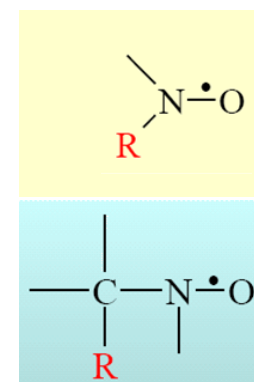


■ Caratteristiche di un buon SPIN TRAP:

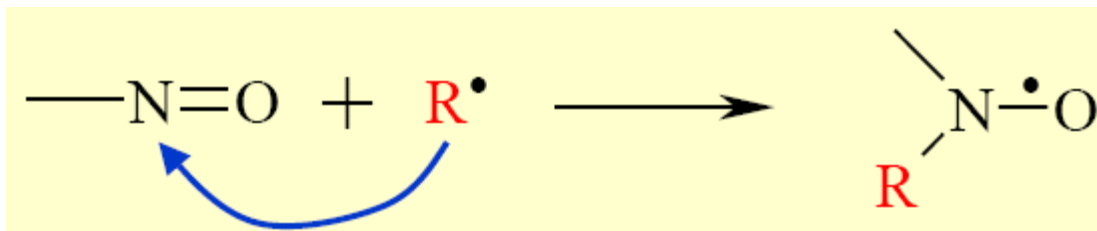
- non deve partecipare e/o dar luogo ad altre reazioni;
- la reazione di addizione deve essere molto veloce e l'addotto sufficientemente persistente;
- l'addotto formato deve essere rappresentativo della reazione oggetto di studio e non provenire da altre;
- lo spettro EPR deve essere interpretabile facilmente e in modo univoco

□ I più comuni sono:

- Nitroso-derivati
- nitroni



■ Nitrosoderivati



□ **Vantaggi:**

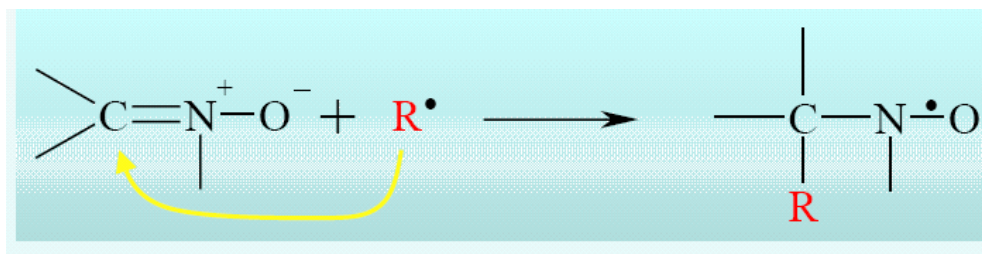
- Dato che **R** è legato in posizione immediatamente adiacente al nitrossido puo' facilmente dare splitting iperfine addizionale, fondamentale per identificare il radicale intrappolato.

□ **Svantaggi:**

- Termolabile, fotosensibile, poco solubile in acqua, dà addotti poco persistenti con spettri che hanno una larghezza di riga elevata.

Nitroso-derivati comunemente utilizzati: **MNP** (specifico per radicali centrati su C), **DMPO** (per radicali centrati su O e C) e **DEPMPO** (specifico per radicali centrati su O)

■ Nitroni (N-ossidi)



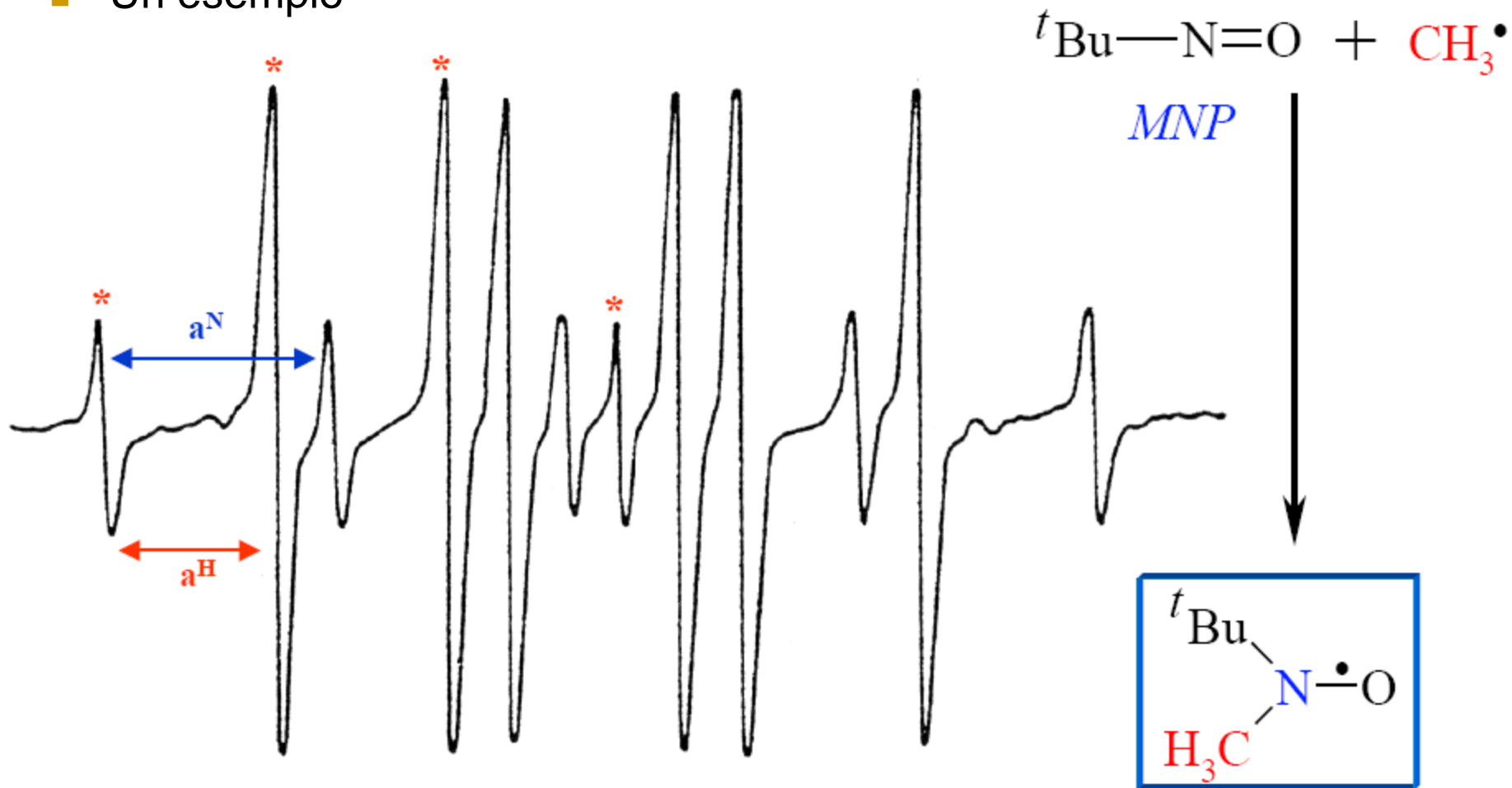
□ **Vantaggi:**

- reattività simile ma con addotti molto più persistenti
- Ragionevolmente idrosolubile e non fotosensibile
- Non particolarmente tossico
- Molto utilizzato in applicazioni biologiche

□ **Svantaggi:**

- **R** non legato direttamente al centro radicalico: i suoi splitting sono meno evidenti e lo spettro è spesso meno strutturato;
- righe piuttosto allargate.

■ Un esempio



■ **Nitroso compounds**

□ **Example:**

■ **2-METHYL-2-NITROSO PROPANE (MNP)**

- MNP was probably the first nitroso compound to be studied extensively and the first to be proposed for use in biology
- MNP is a solid dimer and gives rise in solution to a monomer-dimer equilibrium. Unfortunately the dissociation of dimeric MNP to its active monomeric form is slow and is accompanied by a slow and complicated decomposition even in the dark.

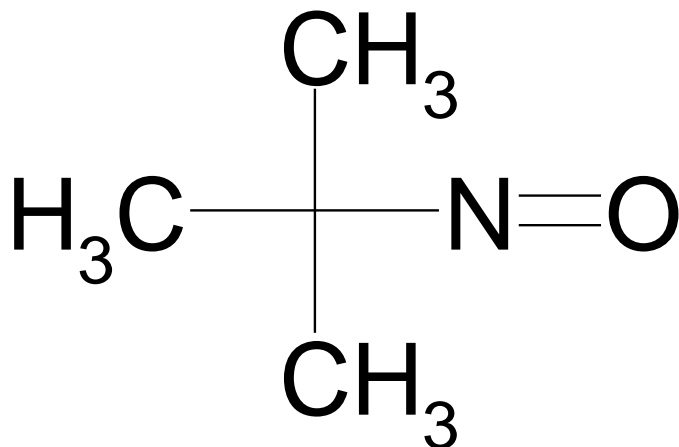


■ Nitroso compounds

□ Example:

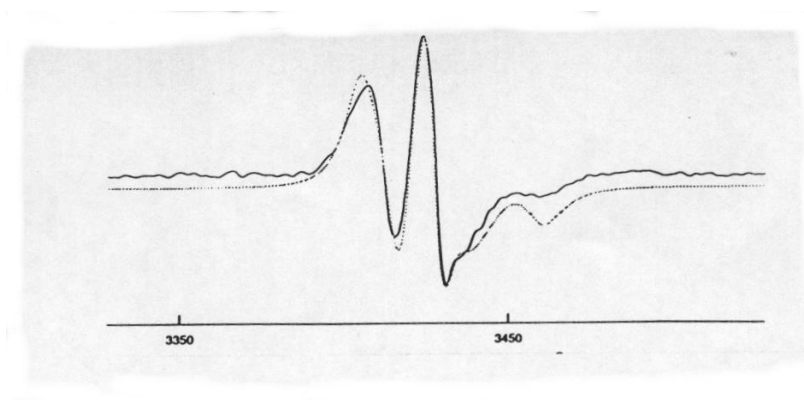
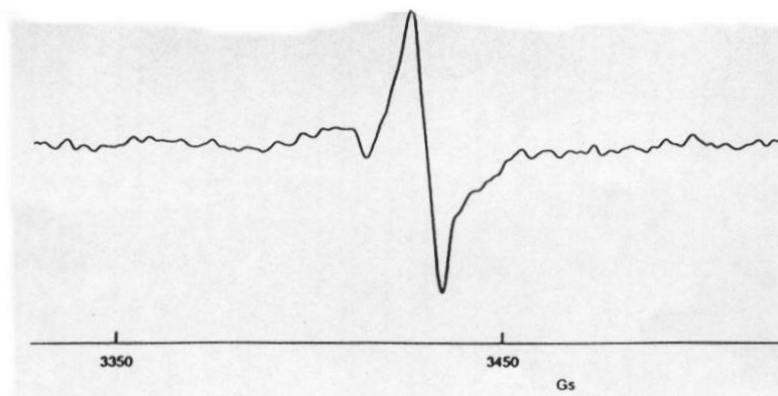
■ 2-METHYL-2-NITROSO PROPANE (MNP)

- MNP is very volatile
- MNP is a specific spin trap for carbon centered radicals. Hydroxyl radicals can not be detected with nitroso spin traps
- MNP gives an –ene addition, usually an unwanted reaction in spin trapping. This non radical reaction forms a hydroxylamine, which in turn may undergo oxidation giving rise to a nitroxide.



Caratterizzazione di specie paramagnetiche ESR di radicali - Spin trap

- EPR spectrum of cytochrome c-MNP adduct in the presence of pyrene ($\nu = 9.6194$ GHz).
- The lineshape of this spectrum is quite different from the spectrum in the absence of substrate :

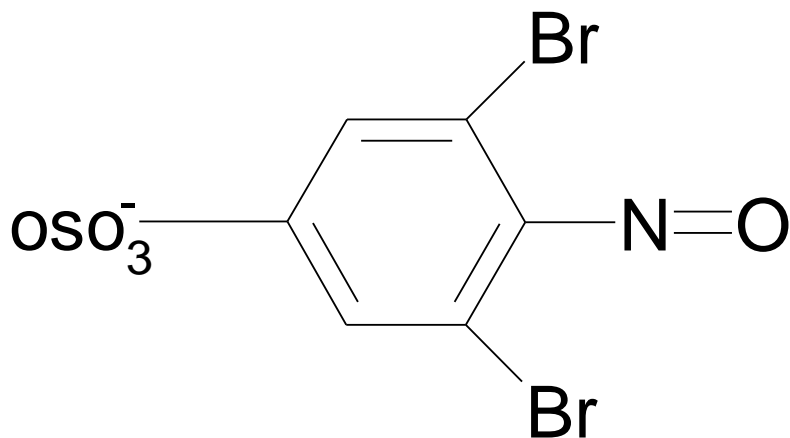


■ Nitroso compounds

□ Example:

■ 3,5-DIBROMO-4-NITROSOBENZENESULFONATE (DBNBS)

- DBNBS is less sensible to light.
- DBNBS is more hydrophilic because the introduction of sulfonate and bromine in 3-5 position.
- DBNBS is a specific spin trap for carbon centered radicals (DBNBS can react with $O_2^{\cdot-}$, for example, but the product formed is unstable and is undetected by EPR spectroscopy..

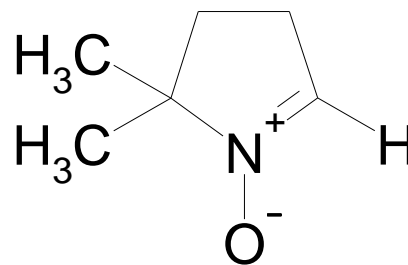


■ Nitroso compounds

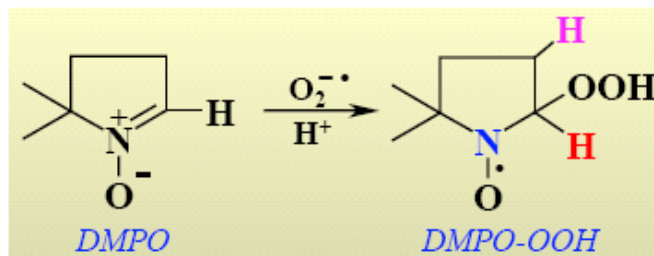
□ Example:

■ 5,5-DIMETHYL-1-PYRROLINE-N-OXIDE (DMPO)

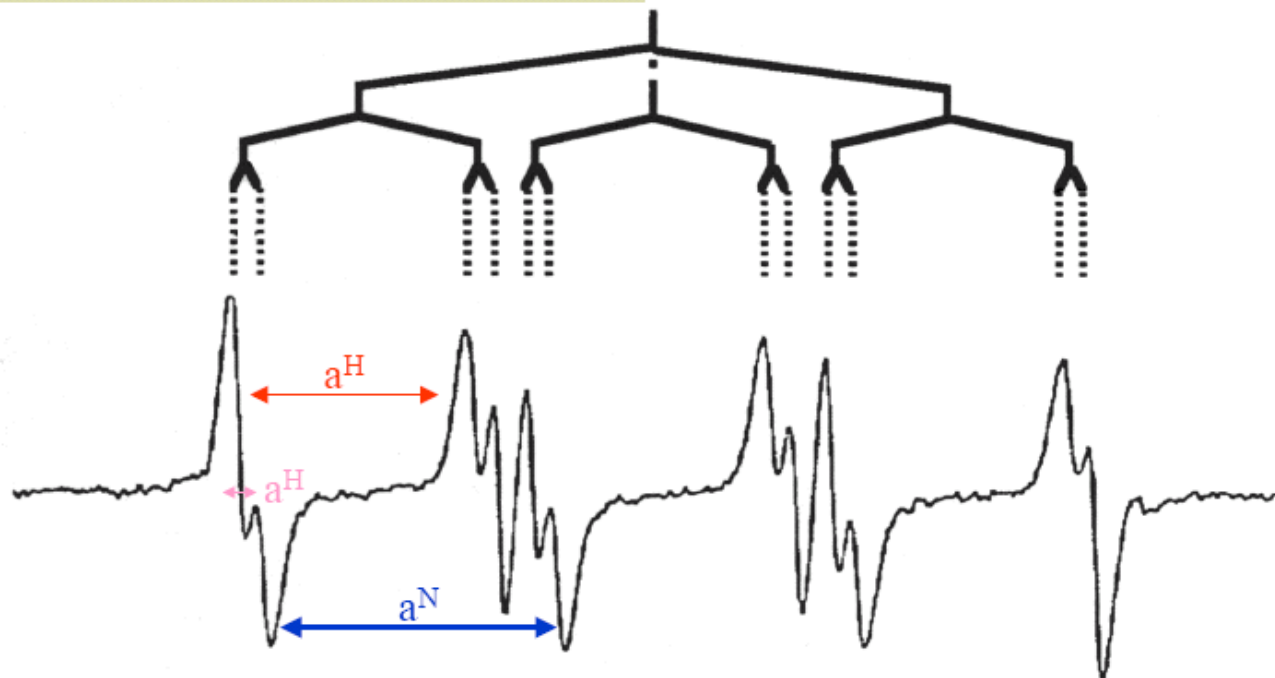
- DMPO is the most useful trap for the study of oxygen-centered free radicals.
- The DMPO-superoxide anion and DMPO-hydroxyl adduct are unique.
- The DMPO alkoxy and peroxy radical adducts are well distinguishable and distinct from carbon-centered radical adducts.
- Reaction of DMPO with superoxide is rather slow
- Furthermore, DMPO-superoxide adduct is relatively unstable and undergoes a rapid chemical conversion to DEPMPO-OH.
- Several cellular components and even superoxide itself are able to reduce DMPO-OOH and DMPO-OH into diamagnetic species.
- Finally, in aqueous solution, DMPO is susceptible to metal-ion-catalyzed addition of water leading via a non radical reaction to a variety of unwanted pyrrolidinoxyl radicals, including DMPO-OH.



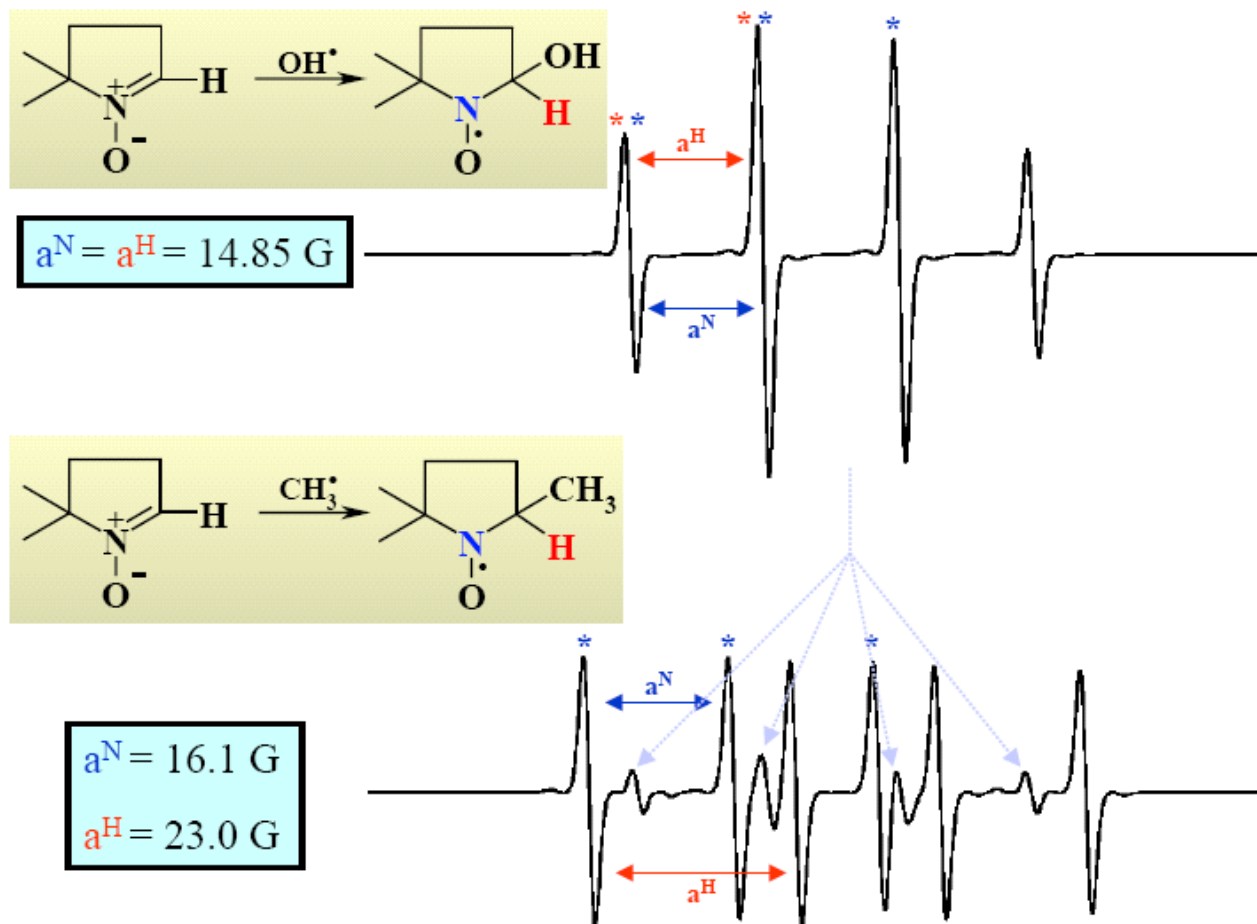
■ DMPO-adducts



$a^{\text{N}} = 14.2 \text{ G}$
 $a^{\text{H}} = 11.3 \text{ G}$
 $a^{\text{H}} = 1.4 \text{ G}$



■ DMPO-adducts

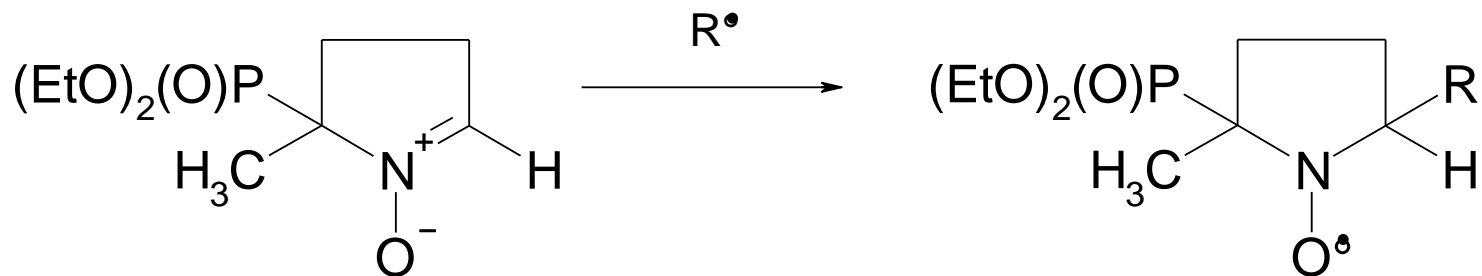


■ Nitroso compounds

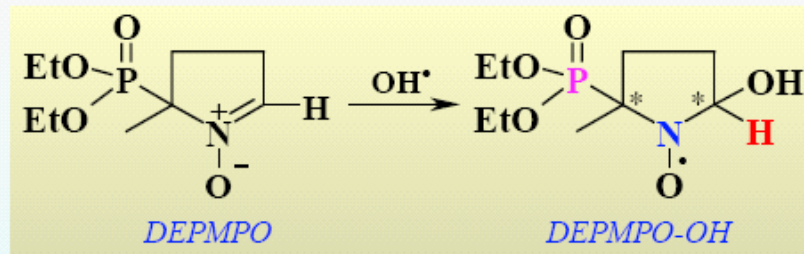
□ Example:

■ 5-(DIETHOXYPHOSPHORYL)-5-METHYL-1-PYRROLINE-N-OXIDE (DEPMPO)

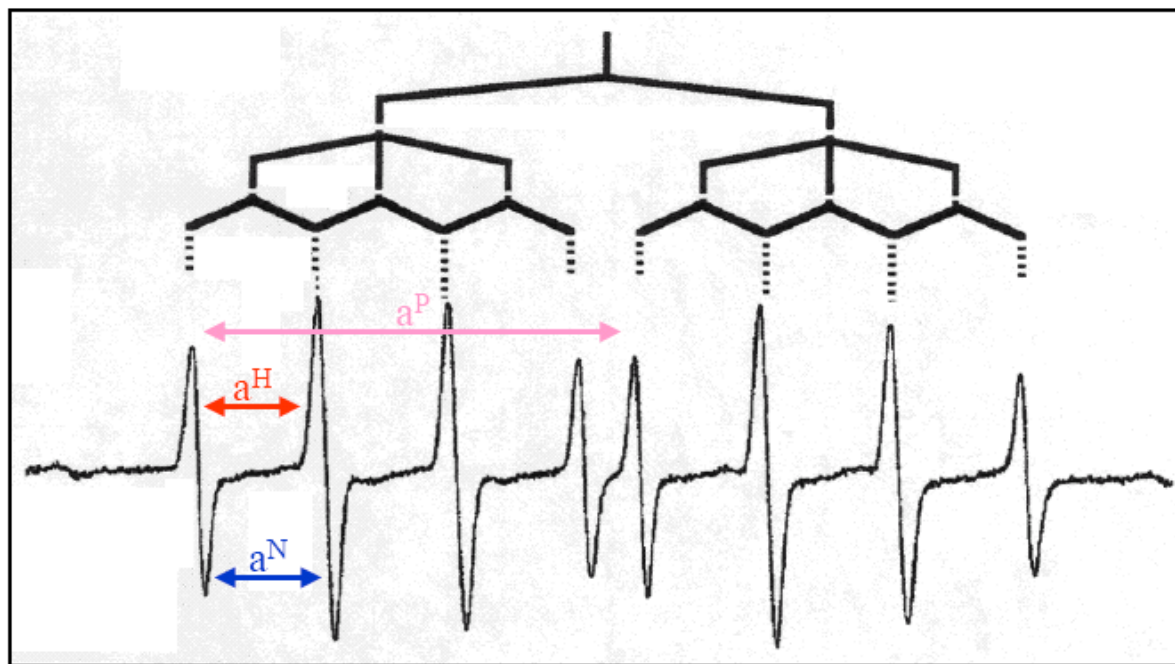
- DEPMPO spin trap is more reactive with a various kind of oxygen radical species and gives adducts stable for long time.
- DEPMPO enables specific and sensitive detection and quantification of superoxide generation.
- In addition, spontaneous decomposition of the -OOH adduct to the -OH adduct was not observed, and in physiological buffers in the presence of metal ions no significant artifacts signal were seen.
- The phosphorus nucleus ($I=1/2$) in DEPMPO spin adducts has a large hyperfine splitting ($A_p \approx 45\text{-}53\text{ G}$) that is sensitive to the nature of the radical trapped and to the nitroxide spin adduct conformation.



■ DEPMPO-adducts

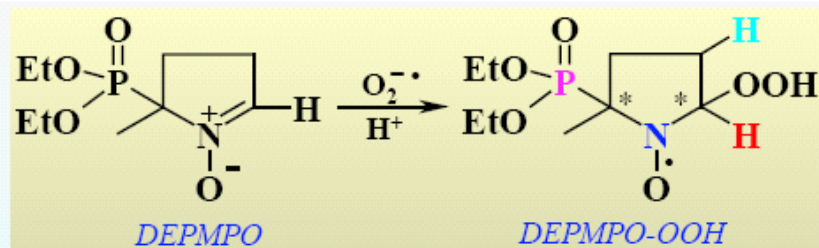


$a^{\text{N}} = 14.1 \text{ G}$
 $a^{\text{H}} = 13.8 \text{ G}$
 $a^{\text{P}} = 47.6 \text{ G}$

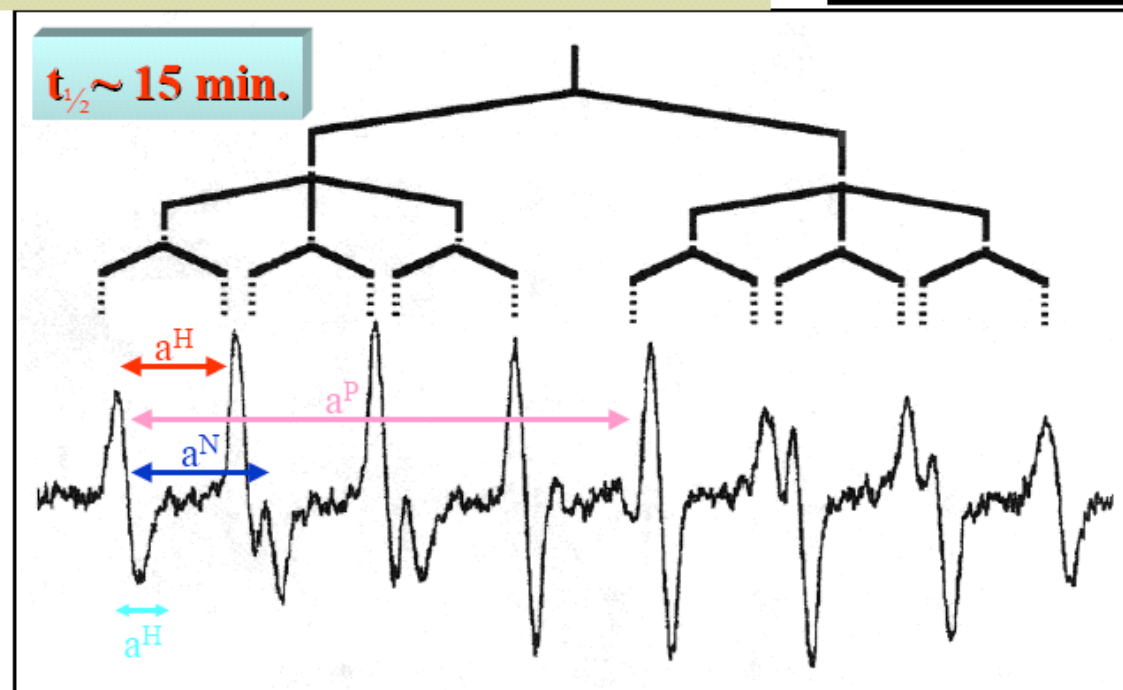


Caratterizzazione di specie paramagnetiche ESR di radicali – Spin trapping

DEPMPO-adducts



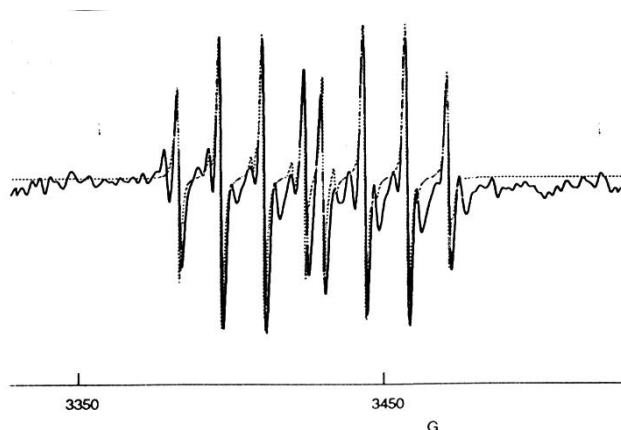
$a^N = 13.1$ (13.3) G
$a^H = 11.1$ (9.9) G
$a^P = 49.0$ (40.5) G
$a^H = 0.9$ (1.5) G



Caratterizzazione di specie paramagnetiche

ESR di radicali - Spin trap

- EPR spectrum of the DEPMPO adducts obtained in the cytochrome c/H₂O₂ system paired with its simulation. The simulation was obtained by the linear combination of DEPMPO-OH (90%) e DEPMPO-OOH (10%) adducts ($\nu = 9.6215$ GHz).
- The third species ($g = 2.0061$, $A^P = 55.6$ G, $A^N = 13.5$ G, $A^H = 13.5$ G) has been attributed to the SECOND CONFORMER OF THE DEPMPO-OOH radical adduct.



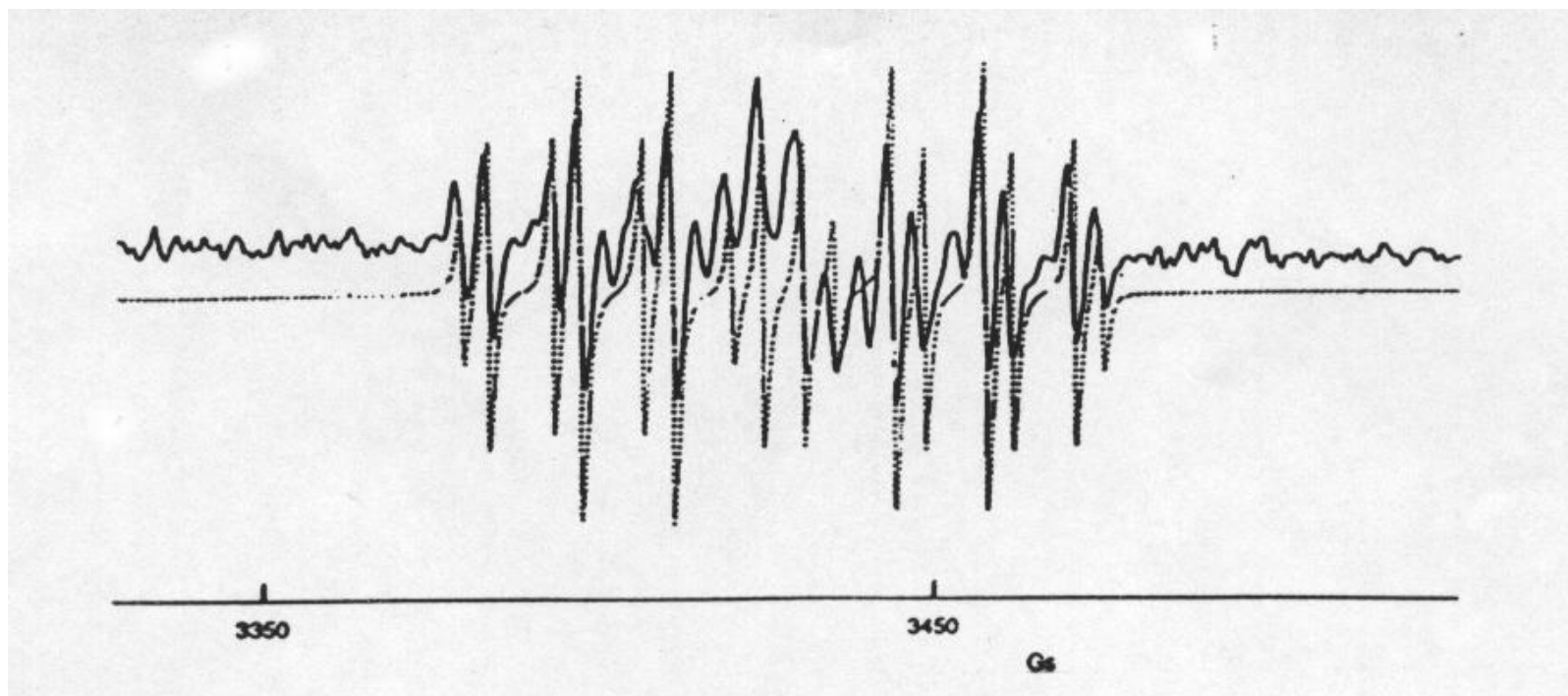
Species A (90%)	Species B (10%)
DEPMPO-OH	DEPMPO-OOH
2.0061	2.0061
47.3	52.5
14	13.4
13.2	11.9

E.BUSI, B.D.HOWES, R.POGNI, R.BASOSI, R.TINOCO e R.VAZQUEZ-DUHALT "Modified Cytochrome H₂O₂ Systems: Spectroscopic EPR Investigation of the Biocatalytic Behaviour" J. Mol. Catal. B: Enzymatic, vol. 9, 39-48 (2000).



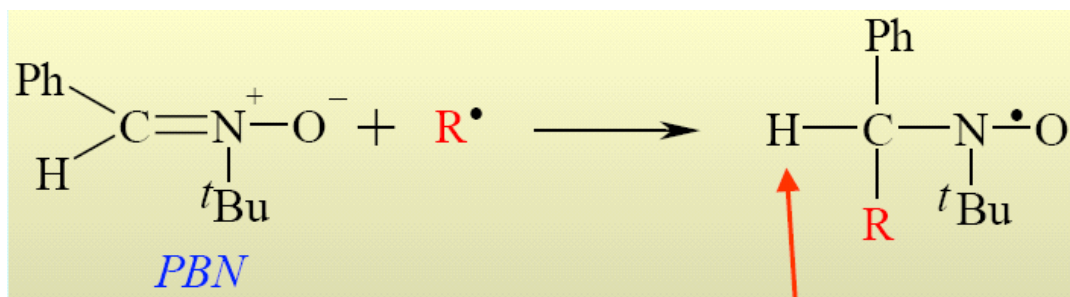
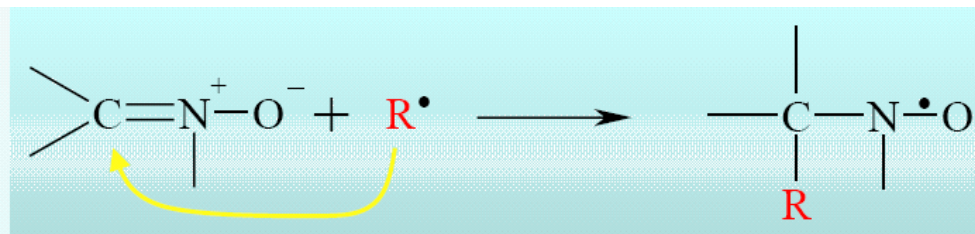
Caratterizzazione di specie paramagnetiche ESR di radicali - Spin trap

- EPR spectrum of the DEPMPO adduct in the Cytochrome c/H₂O₂ system in the presence of pyrene ($\nu = 9.6221\text{GHz}$) paired with its best simulation.



Caratterizzazione di specie paramagnetiche ESR di radicali – Spin trapping

- Nitroni (N-ossidi)
 - reattività simile ma con addotti molto più persistenti

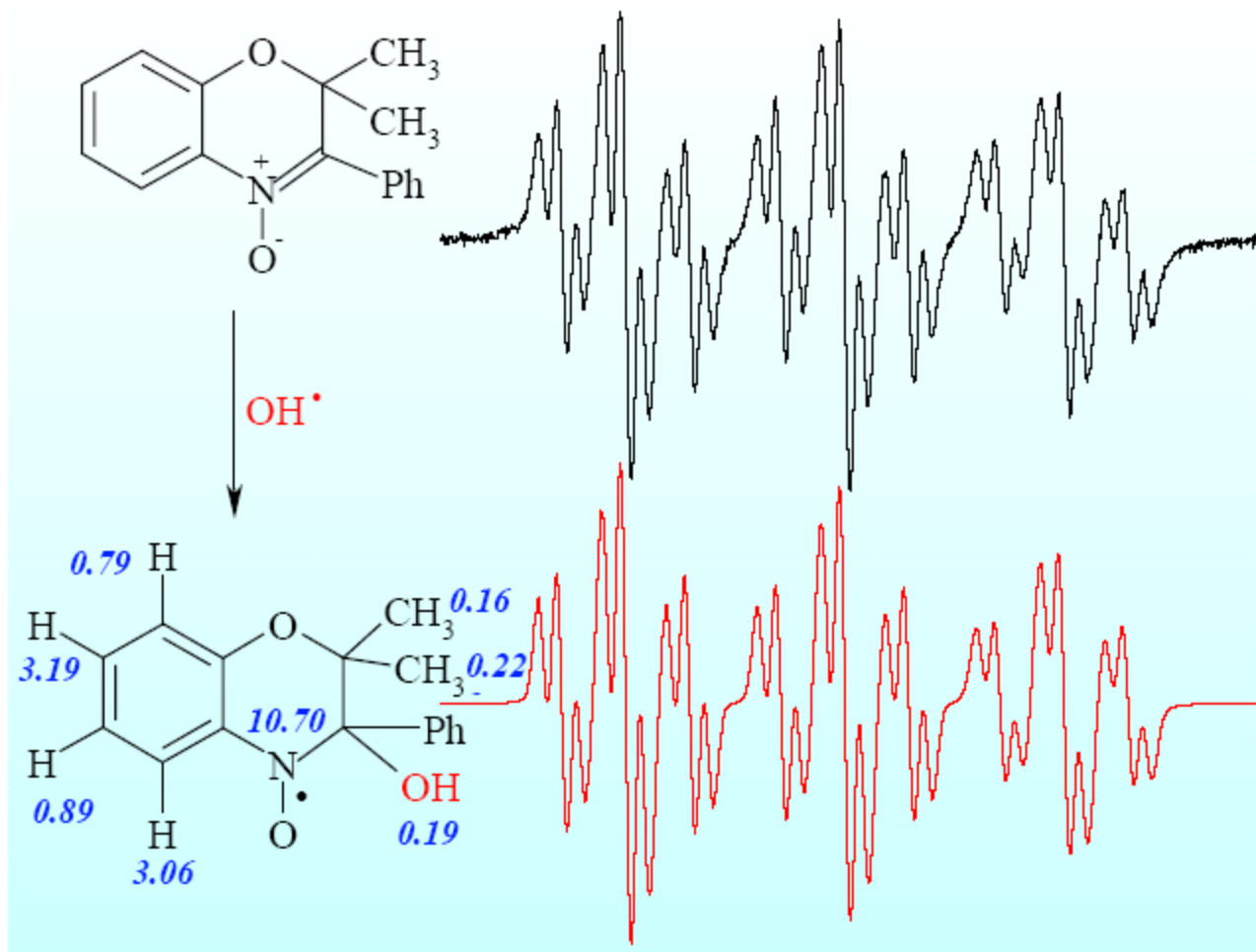


- addotti non particolarmente persistenti (β -H);
- R non legato direttamente al centro radicalico: i suoi splitting talvolta sono meno risolti;
- righe piuttosto allargate (tBu).



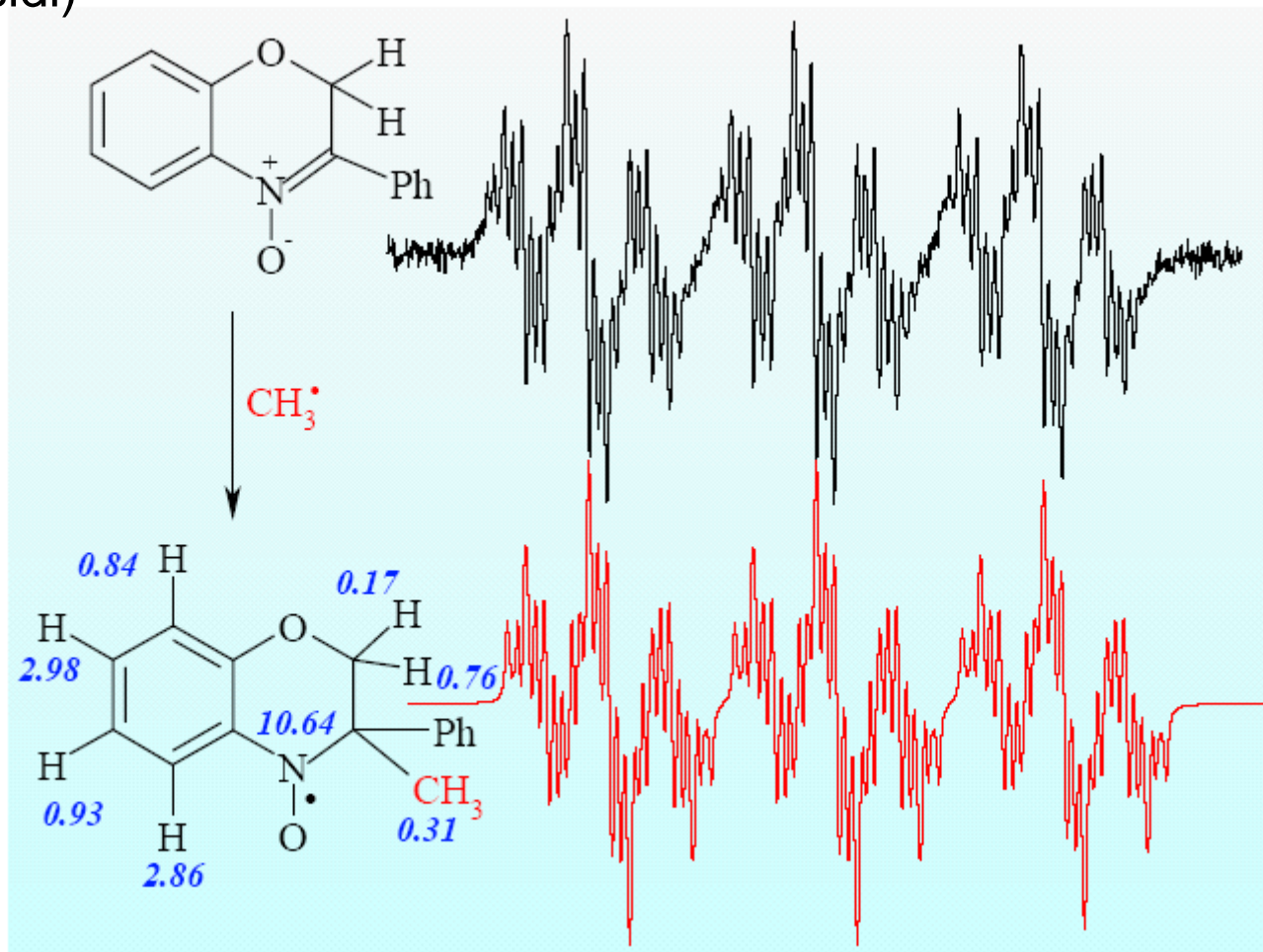
■ Nitroni (N-ossidi)

NOME!!!!

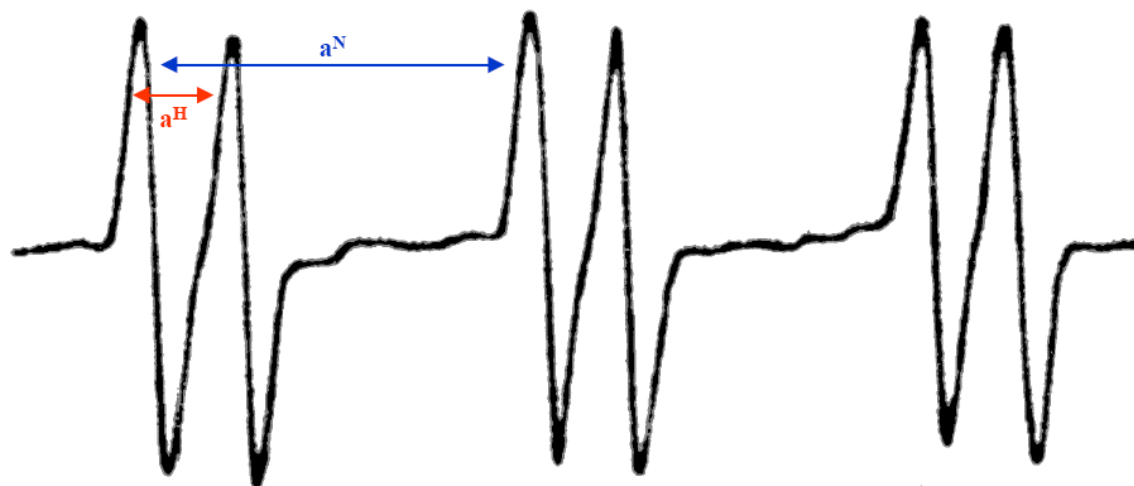
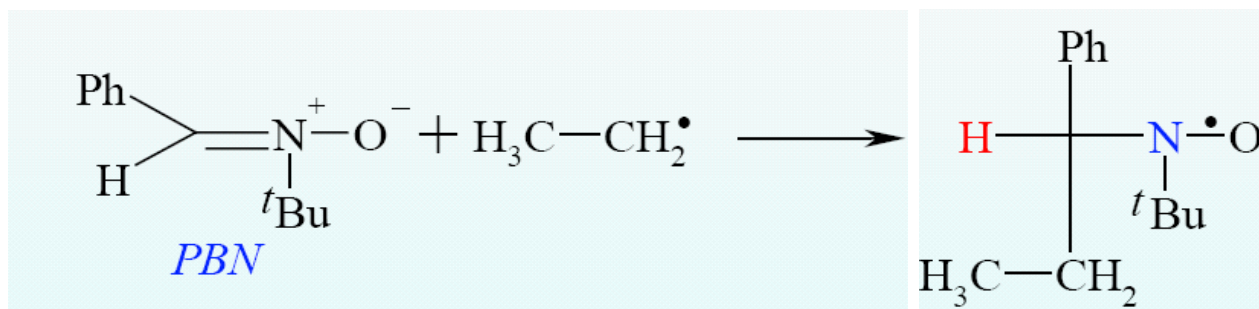


■ Nitroni (N-ossidi)

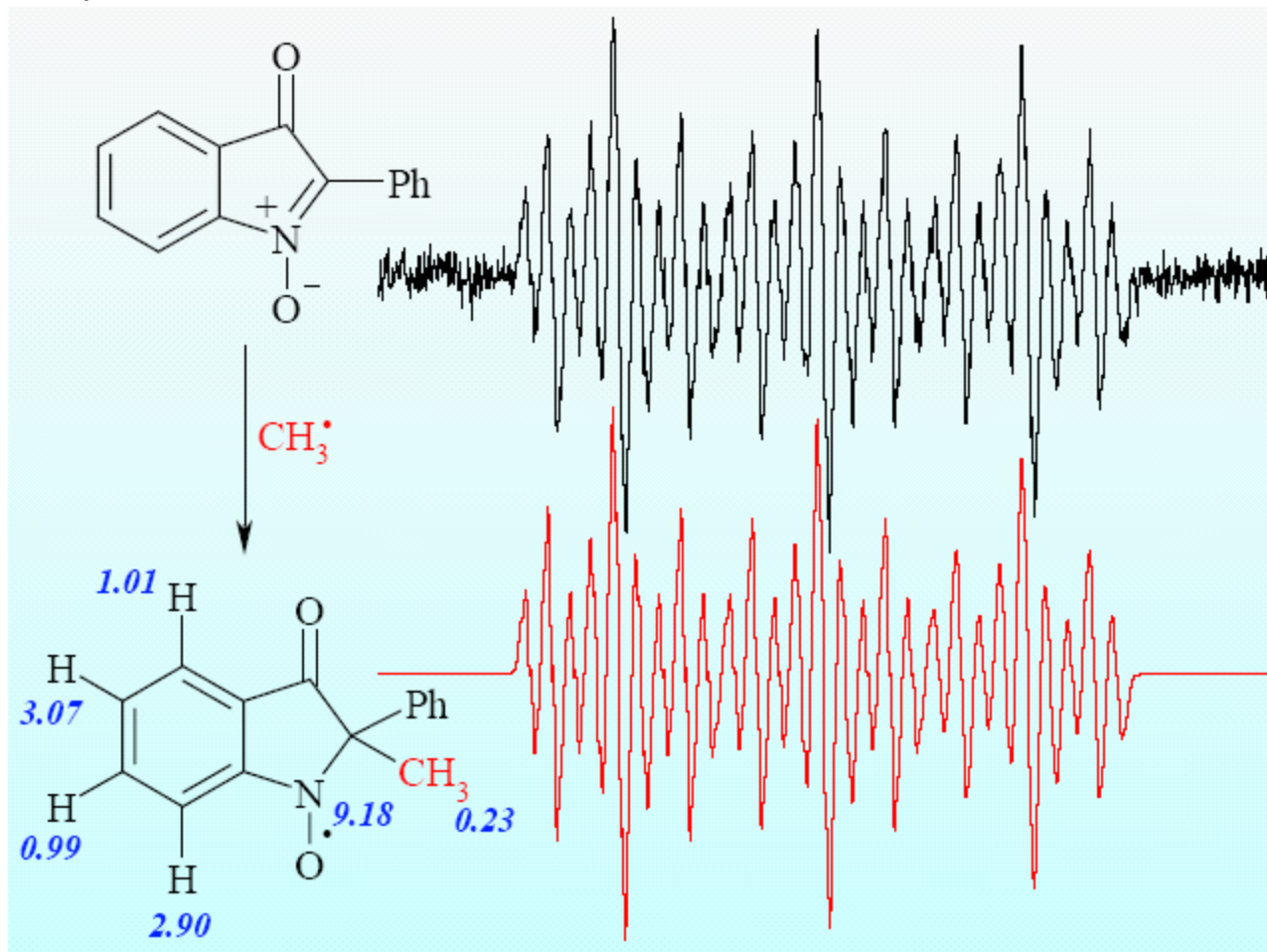
NOME!!!!



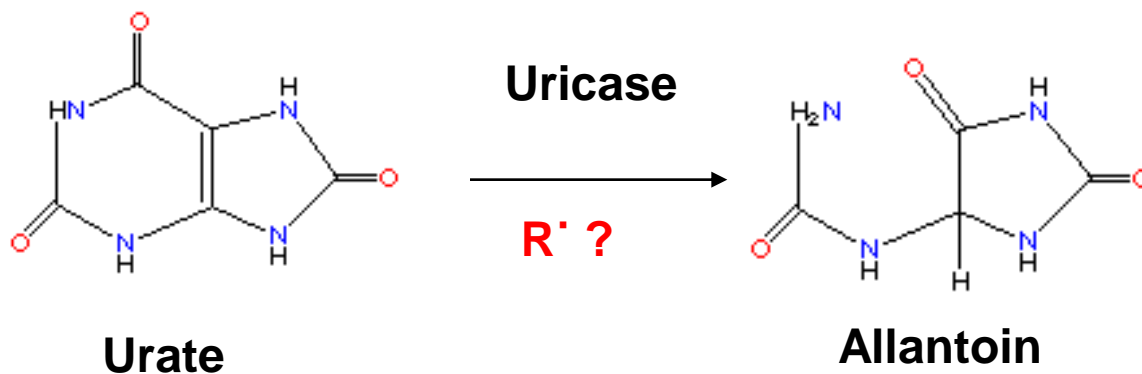
■ Nitroni (N-ossidi)



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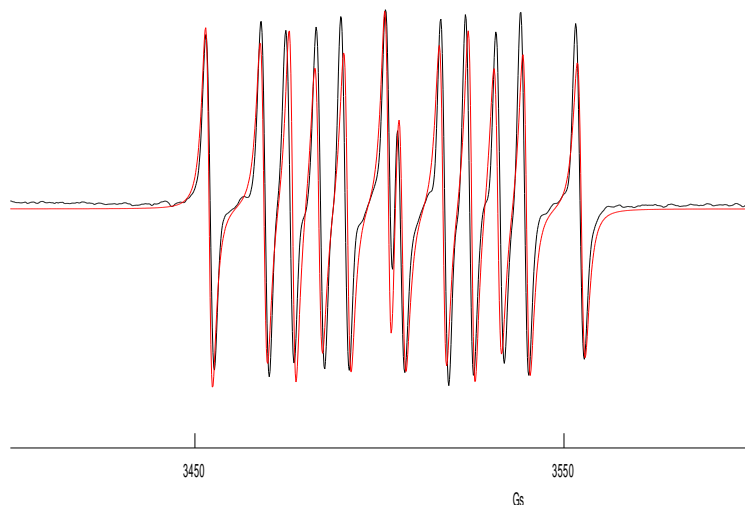


- DEPMPO in the urate oxidation by uricase



Does the reaction proceed through a radical intermediate?

- DEPMPO in the urate oxidation by uricase



EPR spectrum of DEPMPO-radical adduct entrapped in the reaction of uricase from *Candida Utilites* and urate (black line) paired with its simulation (red line). Several uricases have given the same EPR spectrum (*Arthrobacter Globiformis*, *Bacillus Fastidious*, *Porcine Liver*)

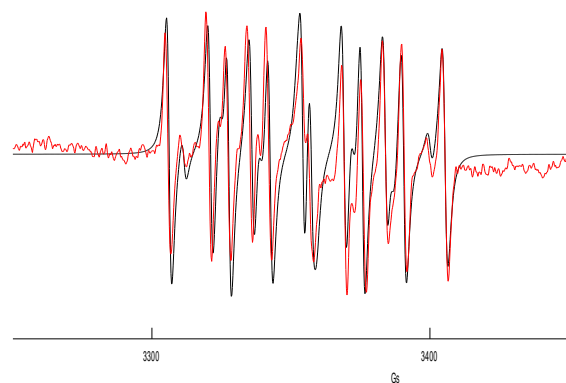
g (±0.0002)	A_N (±0.2G)	A_H (±0.2G)	A_P (±0.2 G)
2.0087	14.9	22.6	48.0

The reaction proceed through a radical intermediate. Which is the structure of the entrapped radical?

Caratterizzazione di specie paramagnetiche ESR di radicali – Spin trapping

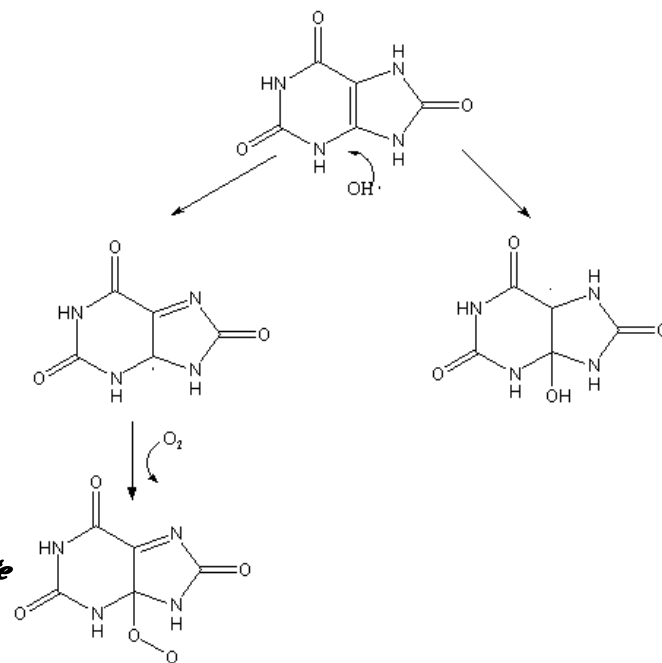
■ DEPMPO in the urate oxidation without uricase

The spin trap experiment was then performed in the absence of uricase generating the urate radical via a Fenton reaction (hydrogen peroxide and Fe(II)). Also in this case the EPR spectrum of DEPMPO radical adduct was detected

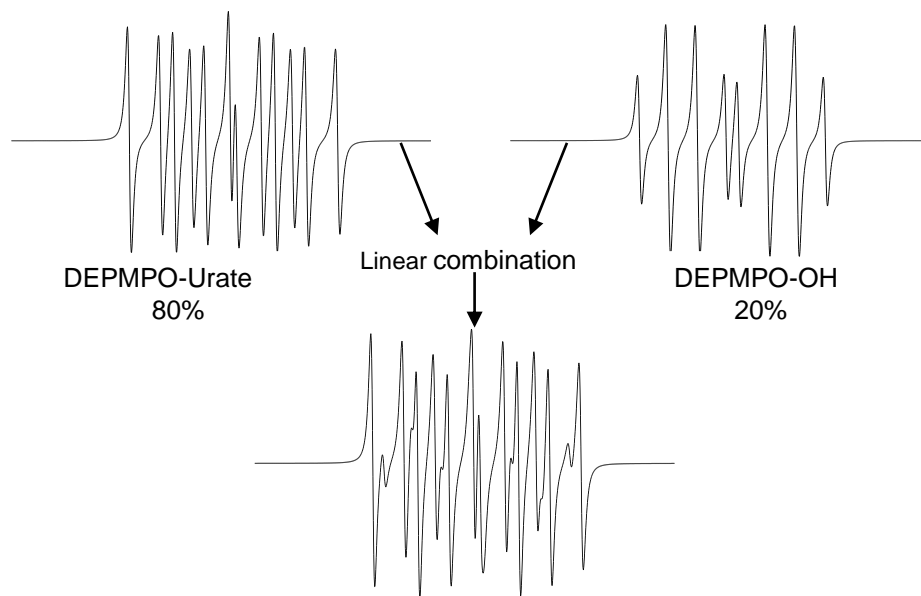
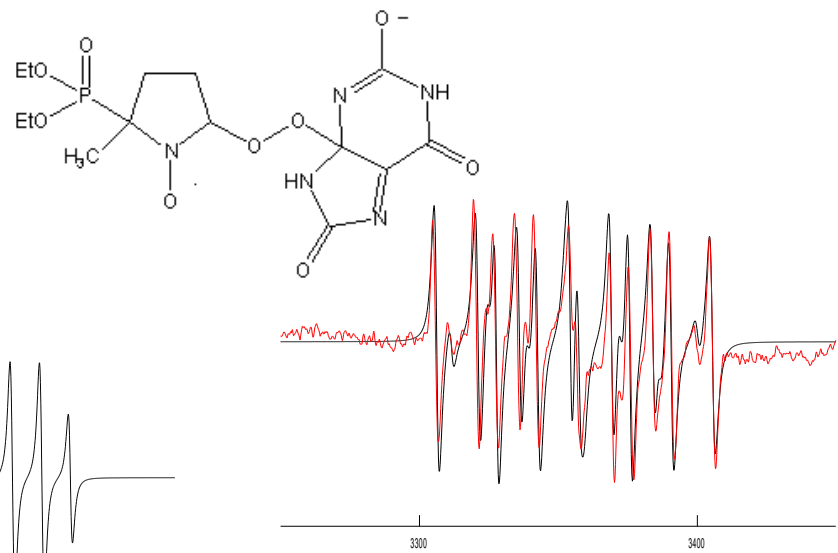


Hypothesized radical intermediate

Proposed mechanism for the generation of a urate radical by radiolysis Hicks, M., Wong, L.S., Day, R. O., (1993) *Free Rad. Res. Commun*, 18(6), 337-351



■ DEPMPO-urate adduct
without uricase



Simulated spectrum (see red line above)

This spectrum was simulated by the linear combination of two simulated spectra: 20% of the simulated spectrum obtained with the parameters of DEPMPO-hydroxyl radical adduct and 80% of the simulated spectrum obtained with the parameters of DEPMPO adduct found in the presence of uricase.

The structure of the DEPMPO radical adduct was definitively confirmed by DFT computations