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Radical trapping properties of imidazolyl nitrones

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Abstract

The ability of ten imidazolyl nitrones to directly scavenge free radicals ($R^{\cdot}$) generated in polar ($^{\cdot}OH$, $O_2^{\cdot-}$, $SO_3^{\cdot-}$, cysteinyl, $^{\cdot}CH_3$) or in apolar ($CH_3-{}^{\cdot}CH-CH_3$) media has been studied. When oxygen or sulfur-centered radicals are generated in polar media, EPR spectra are not or weakly observed with simple spectral features. Strong line intensities and more complicated spectra are observed with the isopropyl radical generated in an apolar medium. Intermediate results are obtained with $^{\cdot}CH_3$ generated in a polar medium. EPR demonstrates the ability of these nitrones to trap radicals to the nitrone $C_{(\alpha)}$ atom (alpha radical adduct) and to the imidazol $C_{(5)}$ atom (5-radical adduct). Beside the nucleophilic addition of the radical to the $C_{(\alpha)}$ atom, the EPR studies suggest a two-step mechanism for the overall reaction of $R^{\cdot}$ attacking the imidazol core. The two steps seem to occur very fast with the $^{\cdot}OH$ radical obtained in a polar medium and slower with the isopropyl radical prepared in benzene. In conclusion, imidazolyl nitrones present a high capacity to trap and stabilize carbon-centered radicals.

Keywords: *Imidazolyl nitrones, carbon- and hetero-centered radicals, spin traps, EPR*

Introduction

Based on the potentially promising therapeutic potential of compounds such as α-phenyl-*tert*-butyl-nitrone (PBN) [1–4] some of us have recently undertaken the design, synthesis and pharmacological evaluation of a nitrone series possessing a substituted imidazole moiety as the salient feature of their chemical structure [5,6] (Figure 1). These novel nitrone-based spin traps have been shown to possess neuroprotective properties in animal models, their side effects and toxicity being lower than those observed for the archetypal PBN [5–8]. Moreover, pharmacological properties could be modulated by the nature and position of substituents. The operational mechanism of these nitrones is still not clear and might reside in their capacity to scavenge free radicals or in the inhibition or degradation of enzymes and proteins, may be in their nitroxide forms (consecutive to radical trapping) [6]. The aim of the present work was to study the capacity of this novel nitrone family to directly scavenge free radicals *in vitro* comparatively to PBN. The spin trapping properties of ten imidazolyl nitrones (Figure 1) have been investigated towards oxygen, sulfur and carbon-centered free radicals in order to evaluate the effect of imidazolyl moiety comparatively to PBN.

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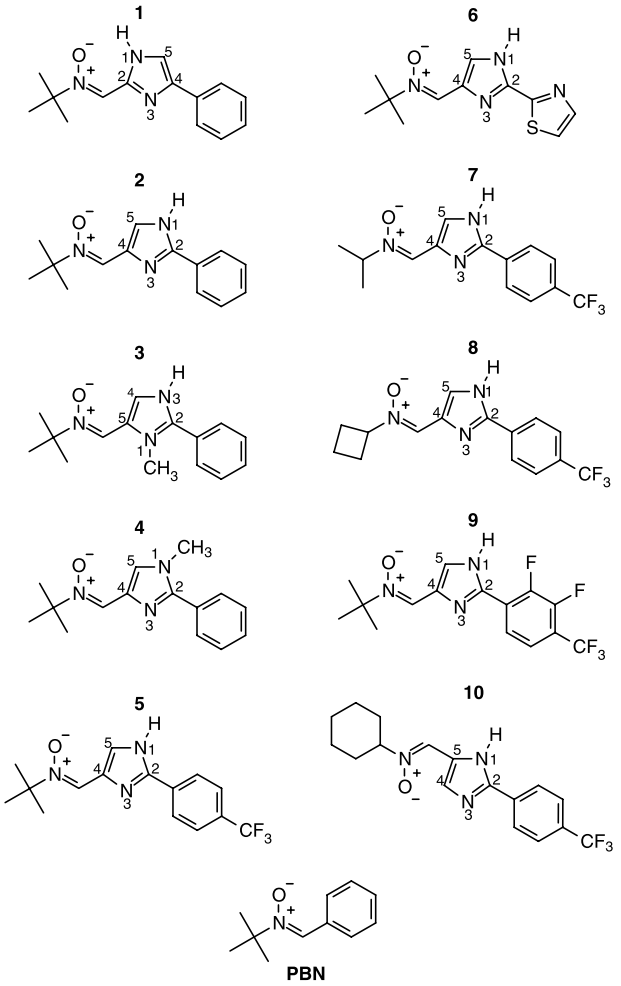


Figure 1. Structure of the imidazolyl nitrones: **1**, (*Z*)-α-[4-phenyl-1*H*-imidazol-2-yl] *N-tert*-butyl nitrone; **2**, (*Z*)-α-(2-phenyl-1*H*-imidazol-4-yl) *N-tert*-butyl nitrone; **3**, (*Z*)-α-[1-methyl-2-phenyl-1*H*-imidazol-5-yl] *N-tert*-butyl nitrone; **4**, (*Z*)-α-[3-methyl-2-phenyl-1*H*-imidazol-5-yl] *N-tert*-butyl nitrone; **5**, (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl] *N-tert*-butyl nitrone; **6**, (*Z*)-α-[2-(2-thiazolyl)-1*H*-imidazol-4-yl] *N-tert*-butyl nitrone; **7**, (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N*-isopropyl nitrone; **8**, (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N*-cyclobutyl nitrone; **9**, (*Z*)-α-[2-(2-fluoro-3-fluoro-4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N-tert*-butyl nitrone; **10**, (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N*-cyclohexyl nitrone.

Materials and methods

Materials

$Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, Na_2SO_3, $KHPO_4$, $NaHPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}H_2O$, benzene, eppendorfs, borosilicate glass micropipettes for EPR measurements were purchased from VWR International (Strasbourg, France). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), PBN and other chemicals were purchased from Sigma–Aldrich–Fluka Co (Saint Quentin Fallavier, France).

The imidazolyl nitrones were synthesized by the Chemistry Research Division of the Institut de Recherches Servier (Suresnes, France). The X-ray structural determination was carried out at the Department of Chemistry of the Facultés des Sciences, Notre-Dame de la Paix (Namur, Belgique) [9,10]. The nitrones studied were: **1**: (*Z*)-α-[4-phenyl-1*H*-imidazol-2-yl]-*N-tert*-butyl nitrone, **2**: (*Z*)-α-(2-phenyl-1*H*-imidazol-4-yl)-*N-tert*-butyl nitrone, **3**: (*Z*)-α-[1-methyl-2-phenyl-1*H*-imidazol-5-yl]-*N-tert*-butyl nitrone, **4**: (*Z*)-α-[1-methyl-2-phenyl-1*H*-imidazol-4-yl]-*N-tert*-butyl nitrone, **5**: (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N-tert*-butyl nitrone, **6**: (*Z*)-α-[2-(2-thiazolyl)-*1H*-imidazol-4-yl]-*N-tert*-butyl nitrone, **7**: (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N*-isopropyl nitrone, **8**: (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N*-cyclobutyl nitrone, **9**: (*Z*)-α-[2-(2,3-difluoro-4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N-tert*-butyl nitrone, **10**: (*Z*)-α-[2-(4-trifluoromethylphenyl)-1*H*-imidazol-4-yl]-*N*-cyclohexyl nitrone.

Methods

Solutions of imidazolyl nitrones which are poorly soluble in water were prepared in water/DMF mixture with a ratio (v/v) ranging from 90/10 (hydroxyl, cysteinyl, sulfite and methyl radicals) to 90/30 (superoxide generated from xanthine oxidase). DMF 10% in the medium gives rise to an intensity decrease of the DMPO–CH_3 adduct of about 30% whereas no effect is noted in the case of DMPO–OH. Despite the radical scavenging capability of DMF, a relative comparison of the spin trapping properties can be done in the series keeping the same water/DMF ratio in experiments devoted to one specific radical.

Generation of radicals

$OH^{\cdot}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100/100 μM) in H_2O/DMF: 90/10 (v/v), 10 mM spin trap. $O_2^{\cdot-}$ was obtained with a mixture of xanthine/xanthine oxidase (700 μM/0.8 units/ml) in HEPES buffer/DMF: 70/30 (v/v), 30 mM spin trap or with a mixture of KO_2/1,4,7,10,13,16-hexaoxacyclopentadecane (5×10^{-4} M) in DMSO in the presence of 3% water (v/v), 30 mM spin trap. Cysteinyl radical was obtained from a mixture of cysteine (20 mM) and $K_2Cr_2O_7$ (0.3 mM) in phosphate buffer, pH 7.0/DMF (9:1), 30 mM spin trap.

$SO_3^{\cdot-}$ was prepared using Fenton's reagents (Fe^{2+}/H_2O_2:100/100 μM) in a mixture of 70% of phosphate buffer (67 mM, pH = 7) (v/v), 10% DMF (v/v), 17% of an aqueous solution of Na_2SO_3 (270 mM) (v/v) and 10 mM spin trap.

$^{\cdot}CH_3$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100/100 μM) in H_2O/DMSO/DMF: 30/60/10 (v/v), 10 mM spin trap. Isopropyl radical was prepared in benzene from isobutyric acid and lead tetraacetate (45/45 mM) according to Colonna *et al.* [11]. Isobutyric acid (12 mg), $(AcO)_4Pb$ (59 mg) and

nitrone (30 mM) were mixed in 3 ml of benzene and homogeneized at room temperature for 5 min, then filtered on 0.22 μm filters and partially evaporated to 500 μl. The solution obtained was filtered again and immediately analyzed. *Caution:* Benzene is a health hazard and has to be manipulated with precaution. Benzene was selected rather than toluene which did not give reproducible results.

EPR experiments

EPR spectra were obtained at X-band at room temperature on a Bruker EMX-8/2.7 (9.86 GHz) equipped with a high-sensitivity cavity (4119/HS 0205) (Bruker, Wissembourg, France). A flat quartz cell FZK160-5 × 0.3 mm (Magnettech) was used to perform analysis. Processing of EPR data and spectrum computer simulation were performed using WINEPR and SIMFONIA software (Bruker). Typical scanning parameters were: scan rate, 0.6 G/s; scan number, 1; modulation amplitude, 1 G; modulation frequency, 100 kHz, microwave power, 20 mW; time constant, 40.96 ms. The diphenylpicrylhydrazil radical (DPPH) was used as reference for the determination of the *g* values. EPR signal intensities have been compared at the same gain. For reason of clearness, spectra on figures have been expanded on the *y*-axis when necessary.

Results

Structures confirmed by X-ray structural determination [9,10] and atom numbering of the imidazolyl nitrones studied are given in Figure 1. PBN was introduced in the study for comparison. DMPO was used as control to check solution preparation and instrumental settings. Spin trapping properties were investigated against oxygen-, sulfur- and carbon-centered radicals. Hyperfine splitting (HFS) constants for the different spin adducts are reported in Table I. The *g* values are in the range 2.0054–2.0058 with the superoxide anion, 2.0055–2.0059 with the sulfite radical, 2.0060–2.0062 with the methyl and isopropyl radicals.

Oxygen-atom-centered radical trapping

When introduced at 10 mM in an aqueous mixture containing Fenton's reagents (Fe^{2+}, H_2O_2) to produce the radical $^{\cdot}OH$, only nitrone **4** gave a strong EPR signal with two distinct triplets (Figure 2). Nitrone **6** gave a very weak signal while no signal was observed for nitrones **1**–**3**, **5**, **7**–**10**. In the same

Table I. Hyperfine splitting constants (Gauss) of EPR spectra of the imidazolyl nitrone and PBN spin adducts. (a) No signal, (b) given in literature [22], (c) difficult to interpret.

Nitrones	$^{\cdot}OH$	$O_2^{-\cdot}$	$SO_3^{-\cdot}$	$^{\cdot}CH_3$	$CH_3{\cdot}CHCH_3$
1	(a)	$a_N = 16.10$	(a)	(a)	$a_N = 13.22$ $a_N = 7.25$
2	(a)	$a_N = 15.00$	(a)	$a_N = 14.33$ $a_H = 2.00$	$a_N = 13.40$ $a_H = 3.60$ $a_H = 1.50$ $a_N = 7.40$
3	(a)	$a_N = 14.17$ $a_H = 2.70$	(a)	$a_N = 14.00$ $a_H = 3.59$	$a_N = 13.65$ $a_H = 2.99$ $a_N = 7.78$
4	$a_N = 17.17$ $a_N = 16.21$	$a_N = 15.00$ $a_H = 2.04$	(a)	$a_N = 14.50$ $a_H = 2.00$	$a_N = 13.65$ $a_N = 7.73$
5	(a)	(a)	(a)	$a_N = 14.17$ $a_H = 1.83$	(c)
6	$a_N = 16.40$	$a_N = 15.30$ $a_H = 1.45$ $a_H = 1.45$	(a)	$a_N = 14.20$ $a_H = 2.10$	$a_N = 13.33$ $a_N = 7.25$
7	(a)	(a)	(a)	$a_N = 14.25$ $a_H = 2.36$ $a_H = 2.53$	$a_N = 11.46$ $a_N = 7.24$ $a_H = 2.70$ $a_H = 1.00$
8	(a)	(a)	(a)	(a)	$a_N = 11.89$ $a_N = 7.36$ $a_H = 1.71$
9	(a)	(a)	(a)	$a_N = 14.25$ $a_H = 2.00$	(c)
10	(a)	(a)	(a)	$a_N = 14.33$	(c)
PBN	$a_N = 14.67$ $a_H = 2.94$	$a_N = 14.80$ $a_H = 2.75$ (b)	$a_N = 15.55$ $a_H = 3.35$	$a_N = 14.17$ $a_H = 2.39$	$a_N = 13.62$ $a_H = 1.92$

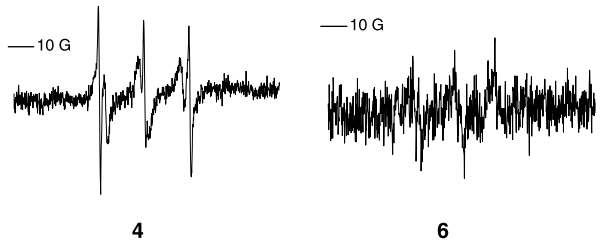


Figure 2. EPR spectra of **4** and **6** (10 mM) with $^{\cdot}OH$ generated by Fenton's reaction (Fe^{2+}/H_2O_2:100/100 μM) in H_2O. Gain: 5.64×10^5.

conditions, PBN gave no signal while the quartet signal was easily observed for the DMPO-OH adduct (data not shown).

When added in the X/XOD system at 30 mM, pH = 7.4, imidazolyl nitrones **1**–**4** and **6** gave a six-line spectrum (Figure 3) with intermediate or weak line intensities. PBN gave no signal in the same conditions. Similar results were obtained when the radical $O_2^{\cdot-}$ was produced from KO_2.

The sodium sulfite oxidation reaction was used to generate the $SO_3^{\cdot-}$ radical. None of the ten imidazolyl nitrones gave an EPR-detectable spin adduct with the sulfite radical. In the same conditions, PBN and DMPO gave a six-line spectrum with a high intensity (data not shown).

The cysteinyl radical was generated by mixing cysteine with $K_2Cr_2O_7$. Adding imidazolyl nitrones to this mixture (10 mM), no EPR signals were observed. In the same conditions, PBN gave no EPR signal while a six-line spectrum was observed with DMPO (data not shown).

Carbon-centered radical trapping

The methyl radical was generated by mixing Fenton's reagents in a solvent mixture containing H_2O/DMSO/DMF (30/60/10). When each of the ten nitrones was added at 10 mM, a methyl spin adduct was observed in each case, with a six-line spectrum clearly observed for nitrones **2**–**4**, **6** and **9** while a nine-line spectrum was observed for nitrone **7**. Weaker intensities were observed for nitrones **1**, **5**, **8** and **10**. In the same conditions PBN gave a six-line spectrum (Figure 4).

The isopropyl radical was synthesized from butyric acid and $Pb(CH_3COO)_4$ in benzene using the protocol

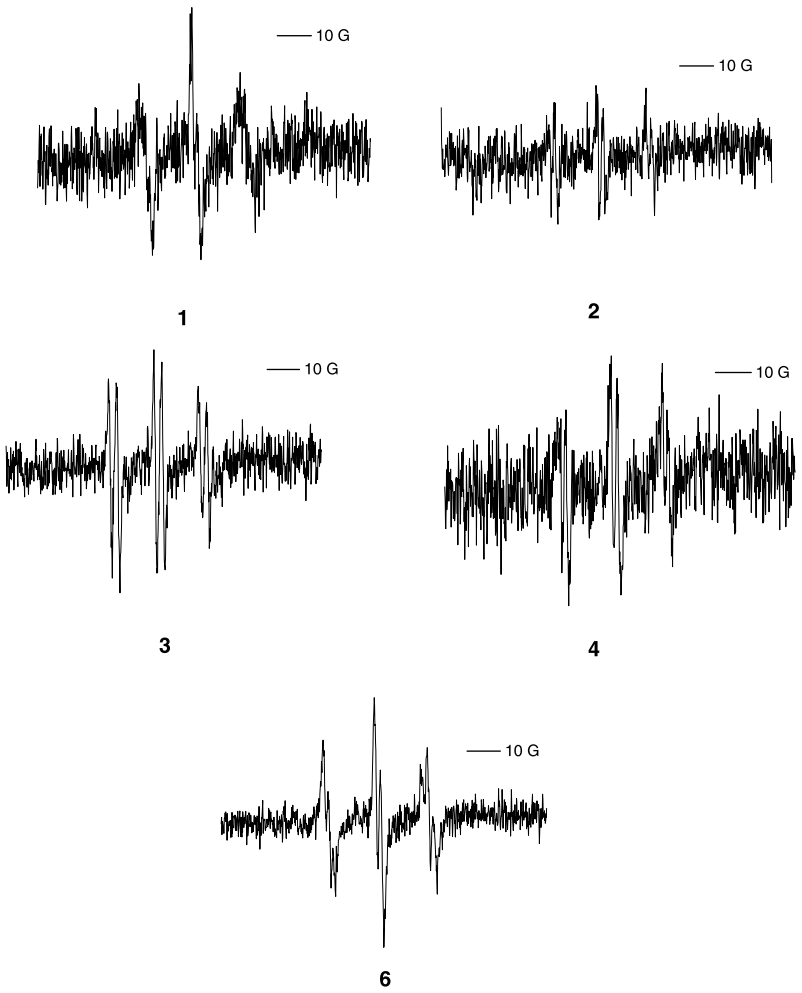


Figure 3. EPR spectra of **1**–**4**, **6** and DMPO (30 mM) with $O_2^{\cdot-}$ generated by the xanthine/xanthine oxidase system. Gain: 5.64×10^4.

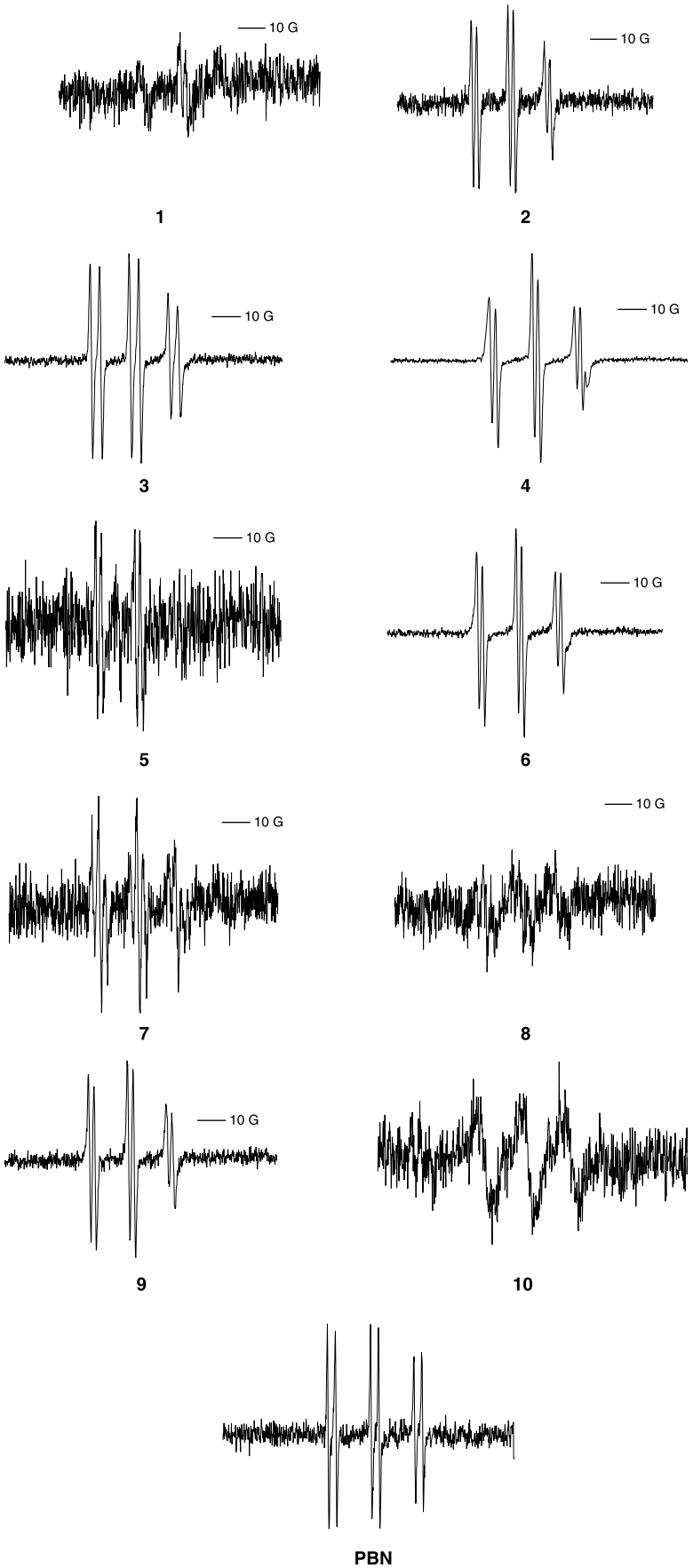
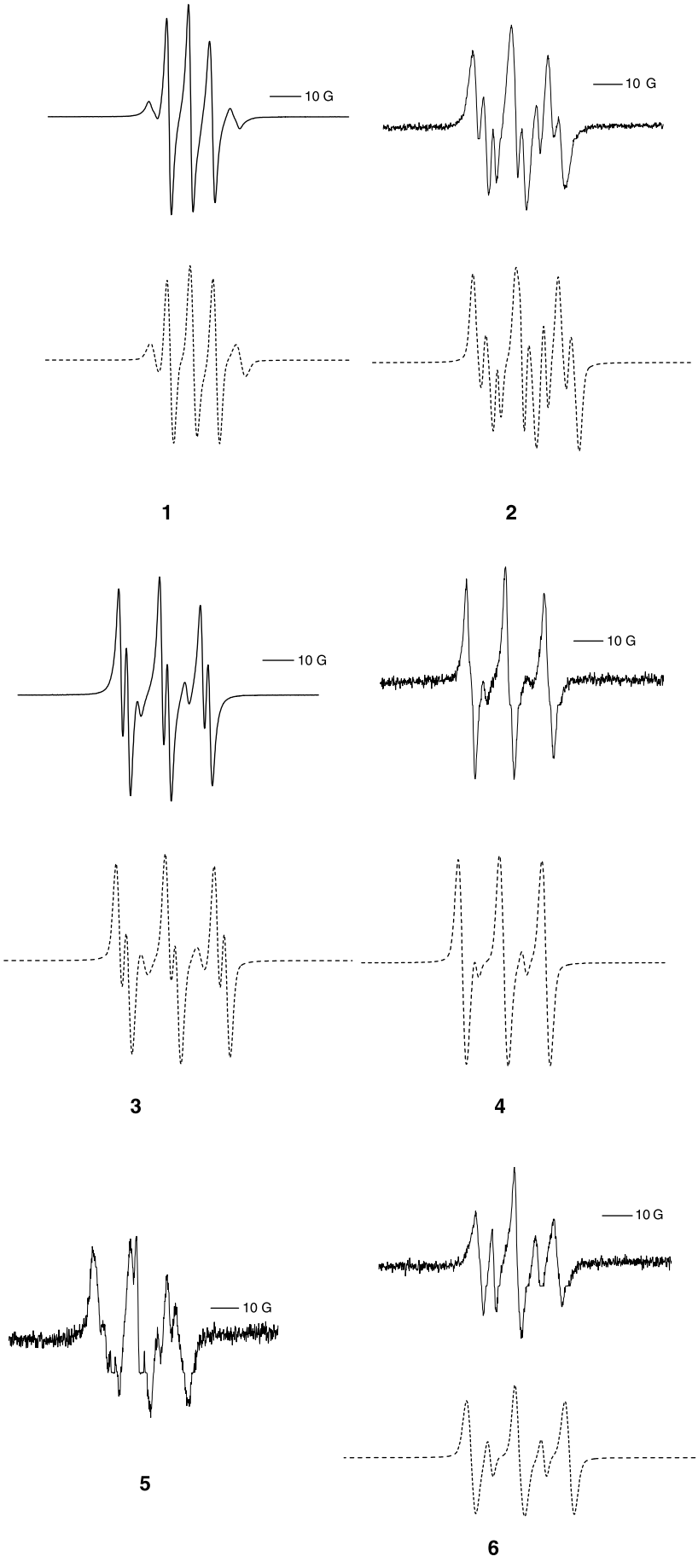


Figure 4. EPR spectra of nitrones **1**–**10** and PBN (10 mM) with $^{\cdot}CH_3$ generated by Fenton's reaction (Fe^{2+}/H_2O_2:100/100 μM) in H_2O/DMSO/DMF: 30/60/10 (v/v). Gain: 5.64×10^4.



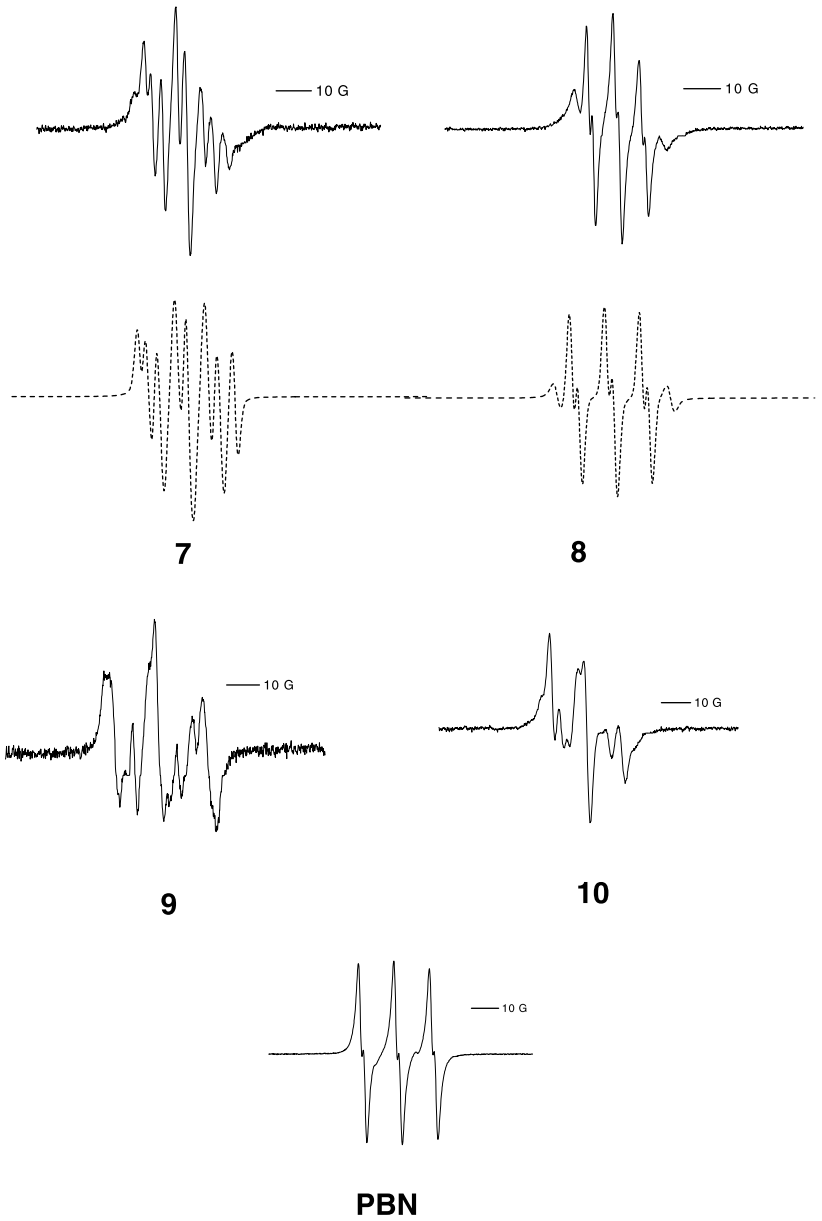


Figure 5. EPR spectra of nitrones **1**–**10** and PBN (10 mM) with $CH_3-\dot{C}H-CH_3$ prepared in benzene from isobutyric acid and lead tetraacetate (45/45 mM). Gain: 5.64×10^3. Solid lines: experimental spectra; dashed lines: simulated spectra.

described by Colonna *et al.* [11]. Each of the ten nitrones tested gave a spin adduct with the isopropyl radical (Figure 5). The EPR spectra exhibit three to nine-lines with high intensity with the simplest feature observed for nitrones **3** and **4**. Adducts from nitrones **1**, **3**, **4** and **6** present symmetrical patterns for which simulated EPR spectra are also given (Figure 5), while nitrone **2**, **5**, **7**–**10** gave unsymmetrical spectra. In the same conditions, PBN presented a six-line symmetrical spectrum.

Discussion

Comparing EPR signal intensities, it immediately appears that imidazolyl nitrones present a strong capacity to trap and give stable spin adducts with carbon-centered radicals (Figures 4 and 5) (methyl and isopropyl radicals) in our experimental conditions, compared to signals obtained with hetero-atom-centered radicals (Figures 2 and 3). Nitrone **4** is the only one to give a relatively intense signal with the $^{\cdot}OH$ radical while other nitrones did not give detectable spin adducts or a very weak one with nitrone **6** (Figure 2), remembering that EPR spectra were recorded about 3 min after adding Fenton reagents in the medium. In the same conditions, PBN gave no EPR detectable signal. Half nitrones of the series gave detectable spin adducts with the superoxide anion, intensities being weak, while PBN gave no signal in the same conditions. Indeed, PBN has poor trapping properties towards hydroxyl and superoxide radicals. At pH = 7.4, the

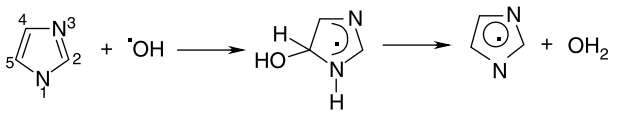
half-life of PBN–OH adduct is 38 s [12,13] while it is even less for the superoxide spin adduct PBN–OOH [14]. No signal was detected when nitrones were reacted with the sulfite or the cysteinyl radical where PBN gave no signal, or a strong signal, respectively. Each of the ten nitrones gives detectable spin adducts with the methyl radical, with variable intensities, while very strong signals are observed in each case when the isopropyl radical is trapped. At this point, we must remember that the methyl radical is produced in a polar medium (DMSO) while the isopropyl radical is prepared in an apolar solvent (benzene). PBN also gave strong EPR signals with both carbon-centered radicals, methyl and isopropyl.

Firstly, we observe the strong capacity of the ten nitrones to trap the isopropyl radical to give EPR detectable spin adducts in an apolar medium. We also note the distinctive feature of nitrone **4** being the only one to give an EPR detectable signal with the highly reactive ˙OH radical. At this point it is interesting to underline that nitrone **4** is the only one in the series to have a methyl group on the imidazol *N-1* atom next to an unsubstituted *C-5* atom. Nitrone **3** which is structurally close to nitrone **4** has an unsubstituted *C-4* atom next to an imidazol atom *(N-3)* bearing no methyl group.

In a second step, comparing the spectral features, simple patterns — triplets or split triplets — are observed with the hydroxyl, superoxide and methyl radicals when they are observed, as for PBN. In contrast, spectral patterns of the isopropyl adducts present broader lines whose assignment needs a simulation procedure while PBN gives a simple six-line spectrum with the isopropyl radical (Figure 5). Nitrones **3** and **4** present a particular behavior giving the simplest spectra with this isopropyl radical.

To explain the differences in spectral features and intensities of these spin adducts, we have to consider the particular chemical structure of this imidazolyl nitrone series. These molecules may trap a radical by nucleophilic addition at the $C_{(\alpha)}$ atom. Supplementary to that, reactions of radical species with heterocyclic compounds are strongly favored [15,16]. In this way, experimental evidence has demonstrated that the ˙OH radical reacts with pyrroles and imidazoles by addition at a carbon adjacent to the nitrogen (*N-1*, *N-3*) [17–19]. The reaction of imidazole with ˙OH could yield three possible adducts, i.e. the 2-, 4-, and 5-hydroxyimidazolyl radicals (Scheme 1). Moreover, both experimental and EPR measurements as well as theoretical studies have shown that in neutral and alkaline (pH = 9–10) aqueous solution, ˙OH specifically adds to the 5-position of imidazole (Scheme 1) [18, 20] and that the 5-hydroxyimidazolyl radical undergoes elimination of water to yield the 1-dehydroimidazolyl radical [21].

Then, considering the chemical structure of these imidazolyl nitrones, there are two possible nucleophilic

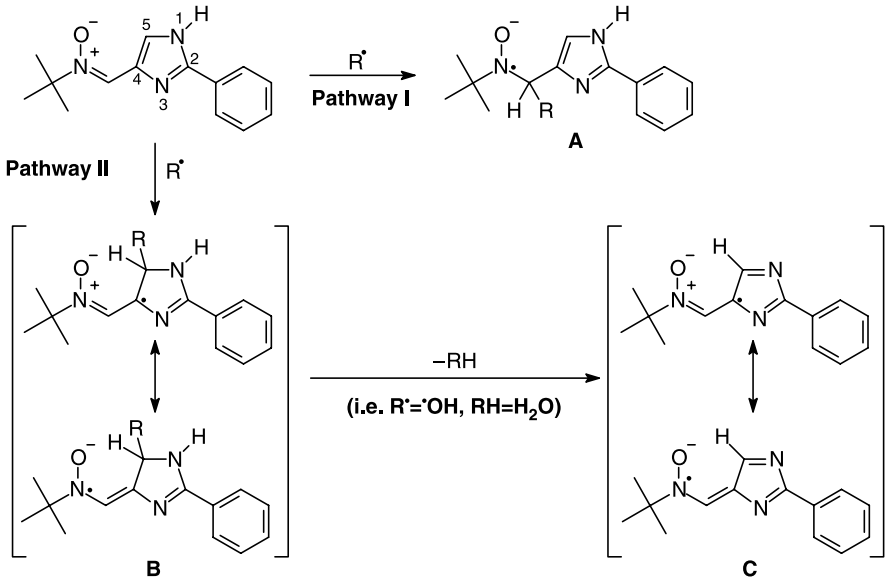


Scheme 1. ˙OH attack on the imidazole core and subsequent dehydration to yield the 1-dehydroimidazolyl radical.

attack sites of the radical, the $C_{(\alpha)}$ atom at the nitrone group and the *C-5* atom of the imidazole group (or at *C-4* atom for nitrones **3** and **10**). The two other sites (*C-2*, *C-4*) cannot be attacked because of the substitution of the core in the series. If we take into account these two possibilities, we can propose Scheme 2 for the radical trapping.

From the two pathways (I and II) proposed in Scheme 2, three possible spin adducts (**A**–**C**) result with three different chemical environments for the free electron. If pathway I was the only one possible, spectral features of the spin adducts obtained with the different radicals generated would be comparable to the ones of PBN, which is not the case in this series. Pathway II gives rise to two different spin adducts **B** and **C**, which could coexist in solution with life times depending on charge, size and steric effects of the trapped radical, structural feature of the imidazolyl nitrone hydrogen bonding, but also on the polarity of the medium. Then, with radicals such as ˙OH, the attack reaction at the *C-5* imidazole carbon atom followed by a water elimination would be kinetically highly favored, particularly in a polar medium, while with bulky radicals such as isopropyl in an apolar solvent, β-elimination of RH should be less favored. Another hypothesis is to have a concomitant radical attack on the $C_{(\alpha)}$-atom and *C-5* atom on the same molecule which could give a transient bi-radical adduct.

If we now examine the whole set of results, we see that nitrone **4** is the only one to give a detectable spin adduct with the very reactive ˙OH radical. Its EPR spectrum exhibits two independent triplets (Figure 1 and Table I). Nitrone **4** is the only one to dispose of a methyl group on the *N-1* imidazole atom next to the unsubstituted *C-5* atom. Then the ˙OH attack and consecutively the possibility to observe the OH spin adduct through pathway II are promoted: (i) the methyl group on the *N-1* imidazolyl atom enhances the electron spin density on *C-5* favoring the radical attack, (ii) the methyl group at the *N-1* atom prevents water elimination (Scheme 1) and the consecutive spin adduct **C** formation. Consequently the intermediate spin adduct **B** is detectable. Nitrone **3** is structurally very close to **4** but the H atom on *N-3* allows the elimination step of a water molecule to reach the **C** type spin adduct. These observations with nitrone **4** and the ˙OH radical confirm the possibility of pathway II with these imidazolyl nitrones. It also shows that the transformation of **B** into **C** by water elimination is very fast leading to transient spin adducts with the ˙OH radical which cannot be



Scheme 2. Radical attack on imidazolyl-nitrones: Possible pathways and mechanisms.

observed on the time scale of these EPR experiments for these nitrones (except for nitrone **4**).

Considering the case where RH elimination is less favored, i.e. a bulky radical like the isopropyl radical, and an apolar medium, benzene as solvent, we observe more complicated spectra which are poorly resolved. Firstly examining the EPR spectrum of the PBN-isopropyl spin adduct, the triplet is clearly defined even though the lines are broader and H-coupling less resolved than in polar medium. This shows that generating isopropyl radical in benzene with isobutyric acid and lead acetate gives rise to less well-resolved EPR spectra. However, compared to PBN-isopropyl adduct spectrum, observation of the imidazolyl nitrone spectra strongly suggests the superimposition of at least two spin adduct spectra.

The question rises now to know which pathway is favored depending on the radical generated in the solution and how many different spin adducts are present in the EPR spectra. In this aim, experimental spectra were simulated in order to get the EPR spectral data reported in Table I [22].

The structural exception of nitrone **4** confirms the possibility of pathway II when the imidazolyl nitrones are attacked by the radical $^{\bullet}OH$. Pathway I may exist but we know that when hydroxyl radicals add to the double bond of PBN, the resulting spin adduct has a half-life of less than 1 min at pH 7.0 [12] and decomposes to release benzaldehyde and *tert*-butyl hydroxylamine. Such instability may be also observed with these imidazolyl nitrones since in both pathways (I and II) the $C_{(\alpha)}$ and the imidazole–hydroxyl spin adducts cannot be observed, except for nitrone **4**.

With the superoxide radical, EPR spectra are poorly resolved indicating a very short life time of the spin adducts which is approximately less than 1 min and comparable to those of PBN. The trifluoromethyl substituent was introduced (nitrones **5**, **7**–**10**) for pharmacological reasons [5–8] and not for spin trapping considerations.

The methyl adducts of the imidazolyl nitrones present spectral data comparable to those of the methyl–PBN adduct indicating an attack of the methyl radical on the *C-α* atom at the nitrone group. This observation is confirmed by the nine-line spectrum observed with nitrone **7** which has an isopropyl group on the nitrone function leading to three coupling constants ($a_N = 14.17$, $a_{H\alpha} = 2.53$, $a_{HCH} = 2.53$). Nitrones **3** and **6** exhibit a small shoulder on the right part of the spectra suggesting traces of secondary adducts. Then, if the methyl radical attacks on the *C-5* atom, spin adducts should have a very short life time in the polar medium used to generate the methyl radical.

Isopropyl adduct spectra present broader lines than those obtained in polar media with other radicals. This may be explained not only by the experimental conditions used to generate the isopropyl radical but also by the superimposition of the spectra of two to three spin adducts in agreement with the two possible pathways I and II. This is confirmed by the spectral simulation which needs to introduce at least two a_N coupling constants (13.3 and 7.5 gauss). Spin adducts **A** and **B** should give higher a_N coupling constants while **C** should give a smaller a_N constant. The EPR spectrum with nitrones **4** for which spin adduct **C** is impossible, is simple and easy to simulate with two a_N coupling constants. Simulation with two a_N constants also gives good results for nitrones **1**, **3**, **6**–**8**. No simulation is possible for nitrones **5**, **9** and **10** with the software used. It is interesting to note that the most complex spectra are obtained with the fluoro-phenyl

functionalised imidazolyl nitrones. The most complex spectra may result from (i) the superimposition of three types of adducts (**A**–**C**), (ii) secondary radicals from decomposition reactions and even (iii) bi-radical adducts which could be favoured in these apolar experimental conditions. Altogether, these results confirm that more than one pathway is involved when imidazolyl nitrones trap the isopropyl radical in an apolar medium.

In conclusion, these studies have shown that imidazolyl nitrones present a high capacity to trap and stabilize carbon-centered radicals which could explain some of their biological properties. We chose to generate the isopropyl radical in benzene in order to bring out the key role of the imidazolyl core in the trapping properties of these nitrones. Further studies, such as theoretical calculations and complementary EPR spectrum simulations are in progress to explain the strong differences observed in the trapping of oxygen-centered or carbon-centered radicals by this imidazolyl nitrone series.

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