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Characteristics and use as Spin Trapping Agent of a β-Phosphorylated Nitroso Compound, DEPNP

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2-(Diethylphosphonate)-nitrosopropane (DEPNP), prepared by oxidation of the corresponding aminophosphonate, was found to essentially exist as monomer in both water and organic solvents. The mechanisms of its degradation under 80°C heating or visible light exposure were studied by EPR spectroscopy: its decomposition gave rise to paramagnetic by-products, which have been identified as DEPNP/ $\cdot C(CH_3)_2[P(O)(OC_2H_5)_2]$ and DEPNP/ $\cdot P(O)(OC_2H_5)_2$ spin adducts. Despite this drawback, DEPNP was successfully used as spin trapping agents to scavenge various carbon – and phosphorus-centred free radicals both in aqueous and organic media, giving rise to intense EPR spectra characteristic of the species trapped.

Keywords: nitroso compound, free radical, spin trapping, spin adduct, aminoxyl radical

INTRODUCTION

The use of nitrones and nitroso compounds in free radical detection became increasingly important, and the merits and drawbacks of these two kinds of spin traps have been reviewed many times[1–3]. Nitroso compounds have an important advantage over nitrones for the addend identification in that the trapped radical is directly bound to the nitrogen, thereby yielding additional hyperfine splittings. However, they are known to be both thermally and photochemically labile, and the life time of the adducts obtained with many heteroatom-centred radicals was found to be exceedingly short. They are also poorly water soluble and have the tendency to form dimers inert in spin trapping experiments.

By far, one of the most popular nitroso compound has been the 2-methyl-2-nitrosopropane (MNP), which has been employed many times to trap carbon-centred free radicals[4–10], but which presents all the drawbacks listed above such as a limited water solubility[11] and the existence of a solid dimer form, which decomposes only slowly to achieve a dimer-monomer equilibrium[12]. Furthermore, the observation in our laboratory that the introduction of a diethoxyphosphoryl group in various nitrones generally enhanced their spin trap performances[13–20] led us to prepare a β-phosphorylated nitroso compound analogue of MNP, the 2-diethylphosphonate-2-nitrosopropane DEPNP. Although this

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compound has been synthesised for the first time in the seventies[21], its physicochemical characteristics, and more particularly its spin trap capacities, have never been fully investigated. Moreover, in previous studies of spin trapping with PBN-type β-phosphorylated nitrones, aminoxyl by -products where detected besides the nitrone spin adducts[20,22]. Since we thus made the hypothesis that they could correspond to free radical adducts of DEPNP, we would like now to verify this. These are the main reasons why we undertook a new study of DEPNP and present in this paper some chemical characteristics of this compound and the first spin trapping results obtained with it.

MATERIALS AND METHODS

General

All chemicals and solvents were purchased from either Sigma or Aldrich Chemical Companies. The solvents were of the highest grade of purity commercially available and twice distilled before use.

DEPNP Synthesis

The 2-(diethylphosphonate) aminopropane **1** was synthesised, purified and identified by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy as described previously[23]. The nitroso compound DEPNP **2** was prepared according to the method of Levin *et al.*[21] modified as follows. A solution of 22.5 g (0.12 mol.) of aminophosphonate **1** in 585 mL pentane was vigorously stirred with a solution of 0.93 g (2.8×10^{-3} mol.) of sodium tungstate dihydrate and 3.75 g (7×10^{-2} mol.) of ammonium chloride in 20 mL water. Addition of a small amount of NH_4Cl made the substrate solubilisation easier. This was followed by the slow addition of 34 mL of H_2O_2 (30%) at a temperature kept below 30°C. Comparing to the method of Levin *et al.*, we have notably divided by two the oxidant concentration to limit the oxidation of the nitroso into the corresponding nitro compound. After stirring the mixture during 4 h, the organic phase was washed with aqueous HCl (10%), dried with $MgSO_4$, and the solvent was evaporated. The DEPNP **2** was obtained as a bright blue liquid (yield 90%) and purified by distillation (Bp 48°C under 0.05 mm Hg) and by column chromatography on silica gel 60 (particle size 0.063–0.200 nm) and using diethylether as elution solvent. During this purification process, the DEPNP was protected as much as possible from ambiant light exposure. This compound was then fully characterised on the basis of its ^{1}H, ^{13}C and ^{31}P NMR spectra, recorded on a Bruker AC 100 spectrometer. The following NMR parameters have been obtained. δ_H (ppm, $CDCl_3$, 100.13 MHz): 4.24 (4H, dq, $J_H = J_P = 7.1$ Hz), 1.55 (6H, d, $J_P = 16.4$ Hz), 1.41 (6H, t, $J_H = 7.0$ Hz). δ_C(ppm, $CDCl_3$, 50.32 MHz): 105.1 (d, $J_P = 145$ Hz), 63.1 (d, $J_P = 7.6$ Hz), 17.9 (d, $J_P = 3.5$ Hz), 16.4 (d, $J_P = 6.4$ Hz). δ_P(ppm, $CDCl_3$, 40.3 MHz): 18.43. Its UV spectrum was also recorded in heptane and in water using a computer controlled UNICAM UV/visible UV4 spectrometer.

K_p Determination

The DEPNP octanol-phosphate buffer partition coefficient K_p was evaluated using a method based on HPLC[19] as follows. A solution of DEPNP was prepared in 0.1 mol.L^{-1} phosphate buffer at a concentration of 0.25 mmol.L^{-1} and oxygen was removed by bubbling argon. Equal volumes (2 mL) of freshly prepared aqueous solution of DEPNP and of n–octanol were thus mixed and vigorously stirred at 37°C during 1 h and afterwards the two phases were separated by a brief centrifugation (1000 g for 20s). A 1 mL sample of each one of the two phases was taken and mixed with 1 mL of a 0.25 mmol.L^{-1} methanolic solution of anthracene used as internal reference. The DEPNP concentration in both

aqueous and octanolic phases was then determined by HPLC, by using a Waters model 600E multisolvent delivery system, equipped with a Waters 2487 UV detector, a Waters Millenium integrator software and a Kromasil 5μm C8 column (25 cm length, 4.6 mm id). HPLC column conditions were as follows: flow rate, 1 mL.min.$^{-1}$; injection volume, 20 μL; elution solvent: 70% methanol, 30% water, degassed by helium bubbling. K_p was then evaluated as the ratio of DEPNP concentration in n-octanol to that in phosphate buffer.

Reduction of DEPNP by $NaBH_4$

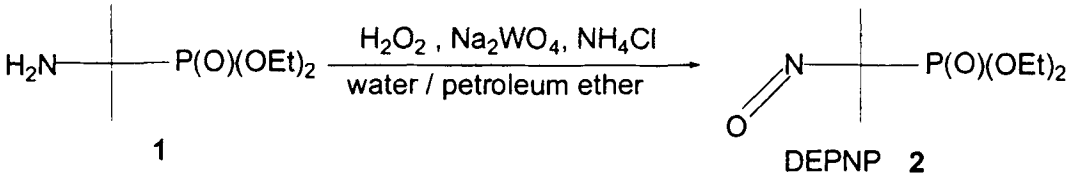
An aqueous solution containing 2 mol.L^{-1} DEPNP and 0.2 mol.L^{-1} of $NaBH_4$ was prepared and vigorously stirred during a few minutes. Autoxidation of the hydroxylamine thus obtained led to the corresponding aminoxyl radical, which can be considered as a « pseudo-DEPNP/ ·H adduct ». When the same reaction was conducted in D_2O, the corresponding « pseudo- DEPNP/ · D adduct » was obtained.

Spin Trapping

In all spin trapping experiments, the DEPNP concentration was 0.1 mol.L^{-1}. In aqueous media, CH_3, $\cdot CH_2OH$, CH_2CH_2OH, $\cdot CH(CH_3)OC_2H_5$ and $\cdot CH_2C(O)CH_3$ were generated in the presence of **2** by a standard Fenton system [0.2% H_2O_2, 2 mmol.L^{-1} ethylenediamine-tetracetic acid (EDTA), and 1 mmol.L^{-1} $FeSO_4$] in the presence of 10% in volume of the appropriate ·OH radical scavenger, *i.e.* dimethylsulfoxide (DMSO), methanol, ethanol, diethylether and acetone, respectively, to yield the corresponding spin adduct. Phenyl and isopropyl radical spin adducts have been obtained in benzene by photolysis of a 1.5 mol.L^{-1}of either phenyl- or isopropyl-iodide in the presence of DEPNP, using a xenon discharge lamp (250W) giving near UV and visible radiations. The spin adduct of the 2 – (diethylphosphonate)-isopropyl radical ($\cdot C(CH_3)_2[P(O)(OC_2H_5)_2]$) was obtained either by heating to 80°C during 3 min. or irradiating with light, using a quartz-iodine 50W lamp equipped with a glass lens, during 10 min. a deoxygenated 0.1 mol.L^{-1} DEPNP solution in toluene, chloroform or water. The spin adduct of the diethoxyphosphoryl radical ($\cdot P(O)(OC_2H_5)_2$) was generated either by heating or irradiating in the same conditions an oxygenated solution containing 0.1 mol.L^{-1} DEPNP in the same solvents. This later aminoxyl was also produced by heating to 60°C a 2 mol.L^{-1} DEPNP solution in diethylphosphite. When both HO· radical and superoxide were produced by a standard Fenton system (see above for the experimental conditions) or by a xanthine (0.4×10^{-3} mol.L^{-1}) – xanthine oxidase (0.4 units. L^{-1}) system, respectively, directly in the presence of DEPNP, no spin adduct was ever observed. In the same manner, when the $SO_3^{-}\cdot$ radical anion was generated in aqueous medium in the presence of DEPNP using a standard Fenton system in the presence of Na_2SO_3 (0.2 mol.L^{-1}), the formation of the corresponding spin adduct was not detected by EPR spectroscopy.

EPR Measurement

EPR assays were carried out at room temperature in EPR tubes by using a computer – controlled Bruker EMX spectrometer operating at X-band with 100 kHz modulation frequency, and equipped with an NMR gaussmeter for magnetic field calibration. The instrument settings were as follows: non-saturating microwave power, 10 mW; modulation amplitude ranging from 0.05 to 0.1 mT; scan time, 180 s; time constant, 0.128 s; receiver gain ranging from 1.2×10^4 to 6.3×10^4. For the various aminoxyl radicals, the hyperfine splitting constants (hfsc) were evaluated by least -squares fitting of the digitised experimental spectra to computer-simulated spectra using the WinSim program elaborated by Duling[24].



SCHEME 1 synthesis of the DEPNP **2** by oxidation of the aminophosphonate **1**

RESULTS

DEPNP Synthesis

Following the method described by Levin *et al.*[21] modified as indicated in the experimental section, the DEPNP **2** was obtained by oxidising the α-aminophosphonate **1** with H_2O_2 in water/petroleum ether biphasic medium, in the presence of ammonium chloride and of a catalytic amount of sodium tungstate, as described in scheme 1. Using only two equivalent of H_2O_2 for one equivalent of **1** permitted to reduce the amount of nitro compound to 4%. The DEPNP **2** was thus obtained in 90 % yield, but always contained an unidentified paramagnetic impurity, which showed a three line EPR signal (a_N= 1.45 mT and a_{13C} = 0.64 mT in toluene). Distillation of **2** under reduced pressure and in absence of light did not permit to eliminate this impurity, but its quantity was largely reduced by purifying the DEPNP by column chromatography. Thus, its EPR signal intensity was low enough to permit the use of DEPNP in spin trapping experiments.

DEPNP Characteristics

The DEPNP UV-visible spectrum has been recorded in heptane and water. The spectroscopic data thus obtained (wavelength, molecular absorption coefficients and type of electronic transition), listed in table I, clearly show that, contrarily to its non-phosphorylated analogue MNP, DEPNP exists essentially as a monomer, even in aqueous medium, since no band was detected for the dimer form of nitroso compounds. In addition, no dimer was ever detected by NMR spectroscopy, as can be seen in the data given in the experimental section.

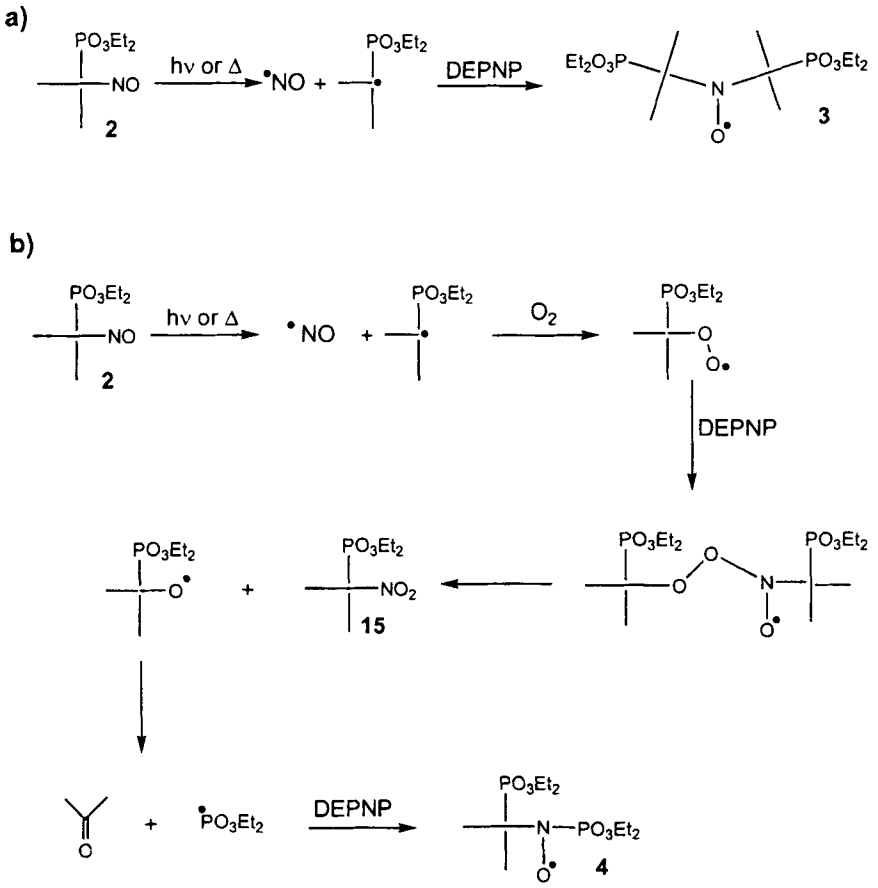
The DEPNP lipophilicity was thus evaluated by the determination of its octanol – phosphate buffer partition coefficient K_P, as described in the experimental section. Because of the presence of impurities in octanolic phase, which may react with DEPNP and distort the results, five experiments were made, which led to the mean value K_P = 7.3 for DEPNP, that is to say lower than for MNP, for which K_P has been evaluated *c.a.* 32[11]. In addition, solubilisation of DEPNP at 0.1 mol.L^{-1} led to bright blue homogenous solutions in both water and organic solvents, which indicated that this compound was soluble enough to be used as spin trap in every kind of media.

TABLE I Wavelength of absorption bands (λ), molecular extinction coefficients (ε_0) and type of electronic transition observed by UV-visible spectroscopy of a 10^{-2} mol.L^{-1} DEPNP solution in water and in heptane

Solvent	λ *(nm)*	ε_0 *(L.mol^{-1}.cm^{-1})*	*transition*
water[a]	215	2800	$\pi \rightarrow \pi^*$
	668	17	$n_N \rightarrow \pi^*$
heptane	222	1700	$\pi \rightarrow \pi^*$
	280	40	$n_0 \rightarrow \pi^*$
	690	20	$n_N \rightarrow \pi^*$

a. The $n_0 \rightarrow \pi^*$ transition was not detected in water, probably because the corresponding absorption band is too close to the large band observed at 215 nm.

Since nitroso compounds have been reported to be heat and light sensitive, it was of crucial importance to examine the DEPNP stability under heating or visible light exposure. Experiments were made in three solvents, water, chloroform and toluene, and the DEPNP decomposition gave rise to two different EPR observable paramagnetic species, depending on the oxygen concentration. When argon was bubbled into the medium to remove molecular oxygen prior to heating or radiation, the formation of the aminoxyl **3**, corresponding to the trapping of the carbon-centred radical $\cdot C(CH_3)_2[P(O)(OC_2H_5)_2]$ by DEPNP and showing the nine line EPR signal given in figure 1a, was observed. When the solutions were heated or irradiated in the presence of oxygen, the aminoxyl radical **4** was observed, corresponding to the trapping of the phosphorus-centred radical $\cdot P(O)(OC_2H_5)_2$ and having a twelve line EPR spectrum (see figure 1b). Note that Mukhtarov *et al.*[25] have previously obtained **3** by heating a DEPNP solution up to 100°C and **4** by heating DEPNP in diethylphosphite. **3** and **4** were noted DEPNP/ $\cdot C(CH_3)_2[P(O)(OC_2H_5)_2]$ and DEPNP/ $\cdot P(O)(OC_2H_5)_2$, respectively, and their EPR parameters in various solvents have been listed in table II. The mechanisms responsible for their formation, which will be discussed later in this text, are given in scheme 2.



SCHEME 2 mechanism of formation of aminoxyl radicals **3** and **4** obtained by heating or irradiating DEPNP a) in deoxygenated solution and b) in the presence of molecular oxygen

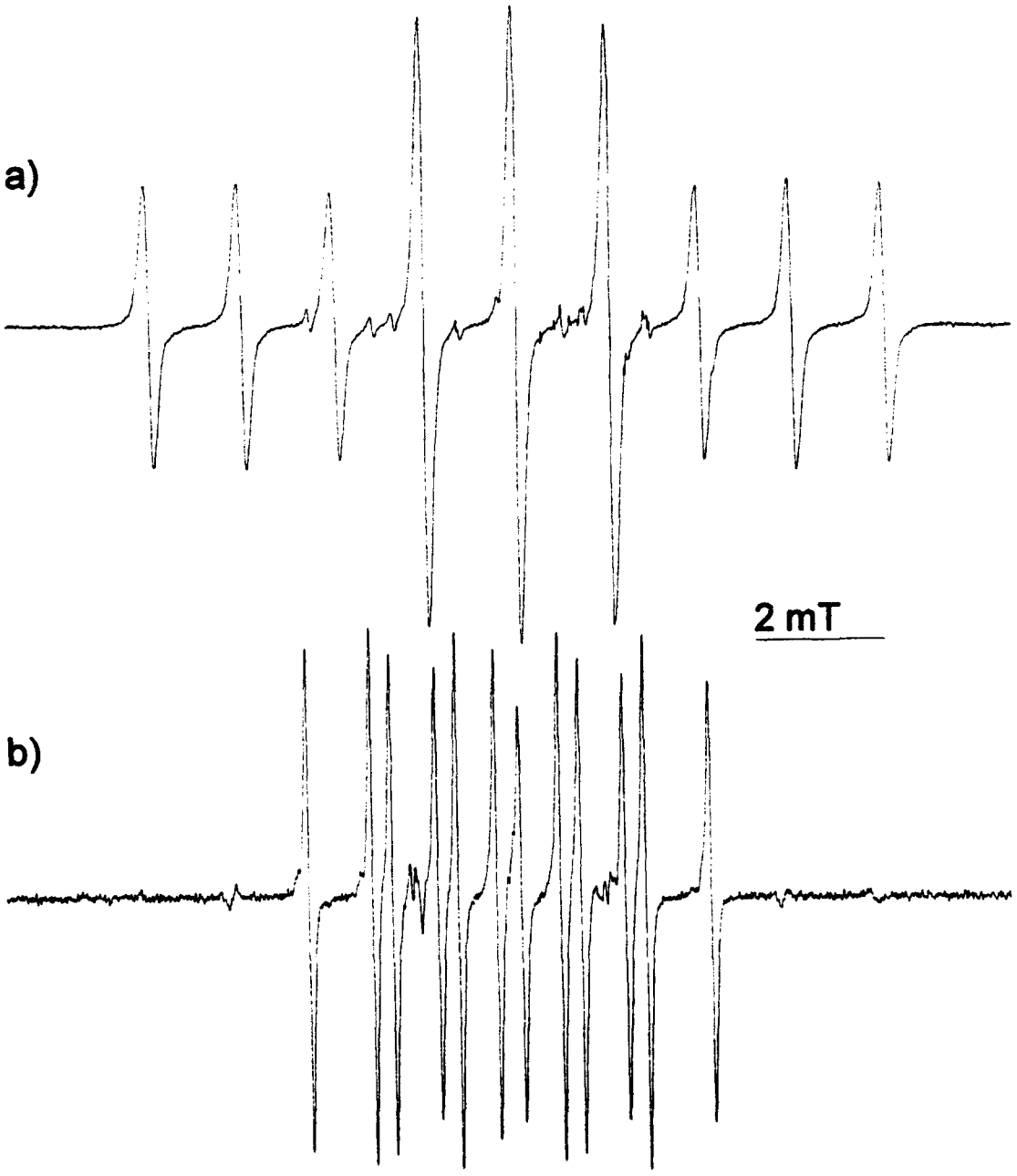
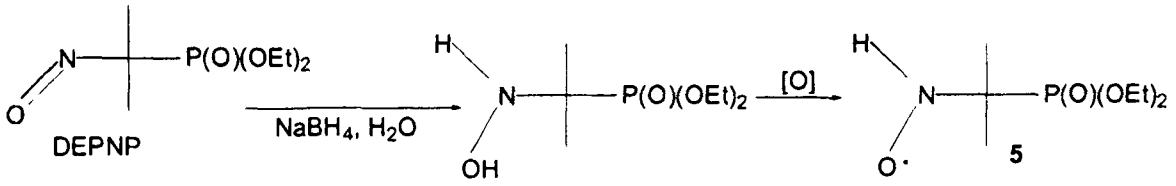


FIGURE 1 a) EPR spectrum of aminoxyl DEPNP/·C$(CH_3)_2$[P(O)$(OC_2H_5)_2$] **3** obtained by light-induced decomposition of DEPNP in deoxygenated toluene (a_N = 1.40 mT and $a_{P\beta}$ = 3.75 mT). b) EPR spectrum of aminoxyl DEPNP – P(O)$(OC_2H_5)_2$ **4** obtained by light-induced decomposition of DEPNP in toluene in the presence of O_2 (a_N = 0.96 mT, $a_{P\beta}$ = 2.80 mT and $a_{P\alpha}$ = 1.26 mT)

TABLE II EPR Parameters of DEPNP/·Y aminoxyl radicals obtained from DEPNP in various media

Aminoxyl radical	*Source*	*Solvent*	a_N *(mT)*	a_P *(mT)*	*others (mT)*
DEPNP/ ·$C(CH_3)_2[P(O)(OC_2H_5)_2]$ **3**	deoxygenated DEPNP solution, visible light or heating	water	1.48	2.80 (2P)	
		toluene	1.40	3.75 (2P)	
		$CHCl_3$	1.41	3.97 (2P)	
DEPNP/ ·$P(O)(OC_2H_5)_2$ **4**	oxygenated DEPNP solution, visible light or heating	water	0.99	3.03	$a_{p\alpha}$: 1.30
		toluene	0.96	2.80	$a_{p\alpha}$: 1.26
		$CHCl_3$	0.96	2.67	$a_{p\alpha}$: 1.27
		$HP(O)(OEt)_2$	0.95	2.89	a_p : 1.26
DEPNP/ ·H **5**	$NaBH_4$	water	1.33	5.15	$a_{H\alpha}$: 1.32
DEPNP/ ·D **6**	$NaBD_4$	D_2O	1.32	5.19	$a_{D\alpha}$: 0.20
DEPNP/ ·CH_3 **7**	Fenton system, DMSO	water	1.55	5.14	$a_{H\beta}$: 1.33 (3H)
DEPNP/ ·CH_2OH **8**	Fenton system, methanol	water	1.39	4.49	$a_{H\beta}$: 0.54 (2H)
DEPNP/ ·CH_2CH_2OH **9**	Fenton system, ethanol	water	1.53	4.95	$a_{H\beta}$: 1.10 (2H)
DEPNP/ ·$CH(CH_3)OC_2H_5$ **10**	Fenton system, diethylether	water	1.42	4.28	$a_{H\beta}$: 0.18
DEPNP/ ·$CH_2C(O)CH_3$ **11**	Fenton system, acetone	water	1.41	4.87	$a_{H\beta}$: 0.79 (2H)
DEPNP/ ·$CH(CH_3)_2$ **12**	I $CH(CH_3)_2$, photolysis	benzene	1.39	4.91	$a_{H\beta}$: 0.15
DEPNP/ ·C_6H_5 **13**	C_6H_5I, photolysis	benzene	1.07	3.76	a_{Ho} : 0.20 (2H)
					a_{Hm} : 0.10 (2H)

In the presence of reducing species, nitroso compounds can also yield aminoxyl radicals that generate artefactual EPR signals, and this has been at the origin of misinterpretation in spin trapping experiments conducted with MNP, as previously mentioned by Kalyanaraman *et al.*[26]. To avoid this problem, it is thus important to study the DEPNP behaviour in chemical reduction processes. By incubating this nitroso compound in a $NaBH_4$ aqueous solution, DEPNP was reduced into the corresponding EPR silent hydroxylamine, which was immediately autoxidised into the aminoxyl **5** noted DEPNP/·H, as described in scheme 3. Its EPR signal recorded in water corresponded to a quartet, due to nitrogen and hydrogen hfscs approximately equal ($a_N = 1.33$ mT, $a_H = 1.32$ mT), split by a large phosphorous coupling ($a_P = 5.15$ mT). Since the same aminoxyl could be obtained by trapping ·H radical, it can thus be considered as a pseudo – DEPNP/·H spin adduct. Similarly, when the same experiment was conducted in D_2O, the pseudo- DEPNP/·D adduct, *i.e.* the aminoxyl **6**, was formed and identified by EPR spectroscopy (see table II).



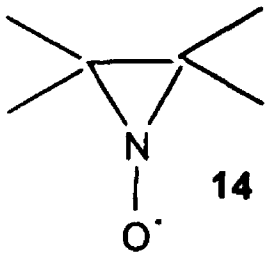
SCHEME 3 formation of the aminoxyl DEPNP/·H **5** by chemical reduction of DEPNP

Spin trapping

In order to appreciate the potential of DEPNP in the detection of short -lived radicals, a series of free radicals have been generated in aqueous or organic media in the presence of this nitroso compound, as described in the experimental section. Since our main purpose was to rapidly assess the DEPNP capacity to act as spin trap, only the trapping of a few radicals has been surveyed. In order to simplify the notation, the aminoxyl obtained by trapping a free radical ·Y by DEPNP will be noted DEPNP/ ·Y. For example, DEPNP/·CH_3 **7** represents the methyl radical spin adduct of DEPNP. This nitroso compound was found to trap very efficiently various kinds of carbon-centred radicals, yielding persistent spin adducts detectable by EPR spectroscopy over a few hours. As an example, the EPR spectrum of the spin adduct DEPNP/ ·$CH(CH_3)OC_2H_5$ **10** recorded in water is shown in figure 2. Whatever the medium was, intense EPR spectra were thus detected and fully analysed by spectral simulation with the aid of a fitting procedure, giving the parameters listed in table II for the spin adducts **7–13**. The spin trapping by DEPNP of the phosphorus -centred radical ·$P(O)(OC_2H_5)_2$ also gave rise to an intense EPR spectrum in various media. However, no EPR signal was ever detected when various heteroatom-centred radicals, such as $O_2^{\cdot-}$, HO· or $SO_3^{\cdot-}$, were produced at room temperature in aqueous media in the presence of DEPNP.

DISCUSSION

Although DEPNP is not a commercially available compound, the synthesis pathway described in this paper makes its obtention quite easy. The DEPNP samples thus obtained were found to contain a paramagnetic impurity, showing a three line EPR signal, which has not been clearly identified. Note however that this aminoxyl present exactly the same hfsc (a_N = 1.45 mT and a_{13C} = 0.64 mT in toluene) than a radical detected and identified by Luckurst *et al.*[27] as the 2,2,3,3-tetramethylazidine-1-oxyl **14**. However, this structure identification has been the origin of a controversy[28,29], and we are not able at the moment neither to confirm that the species detected in our experiments actually corresponds to **14** nor to explain its eventual formation.



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However, this paramagnetic impurity, the amount of which could be considerably reduced by column chromatography, was not found to

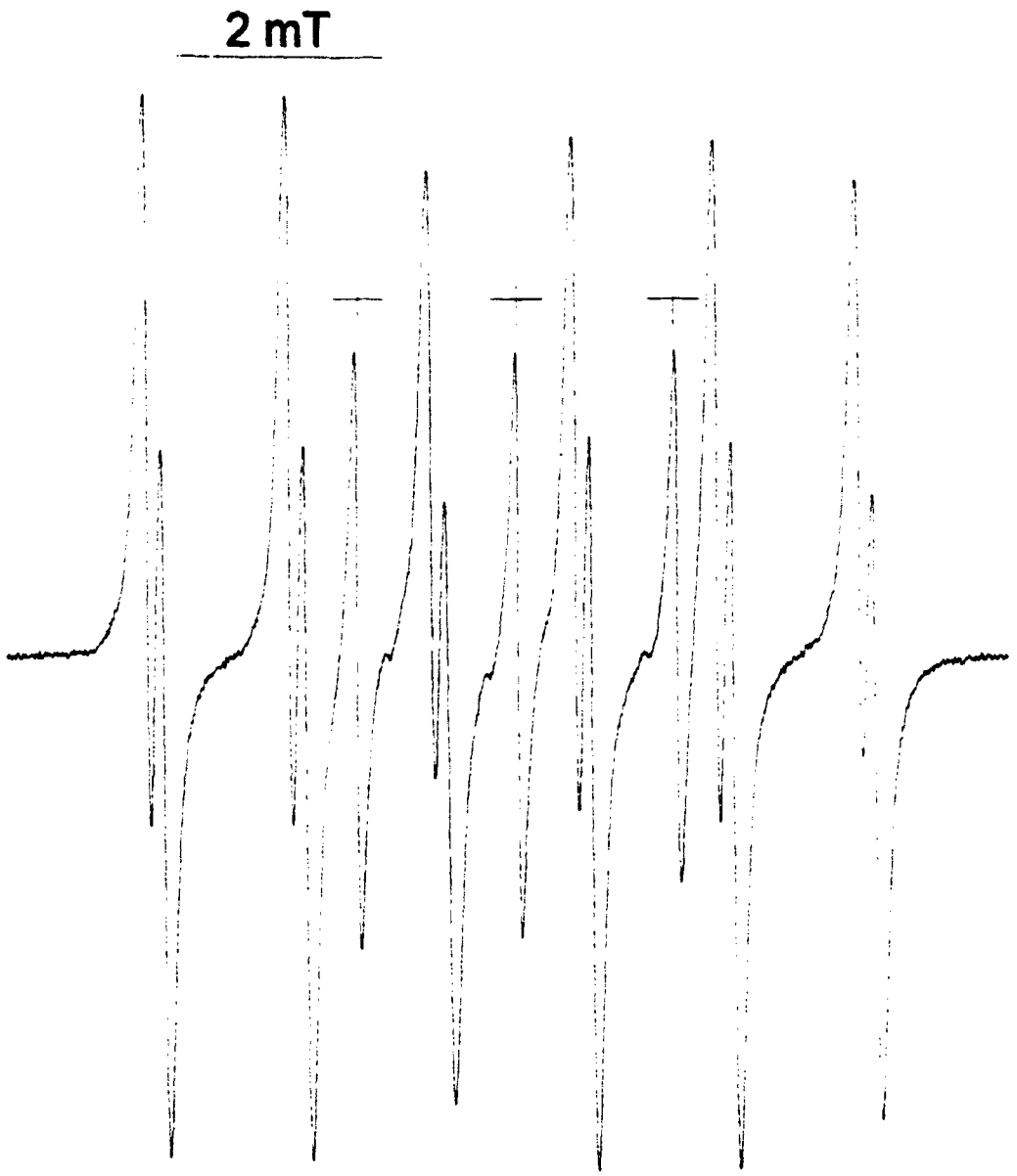


FIGURE 2 EPR spectrum of the spin adduct DEPNP/·CH(CH_3)OC_2H_5 **10** obtained by carrying out a Fenton reaction in water in the presence of DEPNP and diethylether (a_N = 1.42 mT, $a_{P\beta}$ = 4.28 mT and $a_{H\beta}$ = 0.18 mT). The three lines topped by crosses correspond to an unidentified aminoxyl impurity (a_N = 1.60 mT)

really limit the potential applications of DEPNP, since spin trapping experiments were successfully performed with DEPNP despite its presence.

One of the most interesting characteristics of DEPNP consists in the absence of its dimer both in aqueous and organic media. This is certainly

due to the presence of an electronegative group, the diethylphosphonate, which shifts the monomer-dimer equilibrium in favour of the former[30]. Since the dimers are known to be spin trapping inactive, this should be regarded as an important advantage of DEPNP over its non -phosphorylated analogue MNP. In particular, this permits both to use less nitroso compound in spin trapping experiments and to better control the actual concentration of its active form. In addition, DEPNP was found to be soluble in both water and organic solvents and its K_p value indicates that this compound is neither too hydrophilic nor too lipophilic, making its use possible in every kind of media.

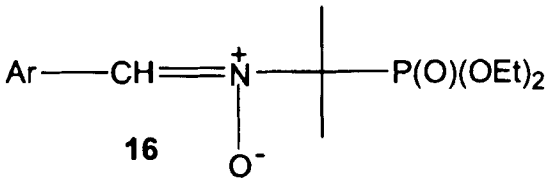
As most of other aliphatic nitroso compounds, DEPNP was found to be thermally and photochemically labile. As mentioned earlier, its decomposition yielded the aminoxyl **3** and **4**, depending on the presence or the absence of molecular oxygen in the medium. When oxygen was removed prior heating or irradiating the solution, DEPNP decomposes following a mechanism similar to that described in the case of MNP: as indicated in scheme 2a, the cleavage of the N-C bond generates NO· and the carbon-centred radical $\cdot C(CH_3)_2[P(O)(OC_2H_5)_2]$, which is subsequently trapped by a second DEPNP molecule, giving the corresponding spin adduct **3**. In the presence of oxygen, the radical $\cdot C(CH_3)_2[P(O)(OC_2H_5)_2]$ produced can be oxidised into a peroxyl radical which is trapped by the DEPNP. The decomposition of the labile adduct thus obtained produces the phosphorus – centred radical $\cdot P(O)(OC_2H_5)_2$ which leads to **4** after its trapping by DEPNP. This second mechanism, fully described in scheme 2 b, is analogous to another one mentioned in a study of photolysis-induced decomposition of MNP in the presence of phosphonates[31]. In order to confirm it, we verified by ^{31}P and ^{1}H NMR and by gas chromatography that acetone and the nitro compound **15** were actually formed during the DEPNP degradation process in the presence of oxygen.

Despite its sensitivity to light and heat, DEPNP was successfully used in various spin trapping experiments without taking too much precautions. Of course, this trap is quite unusable over 50°C, but its decomposition at room temperature is negligible. To avoid the light-induced degradation, one should take care to always use freshly prepared DEPNP solutions and to protect them as much as possible from ambient light. In these conditions, DEPNP was found to trap very efficiently various carbon -centred radicals, giving rise to intense EPR spectra in both water and organic solvents. Note that the time -limited exposure of DEPNP to UV light did not create notable problem when free radicals such as $\cdot C_6H_5$ have been produced by photolysis.

The EPR spectra of DEPNP spin adducts invariably exhibited a main doublet of triplet splitting, due to the interaction of the unpaired electron with the ^{14}N and ^{31}P nuclei. Additionally, these spectra also revealed other splittings arising from magnetic nuclei in the trapped radical, which facilitate the addend identification. This is more particularly interesting when various free radicals can be produced in the medium. For example, when the ·OH radicals are scavenged by ethanol, two different carbon-centred radicals, *i.e.* $\cdot CH(OH)CH_3$ and $\cdot CH_2CH_2OH$, can be formed. The proportion of these two transient species may greatly depend on the ·OH generation system employed, and more particularly on the reagents used in the Fenton system[32,33]. In our case, the use of a nitroso compound as spin trapping agent allowed to identify unambiguously the radical $\cdot CH_2CH_2OH$ as being produced in our experimental conditions, since the spin adduct spectrum thus obtained clearly exhibited a splitting pattern with two equivalent β-hydrogens. In the same manner, we found that ·OH radical reaction on diethylether generated the radical $\cdot CH(CH_3)OC_2H_5$, since in this case the EPR spec-

trum revealed only one β-hydrogen coupling (see figure 2). Since such information could never have been obtained using nitrones as spin traps, this emphasises one of the essential merits of nitroso compounds in free radical detection. Moreover, an important advantage of DEPNP over MNP consists in the presence of a strong phosphorus splitting, very sensitive to the spin adduct conformation, which generally makes the free radical characterisation easier. Actually, it can be seen from the data listed in table II that a_p varies greatly with the species trapped. Thus, this hfsc differs by *ca* 2.4 mT between radicals **4** and **6** in water. The variation of a_p with the solvent, observed for example in the case of aminoxyls **3**, is also noteworthy and reveals in this case important modifications in the spin adduct conformation with the solvent polarity.

As mentioned in the introduction, various PBN-type β-phosphorylated nitrones, corresponding to the general structure **16** given below, have been elaborated in our laboratory a few years ago, and these compounds were found to trap very efficiently various free radicals in aqueous, organic and heterogeneous media[22]. As their non-phosphorylated analogues, when these nitrones were used as spin traps in water in the presence of a Fenton system and eventually of scavengers such as DMSO or methanol, their ·OH radical adduct decomposed giving rise to new aminoxyl radicals. The mechanism thus supposed for this decomposition implied the formation of an aromatic aldehyde and of DEPNP, and the trapping of free radicals present in the medium by this nitroso[19,22]. However, we had not enough information at that time to be absolutely certain of this decomposition pathway. After using DEPNP as spin trapping agent of various free radicals, we are now in the position to confirm the hypothetical mechanism thus described insofar as the aminoxyl byproducts detected were unambiguously identified as radical **5** or as DEPNP spin adducts.



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CONCLUSION

Although DEPNP was found to be heat and light sensitive, just like all the other aliphatic nitroso compounds, it can be efficiently employed in spin trapping experiments to detect carbon- and phosphorus-centred radicals both in aqueous and organic media, giving rise to intense EPR spectra characteristic of the addend. One of its main merits over its non phosphorylated analogue consists in the absence of dimer, both in water and in solvents, which permits to better control the spin trap concentration used. Another important advantage of DEPNP is due to the existence of a strong phosphorous coupling in its various spin adducts, which is very sensitive not only on the radical trapped, but also on the aminoxyl radical environment. As it has been done with phosphorylated nitrones[22], we have now planed to use DEPNP as spin trapping agent in a water-micelles heterogeneous media, since the phosphorus hfsc was generally found to be a good probe of the location of β-phosphorylated aminoxyl radicals in this kind of heterogeneous system[22,33]. Last, since PBN-type β-phosphorylated nitrones have a high potential in spin trapping[18,20], it was of crucial importance to identify the aminoxyl by -products eventually produced by degradation of their spin adducts, and the spin trapping results given in this paper permit to dispose of a set of EPR data concerning various DEPNP spin adducts which may possibly be detected when these nitrones are employed.

References

[1] C.A. Evans (1979) Spin trapping. *Aldrichimica Acta*, 12, 23–29.

[2] M.J. Perkins (1980) Spin Trapping. In Advances in physical organic chemistry (ed. V. Gold and D. Bethell), vol. 17, Academic Press, London, New York, pp 1–64.

[3] M. J. Davies and G. S. Timmins (1996) EPR spectroscopy of biologically relevant free radicals in cellular, *ex vivo*, and in *vivo* systems. In Biomedical applications of spectroscopy (ed. R. J. H. Clark and R. E. Hester), John Wiley & Sons, Chichester, pp 217–266.

[4] G. R. Buettner (1987) Spin trapping: ESR parameters of spin adducts. *Free Radical Biology and Medicine*, 3, 259–303.

[5] R. Borghi, L. Lunazzi, G. Placucci, G. Cerioni and A. Plumitallo (1996) Photolysis of dialkoxy disulfides: a convenient source of alkoxy radicals for addition to the sphere of fullerene C_{60}. *Journal of Organic Chemistry*, 61, 3327–3331.

[6] E. A. Mash, H.-G. Korth and S. M. DeMoss (1997) On the reported intermediacy of vinyl radicals in spontaneous polymerization: an ESR-spin trapping study and its significance for the bond forming initiation theory. *Tetrahedron*, 53, 15297–15320.

[7] L. Bin Luo, D. Yan Han, Y. Wu, X. Yi Song and H. Lan Chen (1998) EPR investigation on radical trap reactions of 2-methyl-2-nitrosopropane encapsulated by cyclodextrins with external organic radicals produced by photolysis of coenzyme B_{12} and its analogues. *Journal of the Chemical Society Perkin Transactions 2*, 1709–1714.

[8] R. P. Mason, B. Kalyanaraman, B. E. Tainer and T. Eling (1980) A carbon-centered free radical intermediate in the prostaglandin synthetase oxidation of arachidonic acid. *Journal of Biological Chemistry*, 255, 5019–5022.

[9] J. B. Feix and B. Kalyanaraman (1989) Spin trapping of lipid-derived radicals in liposomes. *Biochimica Biophysica Acta*, 992, 230–235.

[10] B. Kalyanaraman, J. Joseph and S. Parthasarathy (1992). The use of spin traps to investigate site specific formation of free radicals in low density lipoprotein oxidation. *Biochemical Society Transactions*, 21, 318–321.

[11] E. G. Janzen, M. S. West, Y. Kotake and C. DuBose (1996) Biological spin trapping methodology. III. Octanol-water partition coefficients of spin trapping compounds. *Journal of Biochemical Biophysical Methods*, 32, 183–190.

[12] M. Kuwabara, S. Miyake, T. Jin and S. Sawamura (1995) Reactions and rate constants between hydroxyl radicals and the dimer and monomer of spin trap 2-methyl-2-nitrosopropane determined by the pulse radiolysis method. *Journal of Physical Chemistry*, 99, 14078–14082.

[13] C. Fréjaville, H. Karoui, B. Tuccio, F. Le Moigne, M. Culcasi, S. Piétri, R. Lauricella and P. Tordo (1995) 5-Diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide (DEPMPO): a new efficient phosphorylated nitrone for the *in vitro* and *in vivo* spin trapping of oxygen-centered radicals. *Journal of Medical Chemistry*, 38, 258–265.

[14] S. Barbati, J.L. Clément, G. Olive, V. Roubaud, B. Tuccio and P. Tordo (1997) ^{31}P-Labeled cyclic nitrones: a new class of spin traps for free radicals in biological milieu. In Free radicals in biology and environment (ed. F. Minisci), Kluwer Academic Publishers, Dordrecht, pp 39–47.

[15] V. Roubaud, A. Mercier, F. Le Moigne, G. Olive and P. Tordo (1997) 5-(diethoxyphosphorylmethyl)-5-methyl-4,5-dihydro-3H-pyrrole N-oxide: synthesis and evaluation of spin trapping properties. *Journal of the Chemical Society Perkin Transactions 2*, 1827–1831.

[16] H. Karoui, C. Nsanzumuhire, F. Le Moigne and P. Tordo (1999) Synthesis of a new spin trap: 2-diethoxyphosphoryl)-2-phenyl-3,4-dihydro-2H-pyrrole 1-oxide. *Journal of Organic Chemistry*, 64, 1471–1477.

[17] G. Olive, A. Mercier, F. Le Moigne, A. Rockenbauer and P. Tordo (2000) 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide: evaluation of the spin trapping properties. *Free Radical Biology and Medicine*, 28, 403–408.

[18] B. Tuccio, A. Zeghdaoui, J. P. Finet, V. Cerri and P. Tordo (1996) Use of new β-phosphorylated nitrones for the spin trapping of free radicals. *Research on Chemical Intermediates*, 22, 393–404.

[19] V. Roubaud, R. Lauricella, B. Tuccio, J. C. Bouteiller and P. Tordo (1996) Decay of superoxide spin adducts of new PBN-type phosphorylated nitrones. *Research on Chemical Intermediates*, 22, 405–416.

[20] C. Rizzi, S. Marque, F. Belin, J.C. Bouteiller, R. Lauricella, B. Tuccio, V. Cerri and P. Tordo (1997) PPN-type nitrones: preparation and use of a new series of β-phosphorylated spin trapping agents. *Journal of the Chemical Society Perkin Transactions 2*, 2513–2518.

[21] Y. A. Levin and M. S. Skorobogatova (1976) Nitrosophosphonates. *Izvestiya Akademii Nauk SSSR Seriya Khimicheskaya (Engl. translation)*, 25, 465.

[22] C. Rizzi, R. Lauricella, B. Tuccio, J.C. Bouteiller, V. Cerri and P. Tordo (1997) Spin-trapping of free radicals by PBN-type β-phosphorylated nitrones in the presence of SDS micelles. *Journal of the Chemical Society Perkin Transactions 2*, 2505–2512.

[23] T. Ya. Medved and M. I. Kabachnic (1956) New method for the synthesis of amino phosphonic acids communication 2. Reaction of ketones with dialkylphosphites and ammonia. *Izvestiya Akademii Nauk SSSR Seriya Khimicheskaya*, 684–688.

[24] D. R. Duling (1994) Simulation of Multiple isotropic Spin-Trap EPR Spectra *Journal of Magnetic Resonance B*, 104, 105–110.

[25] A. S. Mukhtarov, A. V. Il'yasov, Y. A. Levin and M. S. Skorobogatova (1976) Nitroxyl radicals with two phosphorous atoms. *Izvestiya Akademii Nauk SSSR Seriya Khimicheskaya (Engl. translation)*, 25, 2625–2627.

[26] B. Kalyanaraman, E. Perez-Reyes and R. P. Mason (1979) The reduction of nitroso-spin traps in chemical and biological systems. A cautionary note. *Tetrahedron Letters*, 50, 4809–4812.

[27] G. R. Luckhurst and F. Sundholm (1971) The synthesis and electron spin resonance spectrum of the nitroxide 2,2,3,3-tetramethylaziridine-1-oxyl. *Tetrahedron Letters*, 8, 675–678.

[28] P. Singh, D. G. B. Boocock and E. F. Ullman (1971) Attempted formation of the unknown tetramethylaziridine-1-oxyl by photolysis and thermolysis of diazetidine-1,2-dioxide. *Tetrahedron Letters*, 42, 3939–3942.

[29] J.F.W. Keana, R. J. Dinersteinand D. P. Dolata (1972) On the recently reported synthesis of the nitroxide 2,2,3,3-tetramethylaziridine-1-oxyl. *Tetrahedron Letters*, 8, 675–678.

[30] J. R. Schwartz (1957) Nature of aliphatic C-nitroso compounds. I. Study of the rate of dissociation of the aliphatic C-nitroso dimer in various solvents. *Journal of the American Chemical Society, 79*, 4353–4355.

[31] Y. A. Levin, A. V. Il'yasov, I. D. Morozova, A. S. Mukhtarov and M. S. Skorobogatova (1975) The formation of phosphorus-containing nitroxyl radicals in the photolysis of 2-methyl-2-nitrosopropane in esters of unsaturated phosphonic acid. *Izvestiya Akademii Nauk SSSR Seriya Khimicheskaya (Engl. translation)*, 24, 251–255.

[32] B. C. Gilbert, R. O. C. Norman, Peter S. Williams and J. N. Winter (1982) Electron spin resonance studies. Part 63. Selective radical oxidation by titanium(IV) complexes. *Journal of the Chemical Society Perkin Transactions 2*, 1439–1445.

[33] J. S. B. Park, P. M. Wood, B. C. Gilbert and A. C. Whitwood (1999) EPR evidence for hydroxyl- and substrate-derived radicals in Fe(II)-oxalate/hydrogen peroxide reactions. The importance of the reduction of Fe(III)-oxalate by oxygen-conjugated radicals to regenerate Fe(II) in reactions of carbohydrates and model compounds. *Journal of the Chemical Society Perkin Transactions 2*, 923–931.

[34] C. Rizzi, C. Mathieu, B. Tuccio, R. Lauricella, J.C. Bouteiller and P. Tordo (1999) Stable β-phosphorylated cyclic aminoxyl radicals in SDS micelles. *Journal of the Chemical Society Perkin Transactions 2*, 2777–2781.