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Investigation of singlet oxygen reactivity towards propofol

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Received 3rd March 2003, Accepted 5th May 2003
First published as an Advance Article on the web 5th June 2003

The reaction between the anaesthetic agent 2,6-diisopropylphenol (propofol, PPF) and singlet oxygen ($^1O_2$) has been investigated in aqueous solution by means of HPLC, GC, absorption spectroscopy and laser flash photolysis with infrared luminescence detection. The rate constants for the physical and chemical quenching of $^1O_2$ by PPF (k_{PPF}) are found to be 2.66×10^5 M^{-1} s^{-1} and ~3.2×10^6 M^{-1} s^{-1} in CD_3OD and D_2O–CD_3OD (75:25 v/v), respectively. The reaction of propofol with singlet oxygen produced by light irradiation of Rose Bengal leads essentially to two reaction products, 2,6-diisopropyl-*p*-benzoquinone and 3,5,3′,5′-tetraisopropyl-(4,4′)-diphenoquinone that are unambiguously identified from comparison with authentic samples.

Introduction

Propofol (2,6-diisopropylphenol, PPF) is the active principle of the intravenous anaesthetic agent DIPRIVAN® currently used for both inducing and maintaining hypnosis during general anaesthesia.[1] Besides its anaesthetic properties, the role of propofol as an antioxidant agent has received particular attention. Many studies have been devoted on its free radical scavenging properties. Indeed, it has been demonstrated to inhibit the lipid peroxidation reaction[2,3] and to react with reactive oxygen species (ROS) such as hydroxyl radicals ($^{\bullet}OH$).[4] Interestingly, recent studies have shown that propofol also reacts with peroxynitrite ($ONOO^-$), a highly reactive molecule which results from the reaction of superoxide anion ($O_2^{-\bullet}$) with nitric oxide ($^{\bullet}NO$).[4–6]

Until now, no studies have been undertaken on the reaction of propofol with singlet oxygen ($^1O_2$). However, singlet oxygen is one of the most important intermediate products of the oxygen metabolism, known to react with various biological molecules and able to cause membrane and tissue damage by inducing unsaturated lipid peroxidation.[7] Furthermore, it has been reported that $^1O_2$ might be generated in ischemia-reperfusion situations.[8] Thus, as an extension of the investigation of free radical scavenging properties of propofol, we have studied its reactivity towards singlet oxygen.

This latter reaction is reminiscent of the effect of some phenolic compounds such as α-tocopherol which prevent lipid peroxidation through radical chain breaking processes and are also considered as $^1O_2$ scavengers.[9] In the same way, propofol, a phenolic tocopherol analogue, was found to prevent lipid peroxidation induced by γ irradiation.[10]

Herein, we investigate the reaction of propofol with $^1O_2$ by absorption spectroscopy using the water-soluble photosensitizer Rose Bengal[11] and other dyes. The overall oxidation products were characterized by absorption spectroscopy, gas chromatography (GC) and high performance liquid chromatography (HPLC). Time resolved detection of the luminescence of singlet oxygen allowed us to determine the rate constant of its reaction with propofol.

Experimental

Chemicals

Propofol (2,6-diisopropylphenol; PPF) from Sigma (Belgium) was stored at 4 °C in the dark. Absolute methanol (HPLC grade), chloroform (HPLC grade) and hydrochloric acid were obtained from Merck (Belgium), Rose Bengal (RB) from Eastman Kodak (USA), disodium hydrogen phosphate-2 hydrate from Riedel-de Haën (Belgium), deuterium oxide (D_2O), sodium azide (NaN_3) and potassium phosphate from Sigma (Belgium). Potassium permanganate came from Noury-Baker (Belgium) and lead(IV) dioxide from Acros (Belgium). $HClO_4$ was obtained by acidification of sodium perchlorate from Aldrich (Belgium). Deuteriated methanol (CD_3OD) was provided from Merck (Darmstadt, Germany). Hematoporphyrin (HP) was purchased from Aldrich and purified as described previously.[12] 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrin (TPPS) was obtained from Porphyrin Products (USA).

Sample preparation

PPF was first dissolved in methanol (18 µl in 1 ml) to obtain a 10^{-1} M solution and then diluted in (1/15 M) phosphate buffer pH 7 (Na_2HPO_4–KH_2PO_4). The experiments were performed with final drug concentrations ranging from 1×10^{-5} to 8×10^{-4} M. Under these conditions, the final percentage of methanol never exceeded 5%.

The RB concentration was determined spectrophotometrically using a molar absorption coefficient of 99 800 M^{-1} cm^{-1} at 548 nm.[13] The dye was used at the concentration of 8×10^{-6} M

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in such a way that the ratio of RB to PPF concentration ranged from 0.01 to 0.8.

When necessary, the samples were deaerated by eight cycles of a freeze-thawing procedure using liquid nitrogen and a two-vacuum pump system (Blazer-Pfeiffer DUO004B Mechanical Pump and TPH055 Diffusion Pump, Germany).

2,6-Diisopropyl-*p*-benzoquinone (PPFQ) was obtained as a yellow brown oil by oxidation of PPF by PbO_2 and $HClO_4$ in acetic acid (yield ~80%) as described by Omura[14] (MS: *m*/*z* 193 (M^+ + 1, base), 164, 121; UV/VIS (MeOH): 256 nm, ε_{256} = 17 000 M^{-1} cm^{-1};[15] IR: 2966, 1656 cm^{-1}).

3,5,3′,5′-Tetraisopropyl-(4,4′)-diphenoquinone (PPFDQ) was prepared using $KMnO_4$ (48 mM) to oxidize PPF (yield ~90%) as reported by Menger and Carnahan[16] (mp: 202–203 °C, lit. 202–203 °C);[17] MS: *m*/*z* 355 (M^+ + 3, base), 340, 256; UV/VIS (MeOH): 423 nm, ε_{423} = 68 000 M^{-1} cm^{-1};[18] ^{1}H NMR ($CDCl_3$): δ 1.2 (d, 24 H, J = 6.5 Hz), 3.22 (septet, 4H, J = 6.5 Hz), 7.64 (s, 4H); IR: 2960, 2870, 1588 cm^{-1}). Note that the molecular mass (M^+ + 3) is probably due to a reduction of the molecule followed by the trapping of a hydrogen atom generated by the technique.

The absorption spectra were recorded using a Kontron double beam spectrophotometer (Uvikon 941). The solutions were placed in a quartz (Suprasil) cell (0.4 cm width × 1cm path length, Hellma, Belgium).

Continuous irradiation

The irradiation of RB solutions was carried out with a slide projector (Pradovit RA 150, Leitz, Germany) as a light source. The halogen lamp (Xenophot 150W, 24V, Osram, Belgium) output was passed through a GG 475 filter (Schott, Germany) to select wavelengths above 475 nm. The solutions were placed at a distance of 11 cm from the projector. Under these conditions, the total volume of the solution was irradiated homogeneously.

Analysis of products

Before the GC analysis, the irradiated solution (for 10 min) of RB (8×10^{-6} M) and PPF (5×10^{-4} M) in phosphate buffer at pH 7 was first acidified with 25 µl HCl (2 M) and extracted with chloroform ($CHCl_3$, 1 ml). The organic layer was then injected into the chromatograph, a Varian Star 34 000 CX (Walnut Creek, USA) fitted with a RTX-5 MS column (Restek, Bellefonte, USA, 30 m length, diameter 0.25 mm). The detector (FID) temperature was 230 °C and injector temperature was 200 °C. The column temperature program began at 90 °C (for 1 min) and was increased thereafter up to 250 °C (for 12 min) at a rate of 12 °C min^{-1}.

The irradiated solution (for 10 min) of RB (8×10^{-6} M) and PPF (5×10^{-4} M) in phosphate buffer at pH 7 was injected without any treatment on a column cc 250/4.4 mm Nucleosil 100–5 C18 (Macherey-Nagel, Düren, Germany). HPLC analyses were performed on a Merck-Hitachi L7000 (LaChrom, Merck, Belgium) fitted with a Merck-Hitachi L7400 UV-visible detector. We used a linear gradient from 0 to 100% of solvent B (trifluoroacetic acid, acetonitrile, methanol, water (0.05 : 50 : 10 : 40)) in solvent A (trifluoroacetic acid, acetonitrile, water (0.05 : 35 : 65)) for 60 min followed by an isocratic elution with solvent B for 25 min. The flow rate was 1 ml min^{-1}.

Time resolved detection of the singlet oxygen ($^1O_2$) infrared luminescence

The global rate constant (k_{PPF}) is the rate constant for the physical and the chemical quenching of $^1O_2$ by PPF. The determination of k_{PPF} was based on the time resolved detection of the singlet oxygen ($^1O_2$) infrared luminescence. The second harmonic (λ = 532 nm, pulse width ~6 ns at half maximum) of the emission from a Quantel Nd/YAG laser was used as the excitation light. The laser energy was monitored after a partial deflection of the excitation light on to a pyroelectric head connected to a joulemeter (models 70810 and 70834, Oriel, Statford, CT). The unfocused beam (8 mm diameter) was incident upon solutions contained in a Suprasil quartz cell (1 × 1 cm). A 3 mm^2 germanium photodiode closely coupled to the cell at a right angle and separated from it by an interference filter was used to detect the infrared radiation at 1270 nm emitted from the sample. After amplification, the signal of the diode was fed to a digital oscilloscope (model 54820, Agilent, Palo Alto, CA). Usually four shots were averaged in order to get a good signal-to-noise ratio. In all cases, the solutions were air-saturated and hand mixed before each laser shot. In CD_3OD, hematoporphyrin (HP) was used as the singlet oxygen source.[19] In D_2O containing 25% (v/v) CD_3OD, the singlet oxygen source was 5,10,15,20 tetrakis(4-sulfonatophenyl)-porphyrin (TPPS).[20] The pD of the D_2O solution was adjusted to 7.4 which corresponds to a pH value of 7.0 in water.[21] Dye absorbances of about 0.5 at 532 nm were employed. All the measurements were performed at 20 °C.

Results

Steady-state irradiation

The absorption spectrum of PPF at pH 7 is illustrated in Fig. 1. PPF solutions followed the Beer–Lambert law between 1×10^{-5} and 8×10^{-4} M (Fig. 1 inset), indicating the absence of self-association of the drug in its ground state. The molar extinction coefficient at 271 nm was found to be about 1600 M^{-1} cm^{-1}, a value close to the molar extinction coefficient of other phenol chromophores.[22]

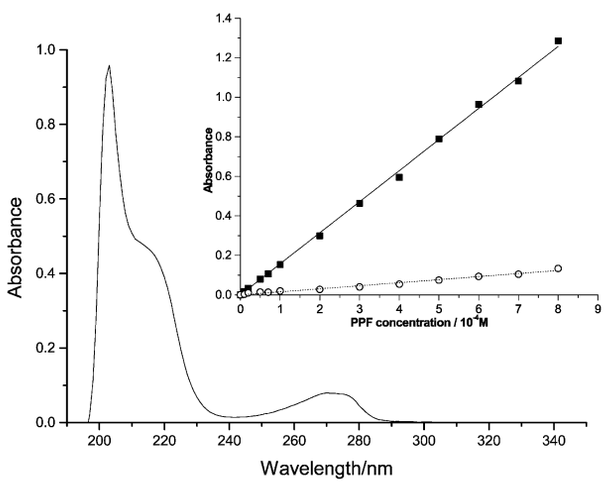

Fig. 1 Absorption spectrum of a 5×10^{-5} M solution of PPF in phosphate buffer pH 7. The inset shows the plot of the absorbance at 271 nm (■) and 241 nm (○) *vs.* PPF concentration.

Rose Bengal (RB) was used as the singlet oxygen ($^1O_2$) source (Φ = 0.75).[11] Preliminary experiments performed on free RB deaerated solutions confirmed that continuous irradiation does not lead to any modification of the absorption spectrum of the dye (data not shown). By contrast, significant evolution of the absorption spectra can be observed in the presence of different concentrations (5×10^{-5}, 5×10^{-4} M) of PPF (Fig. 2(a) and (b)). The amplitude of the absorption changes is correlated with the ratio of the RB/PPF concentration revealing a specific reaction between PPF and the excited RB in deaerated medium. The singlet excited state of the dye is not involved in this process; indeed, the fluorescence lifetime of RB is too short (~100 ps)[23] to be implied in a bimolecular reaction with PPF. Thus, it can be assumed that PPF reacts with the triplet excited state of RB.

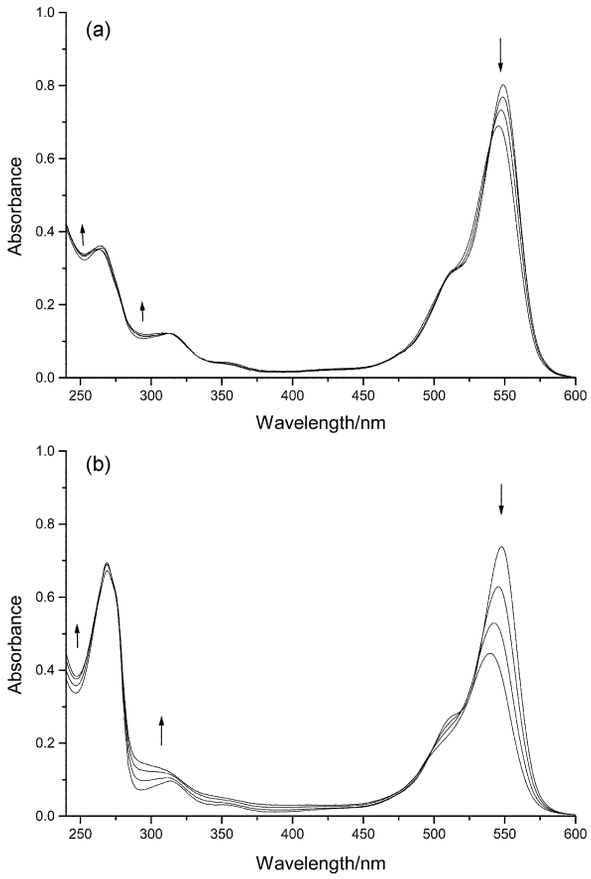

Fig. 2 Absorption spectra of a 8×10^{-6} M solution of RB dissolved in phosphate buffer before irradiation and after exposure to light ($\lambda > 475$ nm) for 5, 10 and 15 min in vacuum condition and in the presence of: (a) PPF (5×10^{-5} M); (b) PPF (5×10^{-4} M).

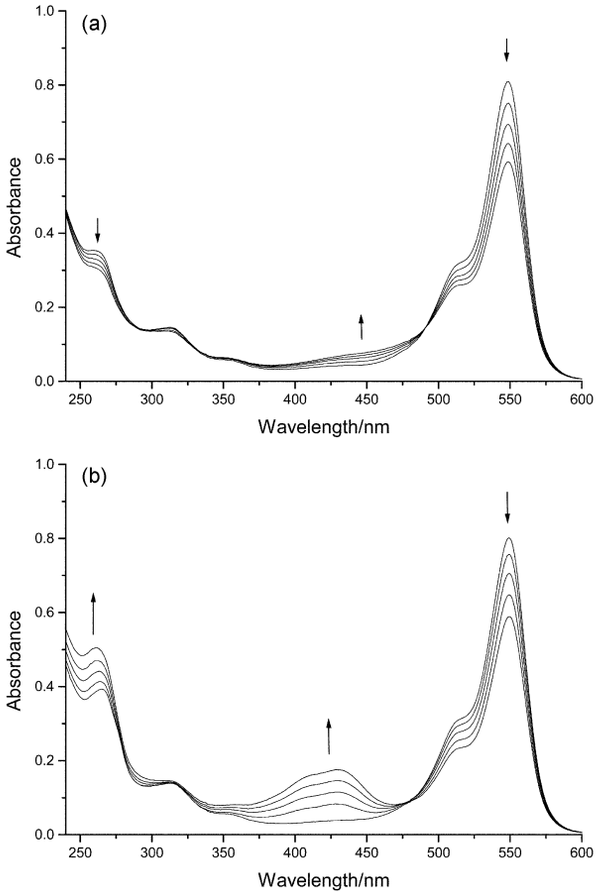

Fig. 3 Absorption spectra of RB (8×10^{-6} M) dissolved in phosphate buffer after continuous irradiation ($\lambda > 475$ nm) in aerated condition: (a) in the absence of PPF; (b) in the presence of PPF (5×10^{-5} M) and for the following times: 0, 2, 4, 6, 8 min.

Phenols have been reported to react with the xanthene triplet state by an electron or a hydrogen transfer process giving rise to the phenoxyl radical and the semi-reduced dye radical.[24,25] Therefore, in agreement with literature data, it may be also assumed in the present work that PPF was able to transfer an electron or a hydrogen to the RB triplet state.

In the following experiment a constant ratio of RB to PPF of 0.2 (Fig. 2(a)) was used in order to minimize the extent of this reaction.

Fig. 3(a) shows the spectral changes observed upon irradiation of an aerated solution of RB (8×10^{-6} M) without added propofol. Intense or prolonged irradiation of xanthene dye in aqueous solution leads to oxidative bleaching involving molecular oxygen and singlet oxygen produced by the dye itself, as reported by different authors.[26–29]

The spectra recorded after the same irradiation times, in the presence of PPF (5×10^{-5} M), show the appearance of a more prominent absorption around 429 nm (Fig. 3(b)) and an increase, broadening and batochromic shift of the transition around 260 nm when compared to the spectra obtained for RB alone (Fig. 3(a)). These results reveal the existence of chemical reactions of molecular oxygen species not only with RB but also with PPF. In order to obtain, at each time interval, the absorption spectra evolution of PPF due to this chemical reaction, the corresponding spectrum of free irradiated RB as well as the initial PPF spectrum were subtracted (Fig. 4). Under these conditions, the spectral variations corresponding to the fraction of oxidized PPF are not taken into consideration. Therefore, the resulting spectrum of Fig. 4 is only qualitative. Nevertheless, it provides information on the shape of the absorption spectra of PPF oxidation products. All the following spectra were treated in the same way.

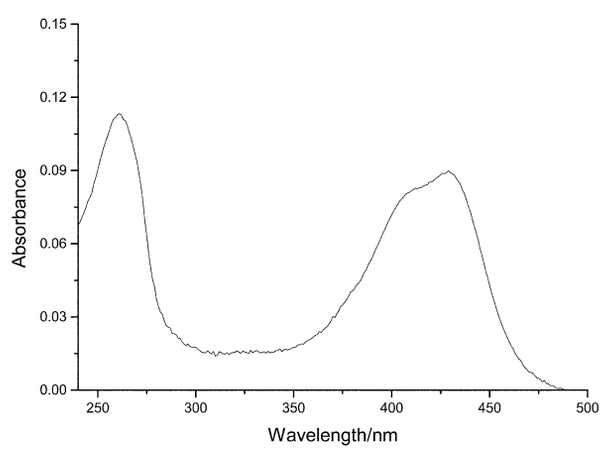

Fig. 4 Absorption spectrum of the oxidation products attributed to PPF, obtained after 4 min of RB irradiation.

The reaction between propofol and $^1O_2$ was then checked by studying the spectral evolution of aerated solutions of RB–PPF mixtures in the presence of NaN_3 (an efficient singlet oxygen quencher)[30] and in D_2O (known to increase $^1O_2$ lifetime).[31] Irradiation of RB (8×10^{-6} M) was performed in the presence of PPF (5×10^{-5} M) and NaN_3 concentrations from 1×10^{-4} to 5×10^{-3} M. As shown in Fig. 5(a), the absorbance increase measured at 260 and 429 nm are considerably reduced, depending on the NaN_3 concentration.

Irradiation in D_2O yields spectra with a shape similar to those obtained in phosphate buffer, except for an increase of the absorption observed at 260 nm and a decrease at 429 nm (Fig. 5(b)).

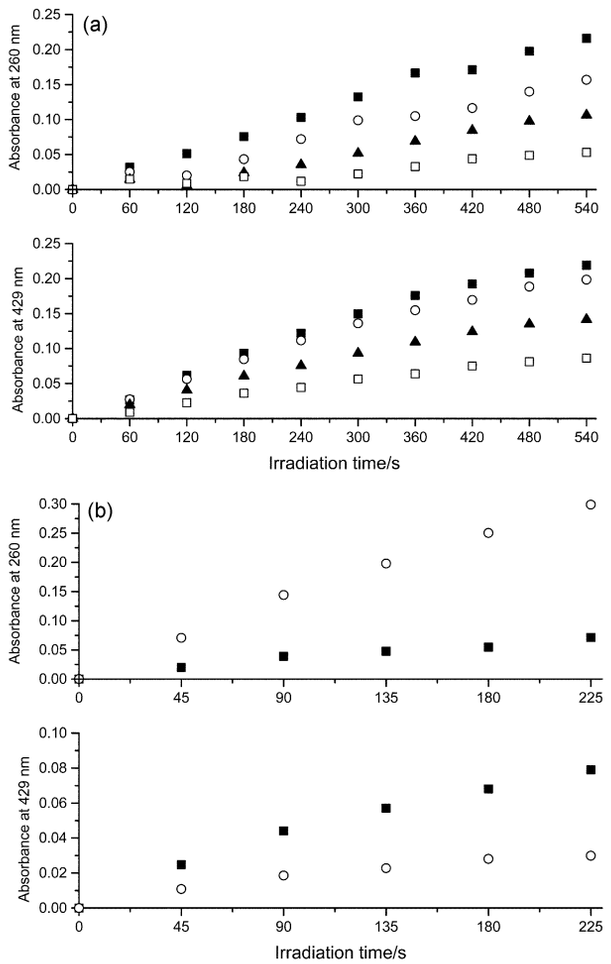

Fig. 5 (a) NaN_3 addition effect monitored at 260 and 429 nm on an irradiated RB (8×10^{-6} M) phosphate buffer solution in the presence of PPF (5×10^{-5} M), in aerated condition: (■) in the absence of NaN_3; (○) in the presence of NaN_3 (1×10^{-4} M); (▲) NaN_3 (1×10^{-3} M); (□) NaN_3 (5×10^{-3} M). (b) Substitution of phosphate buffer by D_2O effect on an irradiated RB (8×10^{-6} M) solution in the presence of PPF (5×10^{-5} M), in aerated condition: (■) in H_2O; (○) in D_2O.

Identification of the reaction products

Two different strategies were applied to characterize the products which give rise to the spectral modifications observed in aerated conditions (Fig. 3(b)).

HPLC and GC results. A solution of irradiated RB (8×10^{-6} M) in the presence of PPF (5×10^{-4} M) was analyzed by two distinct chromatographic techniques: gas chromatography (GC) and high performance liquid chromatography (HPLC). It should be noted that the PPF concentration used was higher in these experiments than in spectroscopic measurements in order to allow the detection of the PPF oxidation products. From literature data [32–35] regarding phenolic compounds, both monoquinone and diquinone derivatives could be expected as oxidation products. Hence, 2,6-diisopropyl-*p*-benzoquinone (PPFQ) and 3,5,3′,5′-tetraisopropyl-(4,4′)-diphenoquinone (PPFDQ) were synthesized from PPF and also analyzed by GC and HPLC methods.

The GC chromatogram of the irradiated RB/PPF solution (Fig. 6(a)) showed two major peaks and a minor one with retention times (R_t) of 6.8, 7.2 and 19.4 min, respectively. The first peak (R_t = 6.8 min) corresponds to the non-oxidized form of PPF as identified on the chromatogram of the stock solution of PPF (data not shown). Each solution of synthesized PPFQ and PPFDQ gave a single peak on the GC chromatogram with

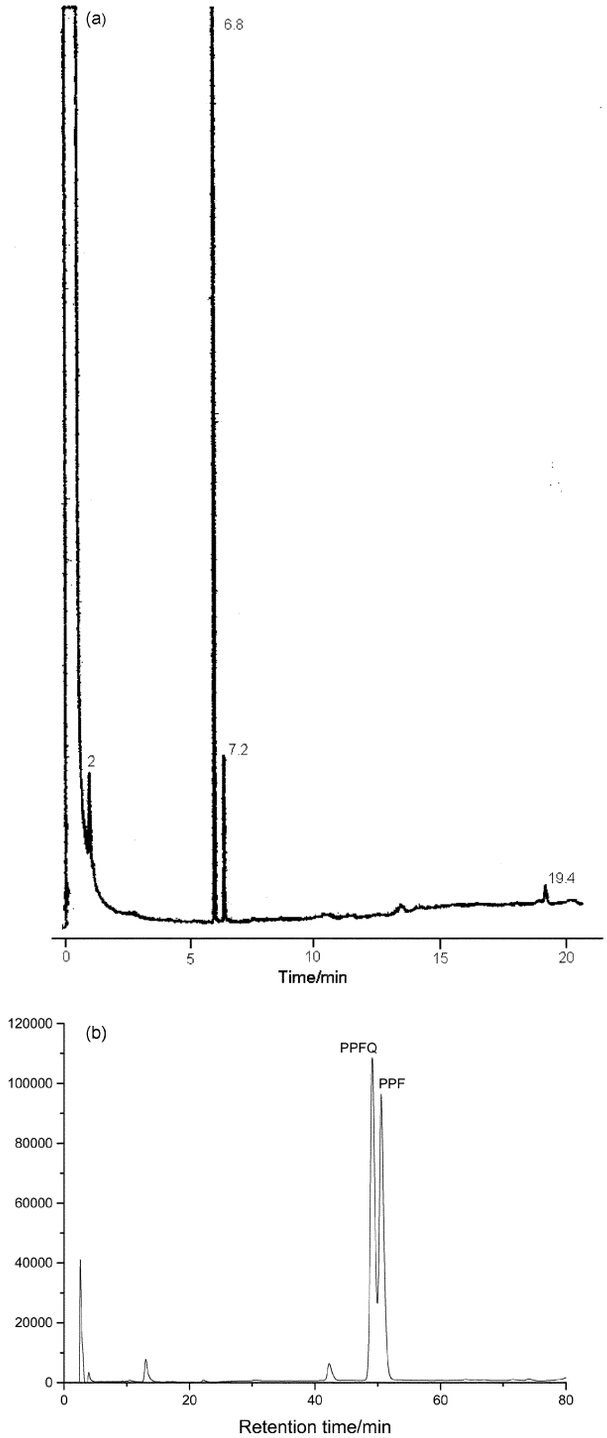

Fig. 6 Chromatography analysis of products formed from the irradiation for 10 min of RB (8×10^{-6} M) solution dissolved in phosphate buffer in the presence of PPF (5×10^{-4} M): (a) GC analysis; (b) HPLC analysis.

a retention time of 7.2 and 19.4 min, respectively (data not shown) which correspond to those measured for the irradiated RB/PPF solution.

On the other hand, the HPLC chromatogram (Fig. 6(b)) showed two peaks at 49.2 and 50.6 min. The first and second peaks were assigned to PPFQ and PPF, respectively, on the basis of chromatograms of authentic samples (data not shown). PPFDQ was not observed by this technique because it was probably converted into its quinol form at the acidic pH of the eluent, as previously reported.[5]

UV/VIS modelisation. The structure and the absorption spectra of PPFQ and PPFDQ are shown in Fig. 7. PPFQ is characterized by a peak at 260 nm and PPFDQ by a peak at 429 nm. By a proper choice of the contribution of each of these two spectra (10% PPFQ, 3% PPFDQ), it was possible to obtain a reconstructed spectrum very close to that shown in Fig. 4 (Fig. 7(c)). Thus, we can associate the absorption spectrum of Fig. 4 to a mixture of PPFQ and PPFDQ.

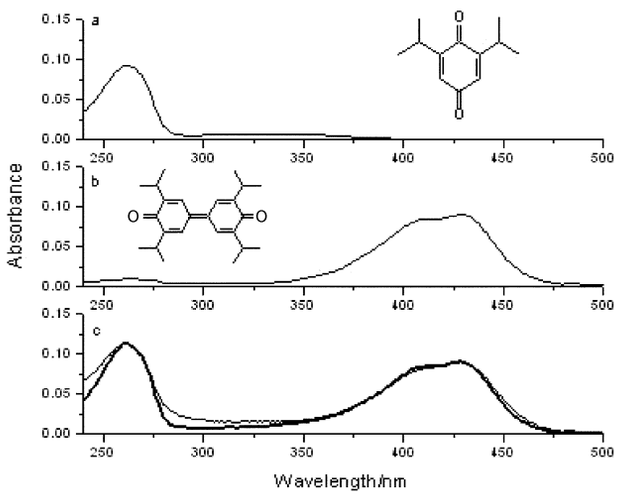

Fig. 7 Absorption spectrum obtained in phosphate buffer of: (a) 2,6-diisopropyl-*p*-benzoquinone (PPFQ, ~5 × 10^{-6} M) synthesized from PPF; (b) 3,5,3′,5′-tetraisopropyl-(4,4′)-diphenoquinone (PPFDQ, ~1.3 × 10^{-6} M) synthesized from PPF. (c) The superimposition of the absorption spectrum due to a combination of PPFQ and PPFDQ (thick line) and the absorption spectrum of the oxidation products attributed to PPF obtained from Fig. 4 (thin line).

Time resolved detection of the singlet oxygen ($^1O_2$) infrared luminescence

In CD_3OD and in D_2O buffered to pD = 7.4 in the presence of 25% CD_3OD (v/v) the lifetime of singlet oxygen was long enough to analyse its decay rate.

Typical decays of the singlet oxygen phosphorescence in the two solvents in the absence and in the presence of PPF (8.9 × 10^{-3} M in CD_3OD; 3.2 × 10^{-3} M in D_2O–CD_3OD (75 : 25 v/v)) are shown in Fig. 8 (insets). They were well fitted by mono-exponential curves yielding the lifetimes of $^1O_2$. The values of k_{PPF} in CD_3OD and in D_2O–CD_3OD solutions were calculated from the lifetimes of $^1O_2$ in the absence (τ_0) and in the presence (τ) of various PPF concentrations. The data were plotted according to a simple Stern–Volmer treatment (eqn. (1), Fig. 8):

$$\frac{1}{\Gamma} = \frac{1}{\Gamma_0} + k_{PPF}[PPF] \qquad (1)$$

These results confirm that PPF reacts with $^1O_2$ in methanol as well as in aqueous medium. The global rate constant (k_{PPF}) was found to be 2.66 × 10^5 M^{-1} s^{-1} and ~3.2 × 10^6 M^{-1} s^{-1} in CD_3OD and D_2O : CD_3OD (75 : 25 v/v), respectively. The deviation from the linearity, observed in Fig. 8(b), is due to the limit of solubility of PPF in aqueous solution of around 4 mM. The initial linear part of the curve was retained to estimate the rate constant. These values are close to the rate constants usually found for other phenol derivatives. The values of k_{PPF} are somewhat larger than those found for phenol (1.3 × 10^6 M^{-1} s^{-1} in D_2O and 2.5 × 10^4 M^{-1} s^{-1} in CD_3OD)[36] as expected from the electron donating properties of the isopropyl substituents. On the other hand, in CD_3OD, the value of k_{PPF} is quite similar to the value found for 2,6-di-*tert*-buthylphenol (k = 2.5 × 10^5 M^{-1} s^{-1}).[36] However, the rate constant for quenching of singlet

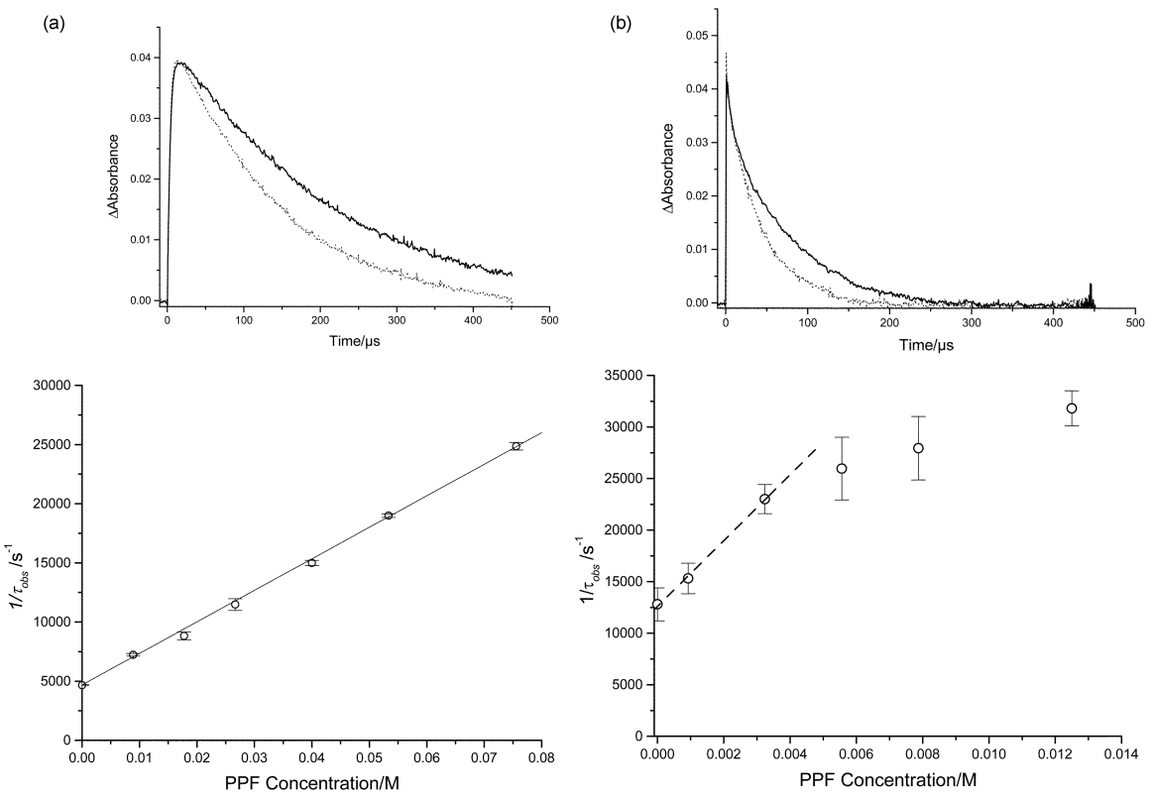

Fig. 8 Plot of ($1/\tau_{obs}$) *vs.* PPF concentration in: (a) CD_3OD; (b) D_2O–CD_3OD (75 : 25 v/v). The deviation from the linearity, observed in (b), is due to the limit of solubility of PPF in aqueous solution of around 4 mM. The insets show the transient absorbance changes from the singlet oxygen luminescence at the end of the laser pulse after 532 nm laser excitation of: (a) a HP solution dissolved in CD_3OD, in the absence (thick line) and in the presence of PPF (8.9 × 10^{-3} M, dotted line); (b) a TPPS solution dissolved in D_2O–CD_3OD (75 : 25 v/v), in the absence (thick line) and in the presence of PPF (3.2 × 10^{-3} M, dotted line).

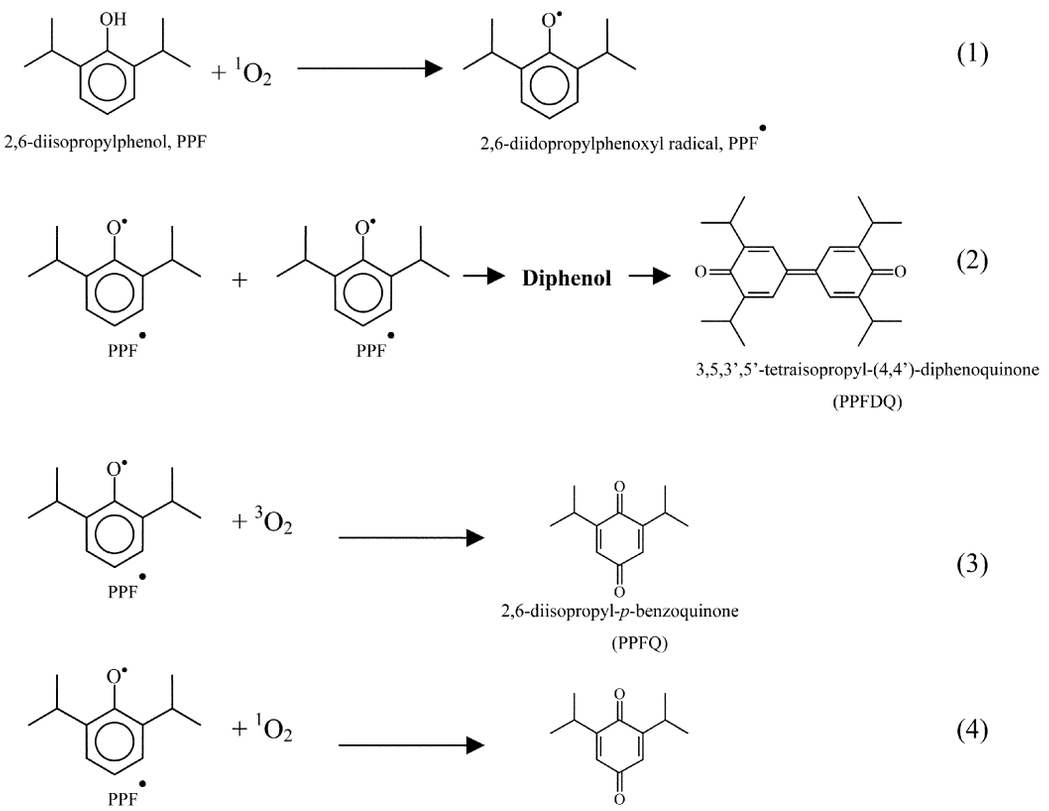

Fig. 9 Proposed mechanism for the reaction between propofol and singlet oxygen.

oxygen by α-tocopherol (vitamin E) in methanol ($k = 6.7 \times 10^8$ M^{-1} s^{-1}) is significantly higher.[9]

Discussion

The study described above illustrates for the first time the ability of PPF to interact with singlet oxygen. The decrease of the formation of PPF oxidation products in the presence of NaN_3 as well as direct measurement of singlet oxygen luminescence quenching by PPF strongly support this conclusion.

The GC and HPLC analysis indicate that PPFQ and PPFDQ are the oxidation products of the reaction between PPF and singlet oxygen. These results are in good agreement with the literature data on other phenols.[32–35]

However, the results obtained with D_2O must be emphasized. The substitution of phosphate buffer by D_2O leads to a $^1O_2$ lifetime increase. If PPFQ and PPFDQ formation result from a direct reaction between $^1O_2$ and PPF, an increase of both PPFQ and PPFDQ formation should be expected in D_2O. In fact, in D_2O an increased formation of PPFQ was observed while the PPFDQ formation was reduced (Fig. 5(b)). A direct reaction between PPFDQ and $^1O_2$ to form PPFQ should be proposed to explain this result. Nevertheless, preliminary experiments have proved that the PPFDQ spectrum remained unchanged in the absence or in the presence of irradiated RB (data not shown) arguing against the validity of this hypothesis. An attractive hypothesis is to speculate on the formation of PPF radicals (phenoxyl radical; PPF$^\bullet$) by reaction with $^1O_2$(reaction (1), Fig. 9). We proposed that species could dimerize to form diphenol which is subsequently oxidized in PPFDQ (reaction (2), Fig. 9). Moreover, the PPF radical could react with both fundamental and singlet oxygen to produce PPFQ (reactions (3), (4), Fig. 9).

This reaction scheme accounts for all our experimental results including the D_2O effect. Indeed, if a reaction between $^1O_2$ and PPF$^\bullet$ occurs to form PPFQ (reaction (4), Fig. 9), one would expect that an increase in $^1O_2$ lifetime favours this reaction at the expense of the bimolecular radical reaction (reaction (2), Fig. 9).

The hypothesis of PPF$^\bullet$ formation is supported by numerous data found on other phenols. Thomas and Foote[34] demonstrated conclusively that a phenolic compound (2,4,6-triphenylphenol) was able to give the corresponding phenoxyl radical subsequently to a $^1O_2$ quenching reaction. The same type of intermediate was suggested by Matsuura *et al.*[32,33] for the sensitized photooxidation of 2,6-di-*tert*-butylphenol. Moreover, Murphy *at al.*[2] demonstrated by electron spin resonance spectroscopy (ESR) that PPF was oxidized by potassium permanganate to form a phenoxyl radical. Mouithys-Mickalad *et al.*[4] proved by the same technique that the oxidation of PPF by both peroxynitrite and radical hydroxyl ($^\bullet$OH) gave rise to the formation of PPF-derived phenoxyl radical (PPF$^\bullet$). More recently, Kohnen *et al.* demonstrated that the reaction between PPF and $ONOO^-$ led to the formation of PPFQ and PPFDQ by a radical pathway.[6]

To conclude, the present results have shown unambiguously the ability of propofol to react with singlet oxygen. 2,6-Di-isopropyl-*p*-benzoquinone and 3,5,3′,5′-tetraisopropyl-(4,4′)-diphenoquinone were identified as the main photochemical products. The rate constant for the physical and chemical quenching of $^1O_2$ by PPF (k_{PPF}) were found to be 2.66×10^5 M^{-1} s^{-1} and ~3.2×10^6 M^{-1} s^{-1} in CD_3OD and D_2O:CD_3OD (75:25 v:v), respectively. These original results offer an interesting prospect regarding the role that PPF could play in biological systems.

Acknowledgements

This work was supported by the CGRI-CNRS-FNRS funds.

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