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Primary process in the photochemistry of fenbufen in acetonitrile

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Abstract

Photochemical properties of fenbufen, a non-steroidal anti-inflammatory agent, in acetonitrile, have been investigated using nano-second laser flash photolysis. The molar absorption coefficient at 282 nm was determined to be 25,000 $dm^3 mol^{-1} cm^{-1}$. Fenbufen showed a very weak fluorescence emission (Φ_f=0.001) centred at 320 nm. The quantum yield for intersystem crossing was determined to be unity and the triplet–triplet absorption spectrum showed a maximum at 420 nm with a molar absorption coefficient of 30,000 $dm^3 mol^{-1} cm^{-1}$. In aerated solution, fenbufen sensitised the formation of excited singlet oxygen with a quantum yield close to unity. These results indicate that fenbufen is a strong photodynamic agent which could cause adverse phototoxic reactions. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Fenbufen; Acetonitrile; Primary process; Photochemistry; Singlet oxygen; Laser; Triplet; Fluorescence; Phosphorescence

1. Introduction

Fenbufen (γ-oxo[1,1′-biphenyl]-4-butanoic acid) (see structure in Scheme 1) is a pro-drug of biphenylacetic acid, a non-steroidal anti-inflammatory drug (NSAID) which is used in the treatment of rheumatoid arthritis [1,2]. Adverse skin reaction, which is common to many NSAIDs such as Naproxen [3], Benoxaprofen [4], Azopropazone [5], is also associated with fenbufen [6]. Fenbufen is known to produce high incidence of skin rashes, especially in females. In-vitro and in-vivo studies have shown the formation of interleukin-1 upon exposure to UV radiation in the presence of the drug [7]. Guido de Guido et al. have demonstrated that fenbufen photosensitises the lysis of erythrocytes and peroxidation of unilamellar phosphatidylcholine [8]. Moreover, they have suggested the involvement of singlet oxygen in these reactions. However, no direct measurements of singlet oxygen or other photophysical parameters of fenbufen have been reported.

In our laboratory, we have determined photophysical parameters such as quantum yields of triplet formation, excited singlet oxygen formation, photoionisation for photosensitive drugs such as Chlorpromazine [9,10], Benoxaprofen and derivatives [11,12], Azapropazone [13] in order to correlate these photophysical parameters with phototoxicity.

In this study, we have investigated the photophysical properties of fenbufen in acetonitrile and have shown the quantum yield of triplet formation and excited singlet oxygen formation to be unity. Moreover, we have also shown that the lowest excited triplet state has π–π* characteristics and not n–π*.

2. Materials and methods

All chemicals including fenbufen were purchased from Sigma. All organic solvents were of super purity solvent grade obtained from Romil Chemicals. Solutions were either saturated with ‘white spot’ nitrogen, oxygen or air as appropriate.

UV–Vis absorption spectra were recorded using a Hewlett–Packard 8451 Diode Array Spectrophotometer coupled to a 7470A plotter and a 98155A keyboard.

Fluorescence and phosphorescence measurements were made on a Perkin–Elmer MPF-43A spectrofluorimeter fitted with a phosphorescence attachment. Fluorescence quantum yields were determined by comparison with tryptophan (Φ_f=0.13 [14]) as standard.

The laser flash photolysis experiments were carried out with a J.K. Lasers System 2000 Neodymium/YAG oscillator with a Neodymium/glass amplifier. The laser delivers up to

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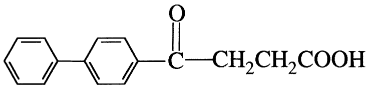

Scheme 1.

100 mJ of 266 or 355 nm radiation in single pulses of 12 ns duration [15]. A quartz prism was used to separate the 266 or 355 nm from the fundamental and other harmonics also produced by the laser system. The detection system consisted of a Xenon arc lamp and Applied Photophysics pulsing unit and monochromator, fitted with a stepper mortar and quartz optics. Optical transmissions (1 cm path length) at various wavelengths selected with the monochromator (bandwidths 1–10 nm) were measured as a function of time before and after the pulse using photoelectric detection. The output of the photomultiplier (Hammatsu 1384) was displayed on a Hewlett–Packard 5451A Digital Storage Oscilloscope. Data acquisition and processing were carried out using a Hewlett–Packard 9000 Series 300 computer.

The luminescence (1270 nm) from excited singlet oxygen ($^1\Delta_g$) produced by photoexcitation of fenbufen in oxygenated solution was detected by a liquid nitrogen cooled North Coast EO-817P germanium Detection System closely coupled to the laser photolysis cell in right-angle geometry. A 1 mm thick (20 mm diameter) piece of AR-coated silicon (II–IV Inc.) was placed between the diode and the cell to act as a narrow band filter for the 1270 nm luminescence. The detection system consists of HP Germanium photodiode EO-817 preamplifier and 823A preamplifier and detector Bias Power Supply unit operated at −350 V. The ac output from the amplifier was fed into the Hewlett–Packard Digitiser in the ac couple mode.

3. Results

3.1. Absorption, fluorescence and phosphorescence measurements

The absorption spectrum of fenbufen in acetonitrile showed two peaks at 202 and 282 nm with extinction coefficients of 33,000 and 25,000 $dm^3\,mol^{-1}\,cm^{-1}$, respectively. The high extinction coefficient is indicative of the long wavelength transition being of π–π* in nature, as in the case of biphenyl where the first band centred at 250 nm is ascribed to π–π* [16]. A red shift of 32 nm in the case of fenbufen compared to biphenyl may be due to the substituent effect. Fenbufen may be considered as a phenyl-substituted acetophenone in which one would expect to see an n–π* transition in the long wavelength region. This may be submerged under the strong π–π* transition. Fenbufen was insoluble in non-polar solvents such as cyclohexane, hence the effect of solvent polarity, which could shed light on the type of transition, could not be investigated. However, there was only a 4 nm blue shift in absorption maximum of the long wavelength band upon going from water to isopropanol. This shift, though small, adds weight to the suggestion that the absorption band centred at 282 nm is of π–π* in nature.

Fenbufen in acetonitrile showed a very weak fluorescence emission spectrum centred at 340 nm, the yield of which was less than 0.001 compared to tryptophan as the standard.

The phosphorescence emission spectrum at 77 K showed two peaks around 475 and 500 nm. From the emission maximum, an estimate for the lowest triplet state was made to be around 270 $kJ\,mol^{-1}$ which is lower than that for biphenyl (278 $kJ\,mol^{-1}$).

3.2. Laser flash photolysis

Fig. 1 shows the transient difference spectra obtained at the end (120 ns) and 41 us after pulsing a nitrogen saturated solution of $1\times10^{-5}\,mol\,dm^{-3}$ fenbufen in acetonitrile. The negative absorption seen below 310 nm is attributable to the depletion of the ground state of fenbufen, the absorption band centred at 420 nm decayed essentially by a second-order process with a rate constant of $5\times10^5\times\varepsilon\,dm^3\,mol^{-1}\,s^{-1}$.

The decay of the absorption centred at 420 nm was accelerated in the presence of air where the decay was essentially a first-order process with a rate of $1.8\times10^6\,s^{-1}$. On the basis of oxygen effect, the transient absorption centred at 420 nm is tentatively assigned to the triplet state of fenbufen.

3.3. Triplet–triplet absorption spectrum sensitised by benzophenone

The assignment of triplet–triplet absorption spectrum could be confirmed by sensitisation with a well known triplet sensitiser with high triplet energy such as benzophenone. Fig. 2 shows the transient absorption spectrum obtained at different times after delivering a pulse of 355 nm laser light to a nitrogen-saturated solution of benzophenone ($2\times10^{-3}\,mol\,dm^{-3}$) containing $4\times10^{-5}\,mol\,dm^{-3}$ fenbufen. Under these conditions, all the laser light is absorbed by benzophenone to produce the triplet state of benzophenone. The spectrum at the end of pulse (80 ns) shows two bands with maxima at 520 and 310 nm, respectively, and the spectrum is in good agreement with the triplet–triplet absorption spectrum of benzophenone in acetonitrile. The spectra at subsequent times show a decrease in absorption of the above two bands with a concomitant increase of a band centred at 420 nm with isobestic points at 360 and 460 nm. The spectrum after 9 μs, when benzophenone triplet has completely decayed, shows a maximum at 420 nm and is identical to the spectrum obtained when fenbufen solution was subjected to 266 nm laser pulse (see Fig. 1). Furthermore, the decay of the absorption at 520 nm matched the build up at 420 nm. It is clear that, as the benzophenone triplet decays, the fenbufen triplet is formed in accordance with reaction (1):

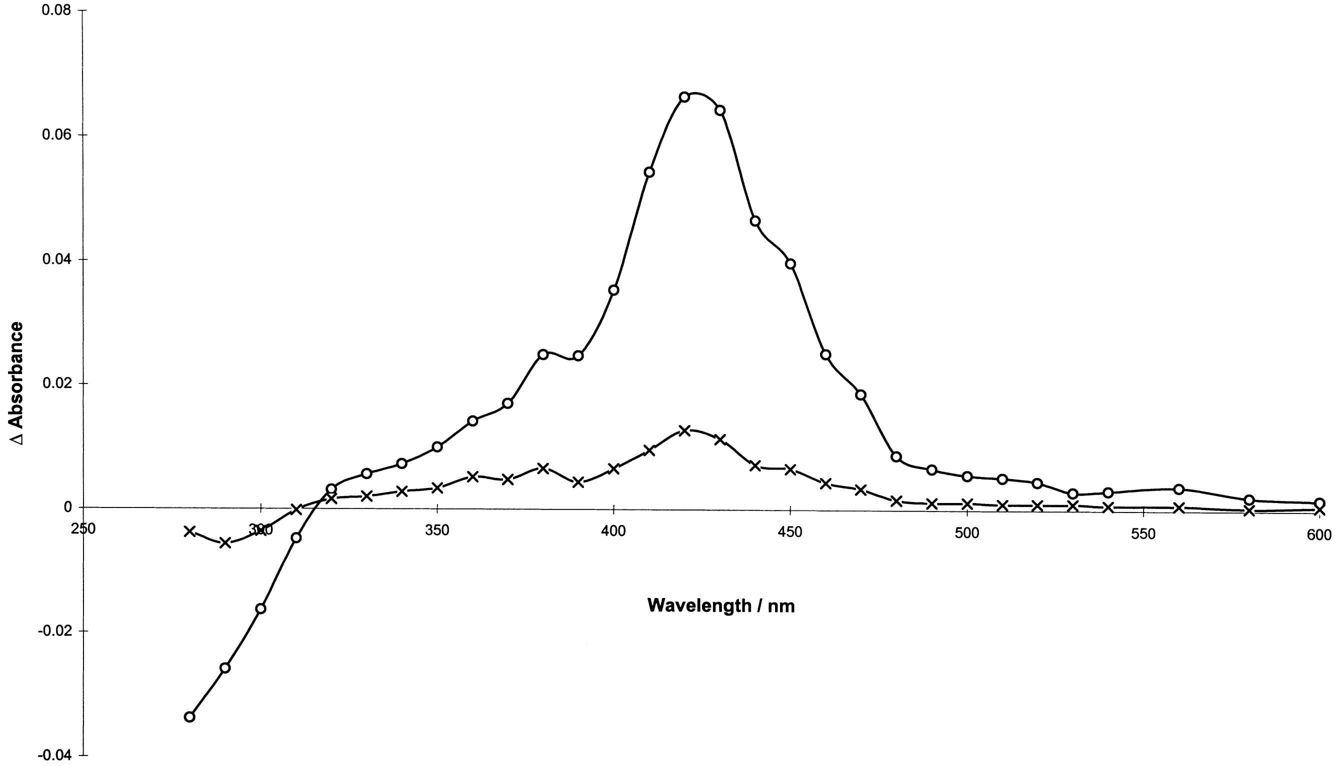

Fig. 1. Transient difference spectra observed at 120 ns (—○—) and 41 μs (—×—) after subjecting a nitrogen-saturated acetonitrile solution of 2×10^{-5} M fenbufen to 5 mJ of 266 nm laser pulse.

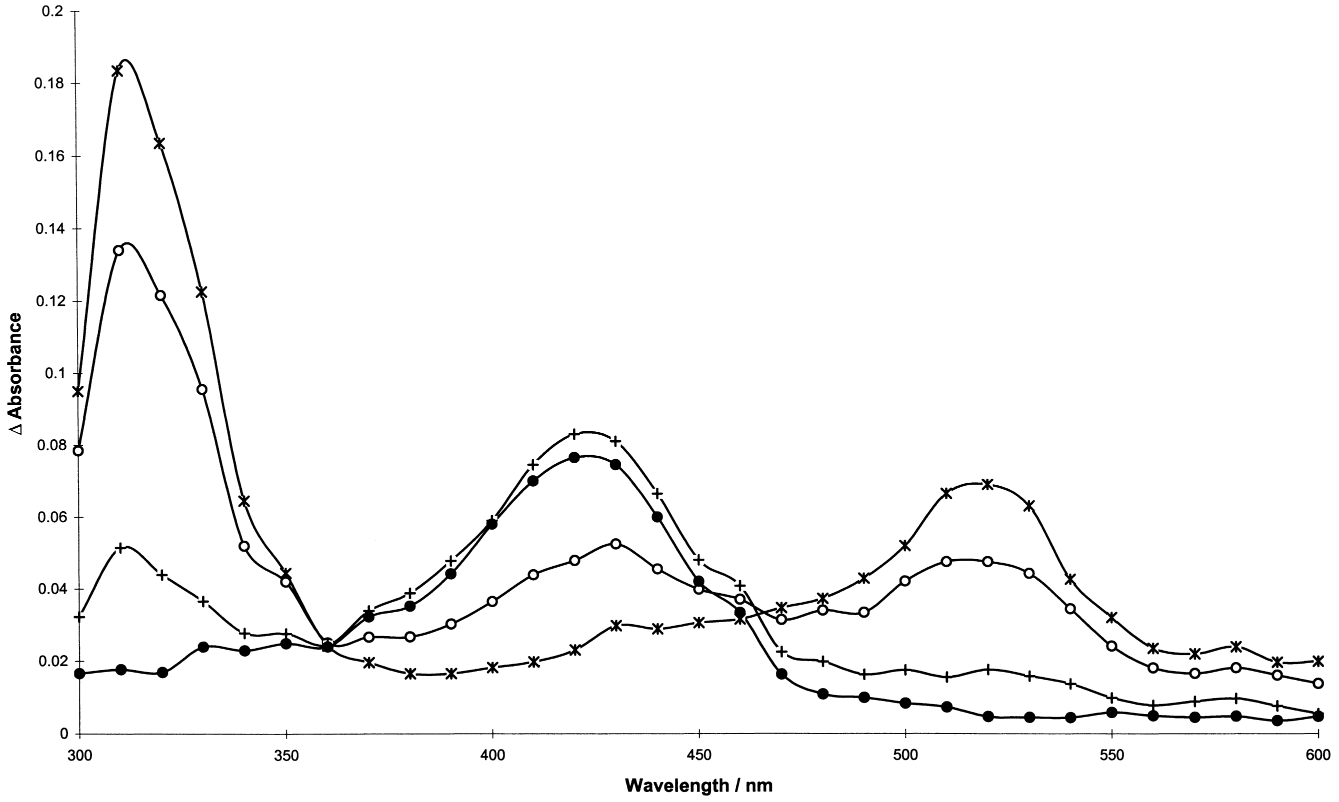

Fig. 2. Transient difference spectra observed at 80 ns (—*—), 800 ns (—○—), 4.3 μs (—+—) and 9 μs (—●—) after subjecting a nitrogen-saturated acetonitrile solution of 2×10^{-3} M benzophenone and 4×10^{-5} M fenbufen to 7 mJ of 355 nm laser pulse.

$$^{3}\mathrm{Bp}^{*} + \mathrm{Fen} \rightarrow {}^{3}\mathrm{Fen}^{*} + \mathrm{Bp} \qquad (1)$$

3.4. Determination of triplet–triplet extinction coefficient

The above experiment was repeated for various concentrations (1.0×10^{-5}–1×10^{-4} mol dm^{-3}) of fenbufen; however, very small laser energies of 1–3 mJ were used. The decay at 520 nm and the build up at 420 nm were determined and both rates were found to be linear with fenbufen concentration. From a plot of the above rates against the concentration of fenbufen, the bimolecular rate constant for reaction (1) was calculated to be $(4.0\pm0.2)\times10^{9}$ dm^3 mol^{-1} s^{-1}.

Fig. 3 shows typical oscilloscope traces observed at 520 and 420 nm in the absence (Fig. 3a) and in the presence of 2×10^{-5} mol dm^{-3} fenbufen (Fig. 3b and c), respectively. Under these conditions, absorption at the end of laser pulse (A_{520})at 520 nm is proportional to the benzophenone triplet formed by laser excitation:

$$[^{3}\mathrm{Bp}^{*}] = \frac{A_{520}}{\varepsilon_{520}}$$

which then sensitises the fenbufen triplet in accordance with reaction (1).

The amount of fenbufen triplet formed is proportional to the maximum absorption (A_{420}) at 420 nm observed under these conditions. Since the rate of decay of fenbufen triplet is not negligible compared to its rate of formation, correction for the decay was made in determining the maximum absorption (A_{420}) at 420 nm.

The fraction of benzophenone triplet that sensitises fenbufen triplet is given by $(k_1-k_0)/k_1$, where k_0 and k_1 are the rates of decay of benzophenone triplet in the absence and in the presence of fenbufen:

$$[^{3}\mathrm{Bp}^{*}]\frac{k_1-k_0}{k_1} = [^{3}\mathrm{Fen}^{*}]$$

$$\frac{A_{520}}{\varepsilon_{520}}\frac{k_1-k_0}{k_1} = \frac{A_{420}}{\varepsilon_{420}}$$

where ε_{520} is the extinction coefficient of triplet–triplet absorption of benzophenone at 520 nm in acetonitrile, which is taken to be 6500 dm^3 mol^{-1} cm^{-1} and ε_{420} is the extinction coefficient of triplet–triplet absorption of fenbufen at 420 nm in acetonitrile, which is calculated to be 30,000±3000 dm^3 mol^{-1} cm^{-1}.

3.5. Determination of quantum yield of triplet formation (ϕ_T)

The quantum yield of triplet formation was determined using the comparative technique. Benzophenone whose ϕ_T and ε_{520} are known is used as the standard.

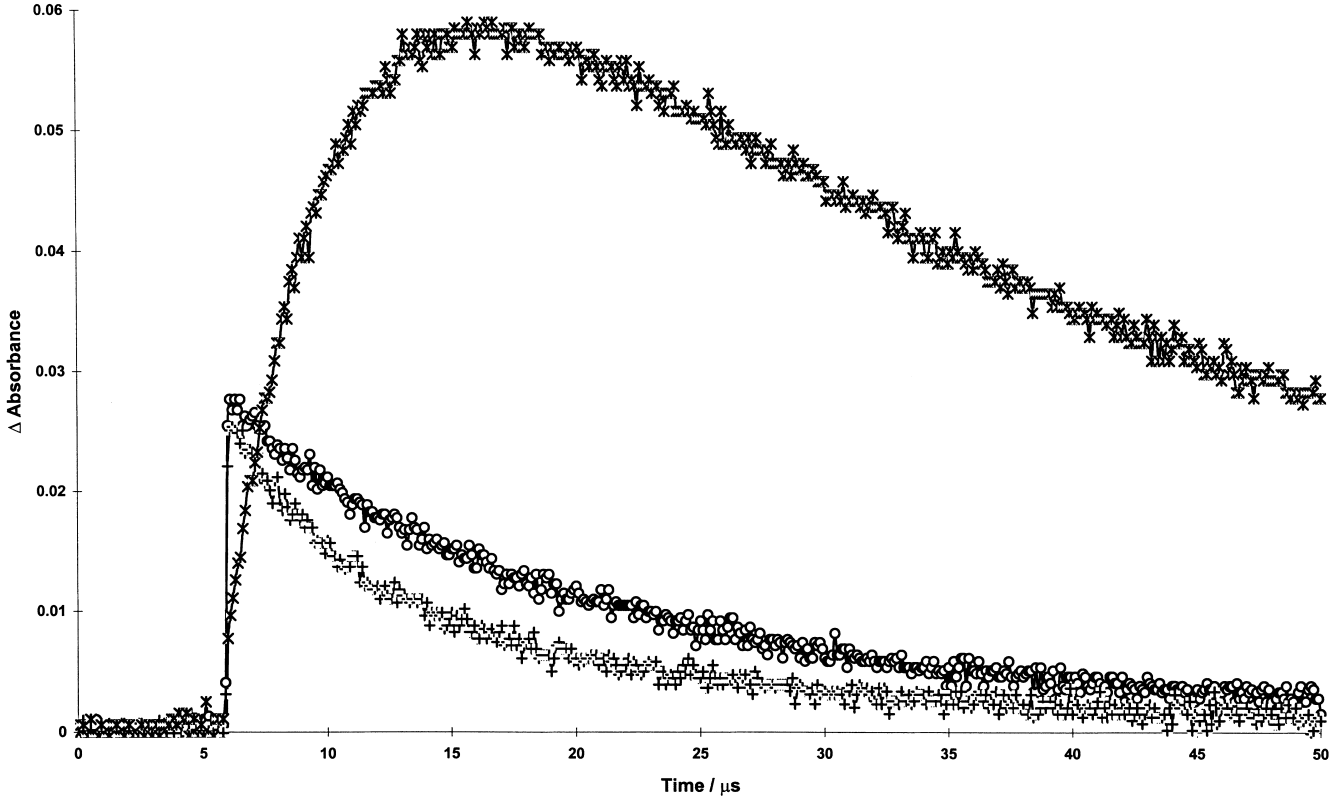

Fig. 3. Kinetic traces observed after subjecting a nitrogen-saturated acetonitrile solution of 2×10^{-3} M benzophenone in the absence (a) at 520 nm (—○—) and presence of 2×10^{-5} M fenbufen (b) at 520 nm (—+—) and (c) at 420 nm (—*—), to 3 mJ of 355 nm laser pulse.

In this experiment, two solutions of benzophenone and fenbufen with identical absorption at the excitation wavelength 266 nm were given laser pulses of a range of energies and the end of pulse absorbencies at 520 and 420 nm, respectively, were measured and found to be linear with energy over the range used. Since the absorbencies of the two solutions are the same at the excitation wavelength, for a given laser energy, equal number of photons are absorbed by the two solutions. Hence, the quantum yield of triplet formation is proportional to the triplet concentration:

$$\frac{\Phi_{T}^{Fen}}{\Phi_{T}^{Bp}} = \frac{[^{3}Fen^{*}]}{[^{3}Bp^{*}]}$$

$$\Phi_{T}^{Fen} = \Phi_{T}^{Bp}\frac{A_{420}\varepsilon_{520}}{A_{520}\varepsilon_{420}}$$

Using the values of 30,000 for ε_{420} (determined above), 6500 for ε_{520} [17] and 1.0 for Φ_{T}^{Bp}, quantum yield of triplet formation by fenbufen in acetonitrile is calculated to be 1.0.

3.6. Effect of oxygen on fenbufen triplet

The effect of oxygen on triplet–triplet absorption was further investigated by saturating the solution under investigation with nitrogen containing varying percentages of oxygen. The decay rate at 420 nm was found to be linear with the concentration of oxygen. From a plot of the decay rate against the concentration of oxygen, a bimolecular rate for reactions (2) and/or (3) is determined to be $9.8\times10^{8}\ dm^{3}\ mol^{-1}\ s^{-1}$:

$$^{3}Fen^{*} + {}^{3}O_{2} \rightarrow {}^{1}O_{2}^{*} + Fen \quad (2)$$

$$^{3}Fen^{*} + {}^{3}O_{2} \rightarrow O_{2}^{\bullet -} + Fen^{\bullet +} \quad (3)$$

where reaction (2) involves energy transfer reaction forming excited singlet oxygen, whilst, reaction (3) involves electron transfer reaction forming superoxide anion and the cation radical of fenbufen.

When an air-saturated solution of fenbufen in acetonitrile was given 266 nm laser pulse, the end of pulse spectrum was similar to that obtained with nitrogen saturated solution which decayed to zero absorption. No absorption corresponding to the cation radical of fenbufen was observed at the end of oxygen reaction. Hence, reaction (3) does not take place under these conditions.

3.7. Singlet oxygen measurements

In the absence of reaction (3), it is reasonable to assume that only reaction (2) occurs with the formation of excited singlet oxygen which could be detected by monitoring the phosphorescence emission from O_2 ($^{1}\Delta_{g}$) as described in Section 2.

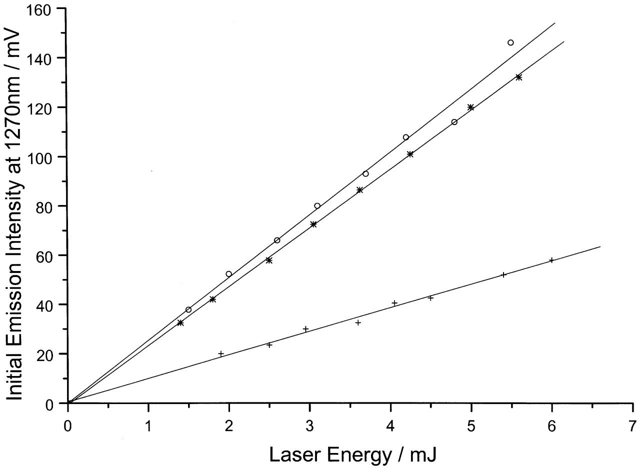

Fig. 4. A plot of initial emission intensity at 1270 nm as a function of laser energy for oxygen-saturated acetonitrile solutions of benzophenone (—+—), perinaphthanone (—*—) and fenbufen (—○—). All solutions had the same absorbance of 0.4 at 266 nm.

Fig. 4 shows the emission intensity (I_e) at the end of pulse when oxygenated solutions of benzophenone, perinaphthanone and fenbufen in acetonitrile (all having the same absorbance at 266 nm) were given a range of 266 nm laser pulses. I_e is linear with laser energy for all three compounds for the range of laser energies used.

For any given laser energy, the number of photons absorbed by any one of the three solutions will be the same since all of them have the same absorbance at the wavelength of excitation (266 nm).

Hence, the quantum of singlet oxygen formation sensitised by fenbufen ($^{Fen}\Phi_{\Delta}$) is calculated from

$$\frac{^{Fen}\Phi_{\Delta}}{^{Bp}\Phi_{\Delta}} = \frac{^{Fen}I_{e}}{^{Bp}I_{e}}$$

where $^{Bp}\Phi_{\Delta}$ which is taken as 0.36 [18]; $^{Fen}I_{e}$ and $^{Bp}I_{e}$ are initial emission intensities for fenbufen and benzophenone, respectively, at any given laser intensity and is obtained from Fig. 4. Using the above values, a value of 0.85 is obtained for $^{Fen}\Phi_{\Delta}$. Similarly, a value of 1.0 was obtained using perinaphthanone as the standard whose quantum yield is taken as 0.95 [19].

It is clear the quantum yield of singlet oxygen formation is almost unity in the case of fenbufen. The fact that triplet formation is unity with a very very low quantum yield of fluorescence implies that the S_{Δ} is unity. Moreover, this confirms our conclusion that no superoxide anion is formed to any appreciable amount according to reaction (3). Excited singlet oxygen is associated with photosensitised reactions and the value for fenbufen is nearly two and a half times that for well known phototoxic drugs benoxaprofen and its derivatives [11,12] and 50% higher than that for the photosensitiser hematoporphyrin [20]. The high yield of singlet oxygen in aerated solutions of fenbufen could be the cause of its phototoxic reactions.

Acknowledgements

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