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Dyes and Pigments 52 (2002) 199–208

DYES and PIGMENTS

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Diphenylchlorin and diphenylbacteriochlorin: synthesis, spectroscopy and photosensitising properties

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Received 9 January 2001; received in revised form 31 October 2001; accepted 8 November 2001

Abstract

Two new photosensitisers, diphenylchlorin and diphenylbacteriochlorin, were prepared from the reduction of 5,10-diphenylporphyrin. These dyes are characterised by strong light absorption in the red spectral region and afford high yields of photosensitised singlet oxygen, photosensitiser anion radicals, and superoxide anion radical, based on studies using EPR spectroscopy. Consequently, they are potential photosensitisers for photodynamic therapy. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Diphenylchlorin; Diphenylbacteriochlorin; Absorption; Fluorescence; Active oxygen species; Photosensitiser anion radicals

1. Introduction

Photodynamic therapy (PDT) is an innovative and attractive modality for the treatment of cancerous tumors. PDT requires both a selective photosensitiser and a light source that matches the absorption spectrum of the photosensitiser employed [1,2]. Following excitation of the photosensitiser to long-lived excited singlet and/or triplet states, tumors are destroyed by singlet oxygen (type II mechanism), and/or radical species (type I mechanism) [3–5].

A number of new photosensitisers have been developed for PDT in recent years, most of which are porphyrin-based compounds, including chlorins, bacteriochlorins and purpurins [6]. In addition to the first-generation photosensitiser "Photofrin" (a hematoporphyrin derivative), the second-generation drug "Benzoporphyrin derivative monoacid ring A" (BPDMA, Verteporfin) has received approval from the US FDA for clinical studies. Other second-generation photosensitisers are also undergoing clinical trials for PDT, including "Mono-L-aspartyl chlorin e_6" (MACE), "Tin Etiopurpurin" (SnET2), and "meso-tetra(*m*-hydroxyphenyl) chlorin" (m-THPC) [7,8].

5,15-Diphenylporphyrin (DPP), a relatively new porphyrin model compound that combines the features of two classic synthetic porphyrins, 5,10,15,20-tetraphenylporphyrin (TPP) and 2,3,7,8,12,13,17,18-octaethyl-porphyrin (OEP), has long been unavailable in quantities suitable for extensive study [9] because of the difficulties associated with its synthesis. Consequently, the characteristics of this molecule could not be fully explored

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PII: S0143-7208(01)00092-4

until a recent synthetic method was developed [10]. This work has opened the door to the synthesis of other DPP-based photosensitisers for PDT studies. In this regard, DPP-based chlorins such as meso-diphenylbenzochlorins and diphenyl-2,3-dihydroxychlorin have been synthesised as potential agents for PDT [11,12].

The aim of the present paper is to report the synthesis of 5,15-diphenylchlorin (DPC) and 5,15-diphenylbacteriochlorin (DPBC), along with results from the evaluation of their photophysical and photodynamic properties. Specifically, data from absorption, fluorescence, and EPR spectra and bleaching experiments are reported.

2. Experimental

2.1. Synthesis

The reagents and starting materials of analytical grades were purchased from Beijing Chemical Works (China). Pyrrole was distilled from CaH_2 at atmospheric pressure and CH_2Cl_2 was distilled from K_2CO_3. Column chromatography was performed on silica gel 60 (230–400 mesh).

2.1.1. 5-Phenyldipyrromethane (*1*)

A modification of a previous procedure for the synthesis of 5-phenyldipyrromethane (**1**) was employed [9]. In the present case, a mixture of benzaldehyde (5.0 ml, 49.1 mmol) and pyrrole (125 ml, 1.8 mol) was deoxygenated using Ar over 15 min, then TFA (0.38 ml, 4.9 mmol) was added and the solution was stirred under Ar for 15 min at room temperature. Excess pyrrole was removed by vacuum distillation with slight heating, to give a dark brown oil that was diluted with CH_2Cl_2 (50 ml). The resultant solution was washed with NaOH (0.1 M) and then with water, and dried over Na_2SO_4. The CH_2Cl_2 solution was charged onto a flash chromatography column and chromatographed by eluting with CH_2Cl_2, monitoring by TLC (CH_2Cl_2:hexane = 1:1). The fractions having the component with $R_f = 0.37$ were collected and evaporated under reduced pressure on a rotary evaporator to yield a yellow oil. This oil was transferred to a sublimation apparatus and subjected to a vacuum (0.01 mm Hg) for 1 h at room temperature, and then to heating at ~0.75 °C/min until visible sublimation began at 130 °C. After sublimation ceased, the product was collected from the cold finger as colorless crystals (5.7 g, 50%), m.p. 101–102 °C (lit. 102–102.5 °C [10]).

^{1}H NMR (200 MHz, $CDCl_3$): 7.90 (*bs*, 2H), 7.33–7.19 (*m*, 5H), 6.67 (*q*, 2H), 6.13 (*q*, 2H), 5.90 (*m*, 2H), 5.45 (*s*, 1H). MS (EI): *m*/*z* 222 (M^+). Anal. calcd. for $C_{15}H_{14}N_2$: C, 81.05; H, 6.35; N, 12.60; Found: C, 81.26; H, 6.32; N, 12.49.

2.1.2. 5,15-Diphenylporphyrin (*2*)

A modification of previously reported procedures for the synthesis of 5,15-diphenylporphyrin (**2**) was used. Trichloroacetic acid (21.2 g, 130 mmol) in CH_2Cl_2 (600 ml) was added dropwise to a stirred solution of compound **1** (1.2 g, 5.4 mmol) and trimethyl orthoformate (44 ml, 0.40 mol) in CH_2Cl_2 (1.6 l) over 15 min. The resultant solution was stirred in the dark at room temperature for 4 h. Pyridine (40 ml) was added and stirring was continued in the dark for 17 h. The solution was then treated with oxygen for 30 min and stirred under normal lighting conditions for 4 h. The CH_2Cl_2 was evaporated under reduced pressure on a rotary evaporator until it was ~90% removed. Water (150 ml) was added and evaporation was continued with a rotary evaporator until a heavy precipitate appeared and the liquid was colorless. The resultant black solid was collected by filtration, air-dried, then dissolved in 50 ml CH_2Cl_2 and mixed with 10 g silica gel. The slurry was dried to afford a dark powder that was loaded onto the top of a flash chromatography column (100 g). Eluting with a mixture of CH_2Cl_2:hexane (7:3) followed by evaporation of the eluent gave a purple solid that was digested with MeOH. The mixture was filtered to give purple crystals (319 mg) that were recrystallised from toluene (30 ml) containing 1% pyridine to give pure **2** (220 mg, 18%), m.p. > 300 °C (lit. > 300 °C [10]).

UV–vis λ_{max} (log ε) in benzene: 406 nm (5.61), 500 nm (4.25), 536 nm (3.71), 574 nm (3.75), 630 nm (3.16). ^{1}H NMR (200 MHz, $CDCl_3$): δ −3.01 (*brs*, 2H), 7.85–7.90 (*m*, 6H), 8.35 (*dd*, 4H), 8.97 (*d*, 4H), 9.38 (*d*, 4H), 10.31 (*s*, 2H). FAB–MS: *m*/*z* 463 ($M+1^+$). Anal. calcd. for $C_{32}H_{22}N_4$: C,

83.09; H, 4.79; N, 12.11. Found: C, 82.81; H, 4.89; N, 11.90.

2.1.3. 5,15-Diphenylchlorin (3)

A mixture of **2** (200 mg, 0.43 mmol), *p*-toluenesulfonylhydrazine (0.2 g, 1.1 mmol) and anhydrous K_2CO_3 (500 mg) in dry pyridine (50 ml) was heated with stirring at 105 °C under Ar for 2 h. *p*-Toluenesulfonylhydrazine (0.2 g) in pyridine (1 ml) was added and the reaction mixture was heated with stirring at 105 °C under Ar for another 2 h. Benzene (300 ml) and distilled water (150 ml) were added and the mixture was heated on a steam bath for 1 h. The organic layer was collected and washed in turn with cold HCl (2 M), saturated $NaHCO_3$ solution, and distilled water. Chloranil (220 mg, 0.89 mmol) was added in portions to the stirred organic solution at room temperature until the absorption band at 734 nm disappeared. The solution was washed with 5% $NaHSO_3$, 5% NaOH, 50% (w/w) H_3PO_4, saturated $NaHCO_3$, and distilled water, then dried over anhydrous Na_2SO_4. After evaporation of benzene under vacuum, the crude chlorin was charged onto the top of a flash chromatography column and eluted with CH_2Cl_2: hexane (1:1). The fractions having the component with $R_f = 0.80$ were collected and evaporated under reduced pressure. The resultant blue solid (122 mg) was recrystallised from toluene (10 ml) to give **3** (102 mg, 51%) as blue crystals, m.p. > 300 °C.

UV–vis λ_{max} (log ε) in benzene: 363 nm (4.53), 409 nm (5.21), 505 nm (4.11), 532 nm (3.72), 593 nm (3.62), 645 nm (4.63). IR (KBr) cm^{-1}: 3380, 2923, 2849, 1606, 1440, 1417. ^1H NMR (200 MHz, $CDCl_3$): δ 1.8 (*brs*, 2H), 4.38 (*t*, 2H), 4.69 (*t*, 2H),7.77 (*m*, 6H), 7.96 (*m*, 2H), 8.21 (*d*, 1H), 8.33 (*d*, 1H), 8,51 (*m*, 2H), 8.89 (*m*, 2H), 9.11 (*d*, 1H), 9.35 (*d*, 1H), 9.97 (*s*, 1H), 10.78 (*s*, 1H). FAB–MS: *m*/*z* 465 ($M+1^+$). Anal. calcd. for $C_{32}H_{24}N_4$: C, 82.73; H, 5.21; N, 12.06. Found: C, 82.30; H, 5.57; N, 11.74.

2.1.4. 5,15-Diphenylbacteriochlorin (4)

A mixture of **2** (100 mg, 0.22 mmol), *p*-toluenesulfonylhydrazine (0.2 g, 1.1 mmol) and anhydrous K_2CO_3 (2.0 g) in dry pyridine (30 ml) was heated with stirring at 105 °C under Ar for 20 h. Every 1.5 h, a solution of *p*-toluenesulfonylhydrazine (0.15 g) in pyridine (1 ml) was added and the reaction mixture was allowed to stand under Ar at room temperature for 8 h, after the first 10 h of heating. Benzene (200 ml) and distilled water (100 ml) were added to the cooled reaction mixture and the reaction mixture was heated on a steam bath for 1 h. The organic layer was collected and washed in turn with HCl (2 M), 68% (w/w) phosphoric acid (5×100 ml), saturated $NaHCO_3$, and distilled water, and dried over anhydrous Na_2SO_4. After evaporation of benzene under vacuum, the crude bacteriochlorin was purified by column chromatography (eluting first with hexane, and then with a mixture of hexane/CH_2Cl_2 containing gradually higher amounts of CH_2Cl_2 until CH_2Cl_2: hexane (1:1) was achieved. The resultant green solid (27 mg) was recrystallised from CH_2Cl_2/hexane (1:1) to give **4** (20 mg, 19%) as green crystals, m.p. > 300 °C.

UV–vis λ_{max} (log ε) in benzene: 349 nm (5.07), 373 nm (5.16), 506 nm (4.66), 734 nm (5.11). IR (KBr) cm^{-1}: 3372, 2925, 2851, 1606, 1440, 1419. ^1H NMR (200 MHz, $CDCl_3$): δ 1.53 (*brs*, 2H), 4.39 (*t*, 4H), 4.67 (*t*, 4H),7.67 (*m*, 6H), 7.86 (*m*, 4H), 8.19 (*d*, 2H), 8.81 (*d*, 2H), 9.97 (*s*, 2H). FAB–MS: *m*/*z* 467 ($M+1^+$). Anal. calcd. for $C_{32}H_{26}N_4$: C, 82.38; H, 5.62; N, 12.0l. Found: C, 81.98; H, 5.61; N, l1.97.

2.2. Measurements

IR spectra were recorded on a BIO-RAD FTS spectrophotometer. ^1H NMR spectra were recorded on a Varian Gemini-300 (200 MHz) in $CDCl_3$ with TMS as the internal standard. FAB mass spectra were recorded on an AEI-MS 50 Kratos spectrometer and EI mass spectra on a VGTR10-200 spectrometer.

UV–vis absorption spectra were recorded on a Hew1ett-Packard 854l A diode array spectrophotometer. The absorption spectra were recorded in spectroscopic grade benzene from Beijing Chemical Works, China. Molar absorption coefficients were determined from the slope of the Beer-Lambert plots for a series of five dilutions, with each solution having an absorbance value below 1.0.

Emission spectra were recorded on a Perkin-Elmer LS-5 spectrofluorimeter, using spectroscopic grade benzene as the solvent. Air- and nitrogen-

saturated solutions were studied at room temperature. In each case, the Q (0,0)-band absorption values were below 0.05. Excitation was performed at 510 nm, and the fluorescence emission spectra were corrected for instrument response time and temporary variations in light intensity. Fluorescence quantum yields were determined by comparison with the reference compound meso-tetraphenylporphyrin (TPP) ($\Phi_f = 0.13$ in the nitrogen-saturated solution, $\Phi_f = 0.11$ in the air-saturated solution) [13].

2.3. EPR measurements

5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from Aldrich Chemical Company, and purified prior to use using activated charcoal under an Ar atmosphere. 2,2,6,6-Tetramethyl-4-piperidone (TEMP), 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical (TEMPO) and *p*-benzoquinone were also obtained from Aldrich Chemical Company. 1,4-Diaza-bicyclo[2,2,2]octane (DABCO) was purchased from Merck Chemical Company and dithiothreitol (DTT) was purchased from Sigma Chemical Company. The spectroscopic grade solvents, chloroform, acetonitrile and all other reagents were purchased from Beijing Chemical Works (China).

In a series of EPR experiments, singlet oxygen ($^1O_2$) formation was detected using TEMP as a spin trap, superoxide anion radical ($O_2^{\cdot-}$) formation was detected using DMPO a spin trap, and photosensitiser anion radical ($Sens^{\cdot-}$) formation was detected using TEMPO as a spin trap. EPR spectra were recorded at room temperature (22–24 °C), using a Bruker ESP-300E spectrometer operating at 9.8 GHz and equipped with an X-band of 100 kHz field modulation. A xenon arc lamp (150 W) with an intensity of 3.6 W/cm^2 and equipped with UV and IR cut-off filters (transmittance 470–750 nm) was used as the light source. A water-jacketed cooling device was used to eliminate heat emission. Dye solutions were made in cuvettes, which allowed treatment of each medium with O_2 or Ar for 30 min in the dark, or they were irradiated outside the instrument cavity in the cuvettes and then immediately transferred into the quartz capillaries used in EPR experiments.

2.4. Determination of quantum yields for $^1O_2$ generation

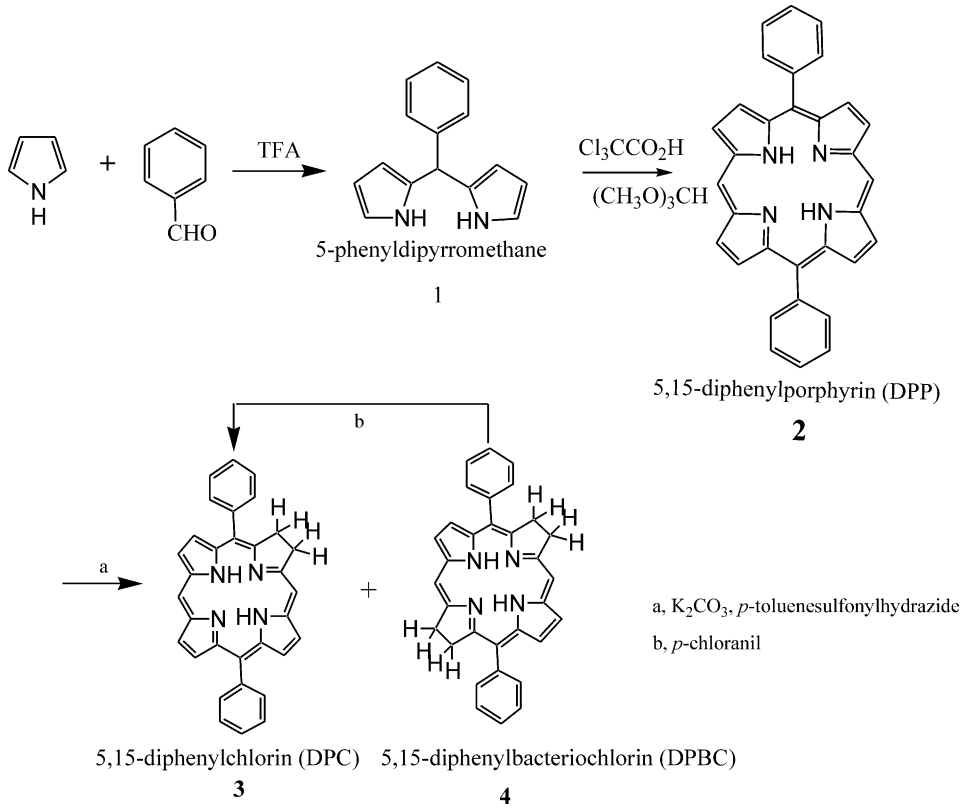
The $^1O_2$ generating efficiency for DPC was determined using the DPA-bleaching method described in an earlier report [14]. 9,10-Diphenylanthracene (DPA) was obtained from Aldrich Chemical Company, and the reference compound haematoporphyrin ($\Phi_\Delta = 0.79$) was purchased from Theodor Shuchardt Gmbh (Germany). The photo-oxidation of DPA sensitized by DPC was carried out on a 'merry-go-round' apparatus, using a medium-pressure rare earth's mercury lamp (400 W) as the 1ight source. Filters were used in combination to isolate the 410 nm emission of the lamp (maximum transmittance = 45%). The reactions were followed spectrophotometrically by observing the decrease in the 374 nm absorption band of DPA as a function of irradiation time.

3. Results and discussion

3.1. Synthesis

The synthesis of DPC and DPBC is summarised in Scheme 1. DPP was prepared as described in the literature [9,10], with modification of the work-up procedures. In the present work-up procedure, water was added to the reaction mixture during the evaporation of CH_2Cl_2. Thus when CH_2Cl_2 was completely removed, leaving pyridine/water, filtration and air-drying gave DPP suitable for chromatography.

The intermediate porphyrin (**2**) has been reduced using diimide, in the method of Whitlock et al. [15]. When DPC was the target compound, this step required 4 h, which was less than the time required in the synthesis of meso-tetraphenylchlorin (TPC) [15], presumably due to the absence of steric hindrance in DPP. The formation of DPBC was quite difficult, requiring a long reaction time and a large amount of *p*-toluenesulfonylhydrazine. Separation of the product mixture was facilitated by differences in the solubilities of the reduced porphyrins in benzene and phosphoric acid. Since the basicity of DPP is higher than that of TPP [9], a dilute H_3PO_4 solution could be used



Scheme 1. The synthesis of DPC and DPBC.

to separate DPC and DPBC from DPP, in contrast to the separation of TPC and meso-tetraphenylbacterichlorin (TPBC) from TPP [15].

3.2. Absorption and fluorescence spectra

3.2.1. Absorption spectra

The absorption spectra of DPC and DPBC in benzene are shown in Fig. 1. DPC gave a broad soret band at 409 nm and a relatively sharp Q(0,0) band ($\varepsilon = 42{,}000\ M^{-1}\ cm^{-1}$) at 645 nm. DPBC gave an intense narrow absorption at 734 nm ($\varepsilon = 130{,}000\ M^{-1}\ cm^{-1}$), a spilt soret band at 349 and 373 nm, and another band of intermediate intensity at 506 nm. The soret band for DPC ($\varepsilon = 162{,}000\ M^{-1}\ cm^{-1}$) was significantly less intense than the corresponding band from TPC ($\varepsilon = 190{,}000\ M^{-1}\ cm^{-1}$) [15]. The absorption spectra of DPC and DPBC were similar, albeit slightly blue-shifted compared with those of TPC and TPBC [15]. The blue-shifts were attributed to the presence of two meso substituents in DPC and DPBC, as a similar blue-shift occurred upon switching from TPP [16] to DPP [10] to porphine [17].

3.2.2. Fluorescence spectra and quantum yields

The fluorescence spectra of DPC and DPBC in nitrogen-saturated benzene are shown in Fig. 2. The chlorin DPC exhibited a strong Q (0,0) fluorescence band near 646 nm and a weak emission band in the 670–740 nm region. The latter exhibited discernible maxima near 687 and 712 nm. DPBC gave an intense Q (0,0) fluorescence band near 737 nm and a broad weak band in the 760–840 nm region. DPC produced fluorescence quantum yields of 0.18 in a nitrogen-saturated solution and 0.15 in an air-saturated solution. DPBC gave fluorescence quantum yields of 0.14 and 0.12 in nitrogen-saturated and air-saturated solutions, respectively.

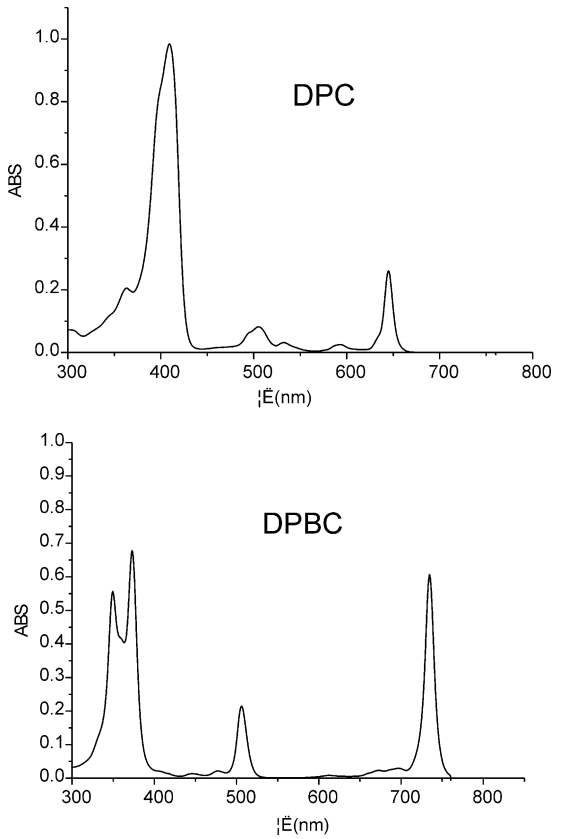


Fig. 1. Absorption spectra of DPC and DPBC in benzene.

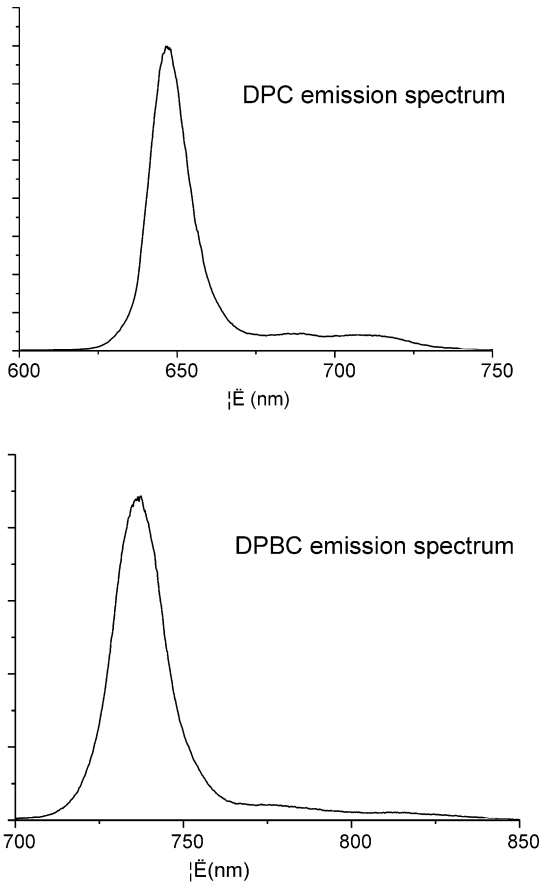


Fig. 2. Fluorescence spectra of DPC and DPBC in nitrogen-saturated benzene.

3.3. Assessment of photodynamic properties

Photosensitisers are believed to function in PDT through the generation of singlet oxygen and radical species such as the superoxide anion radical and photosensitiser anion radicals. With this in mind, EPR measurements and 9,10-diphenylanthracene-induced bleaching experiments were used to evaluate the photosensitizing properties of DPC and DPBC.

3.3.1. Superoxide anion radical generation

When CH_3CN:$CHCl_3$ (95:5) containing DPC or DPBC photosensitiser (0.1 mM) and DMPO (50 mM) was irradiated, EPR signals appeared immediately (Fig. 3). The EPR spectrum was characterized by three coupling constants that can be attributed to the nitrogen atom and the hydrogen atoms in the β and γ positions. The values determined, $g = 2.0056$, $\alpha^N = 13.0$ G, $\alpha_\beta^H = 10.0$ G and $\alpha_\gamma^H = 1.4$ G, are consistent with data reported in the literature for the DMPO–superoxide radical adduct [18]. Control experiments suggested that photosensitiser, oxygen and irradiation were all required to produce the EPR signal shown in Fig. 3. The addition of *p*-benzoquinone (4 mM), an efficient scavenger of $O_2^{\bullet -}$ [19], prior to irradiation reduced the intensity of the EPR signal significantly. These observations confirmed that the EPR spectrum is due to the DMPO–$O_2^{\bullet -}$ adduct.

The results in Fig. 4 suggest that DPC and DPBC efficiently facilitate the generation of $O_2^{\bullet -}$. Also, the addition of electron donors such as DTT (5 mM) significantly enhanced the intensity of the EPR signal for the DMPO–$O_2^{\bullet -}$ adduct, suggesting that anion radicals of these photosensitisers could

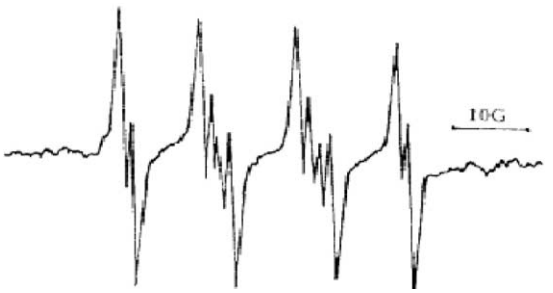


Fig. 3. EPR spectra of DMPO–$O_2^{\bullet-}$ produced from irradiation of an oxygen-saturated 95 CH_3CN:5 $CHCl_3$ solution of DPBC (0.1 mM) and DMPO (50 mM).

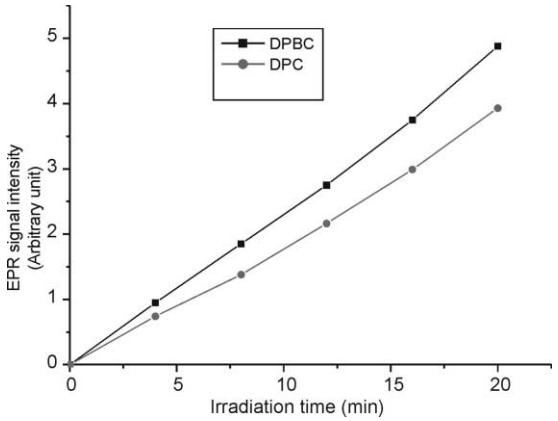
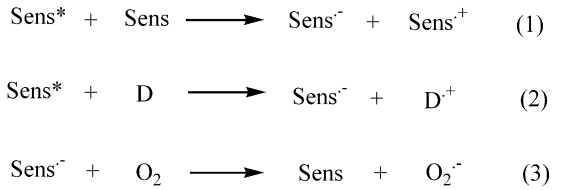


Fig. 4. Signal intensity of the spin-adduct DMPO–$O_2^{\bullet-}$ during illumination of an oxygen-saturated 95 CH_3CN:5 $CHCl_3$ solution of DPC and DPBC (0.1 mM) and DMPO (50 mM).

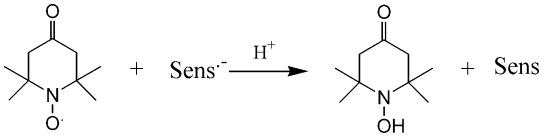
be precursors in $O_2^{\bullet-}$ formation. In the absence or presence of electron donors, $Sen^{\bullet-}$ species could form and then transfer an electron to oxygen to form $O_2^{\bullet-}$, in the presence of oxygen (Scheme 2). The addition of DABCO (10 mM), an $^1O_2$ inhibitor, had no effect on the intensity of the DMPO–$O_2^{\bullet-}$ EPR signal, indicating that $^1O_2$ is not involved in the formation of $O_2^{\bullet-}$ by DPC and DPBC.

3.3.2. Formation of photosensitiser anion radicals

In this aspect of our work, a method developed for the indirect detection of porphyrin anion radicals was employed [20]. This method is based on the observation that under anaerobic conditions the generated porphyrin anion radical reduces TEMPO, eliminating its spin (Scheme 3). We employed this method to determine the ability of DPC and DPBC to produce $Sen^{\bullet-}$ species.



Scheme 2. Formation of $O_2^{\bullet-}$ in the absence or presence of electron donors.



Scheme 3. Elimination of the spin of TEMPO using photosensitiser anion radicals.

A solution of TEMPO (0.05 mM) in anaerobic CH_3CN:$CHCl_3$ (95:5) underwent degradation when exposed to light in the present of photosensitiser (0.1 mM). No significant degradation was observed in solutions exposed in the presence of air. In addition, no degradation was observed when solutions of TEMPO were exposed to photosensitiser in the dark or to light in the absence of photosensitiser. Thus, the observed spin elimination was not mediated by active oxygen species because this process is suppressed in aerated solutions.

The electron donor DTT significantly enhanced the photodegradation of TEMPO in anaerobic solutions. This confirmed that the spin elimination of TEMPO was caused by the reaction of TEMPO with $Sen^{\bullet-}$ species.

The results in Fig. 5 show that DPC and DPBC efficiently eliminated the spin of TEMPO, and their relative efficiency in this regard correlated with their ability to produce superoxide anion radicals in oxygen-saturated solution.

3.3.3. Singlet oxygen formation

$^1O_2$ photosensitization by DPC and DPBC in $CHCl_3$ was also investigated by EPR, using TEMP as a spin trap. It had been reported that TEMP readily reacts with $^1O_2$ to give TEMPO [21], a process detectable by EPR spectroscopy. In our studies, the irradiation of oxygen-saturated $CHCl_3$ solutions containing photosensitiser (0.1 mM) and TEMP (10 mM) afforded the three-line EPR

spectrum expected for TEMPO (Fig. 6) [21]. However, in the absence of photosensitiser, oxygen or light TEMPO formation did not occur. Similarly, the presence of the $^1O_2$ scavenger DABCO suppressed the EPR signal of TEMPO.

We found that DPC was a considerably more effective photosensitiser for $^1O_2$ production in $CHCl_3$ than DPBC (Fig. 7). This was the case despite the intense absorption of DPBC at 734 nm, which is an ideal wavelength for PDT. The reason for the lower effectiveness of DPBC is not well understood. Presumably this is because the energy of the triplet states of DPBC was not high enough to produce $^1O_2$ [22].

The $^1O_2$-induced photooxidation of DPA to the corresponding endoperoxide derivative is often used to detect $^1O_2$ formation during photosensitization [14]. In order to determine the quantum yield for $^1O_2$ generation by DPC photosensitization, the DPA photooxidation method was employed and haematoporphyrin was used as the reference ($\Phi_\Delta = 0.79$). During these experiments, the DPC concentration (10 μM) remained constant and the intensity of the haematoporphyrin absorption band (410 nm) was adjusted to be the same as that for the DPC solution.

The rates of DPC photosensitized oxidation of DPA in $CHCl_3$ (line 2) are shown in Fig. 8. It was clear from the results of control experiments that oxidation of DPA did not occur in the absence of

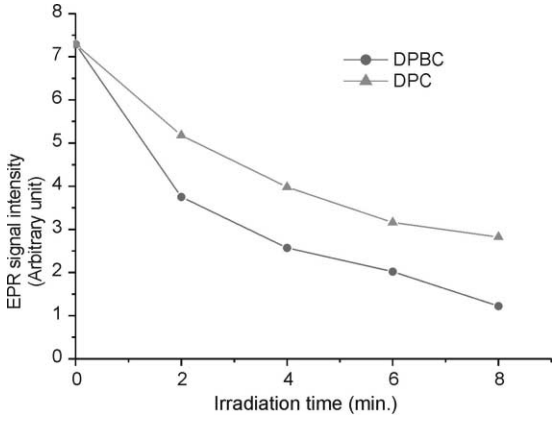


Fig. 5. Spin elimination of TEMPO (0.05 mM) by DPC and DPBC (0.1mM) in anaerobic 95 CH_3CN : 5 $CHCl_3$ as a function of illumination time.

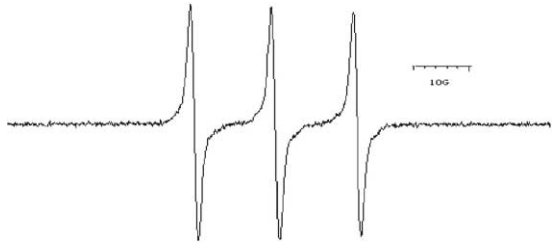


Fig. 6. EPR spectra obtained from irradiation of an oxygen-saturated $CHCl_3$ solution of DPC (0.1 mM) and TEMP (10 mM).

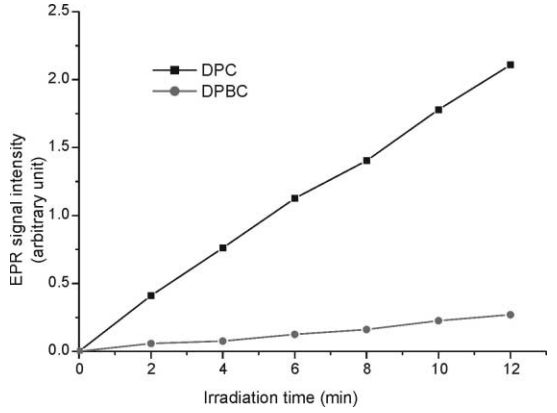


Fig. 7. The EPR signal intensities of TEMPO adducts formed in $CHCl_3$ containing DPC and DPBC (0.1 mM) and TEMP (10 mM) as a function of illumination time.

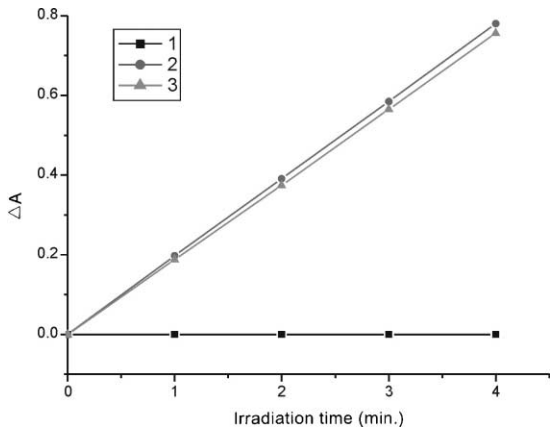


Fig. 8. Photosensitized DPA-bleaching measured via the absorbance decreases (ΔA) of DPA at 374 nm as a function of irradiation time (min).

Table 1
Spectral data and quantum yields of $^1O_2$ from DPC and DPBC

Collected data	DPC	DPBC
λ_{max} (log ε) in benzene	363 nm (4.53), 409 nm (5.21), 505 nm (4.11), 532 nm (3.72), 593 nm (3.62), 645 nm (4.63)	349 nm (5.07), 373 nm (5.16), 506 nm (4.66), 734 nm (5.11)
F_{max} (nm) in benzene	646	737
Φ_F in N_2-saturated benzene	0.18	0.14
Φ_F in air-saturated benzene	0.15	0.12
Φ_Δ in $CHCl_3$	0.81	–

DPC, oxygen or irradiation. We also found that DPC is about 3% more effective than haematoporphyrin in the photosensitized generation of $^1O_2$. The quantum yield for DPC-induced $^1O_2$ generation in $CHCl_3$ is thus estimated to be 0.81 versus 0.79 for haematoporphyrin.

4. Conclusions

Using variations of previously reported methods, the synthesis of DPC and DPBC in quantities suitable for their evaluation as potential sensitisers for PDT can be achieved. Spectral data and quantum yields of $^1O_2$ from DPC and DPBC are summarized in Table 1. These new photosensitisers produce $O_2^{\bullet-}$ via electron transfer from photosensitiser ion radicals to molecular oxygen, and $^1O_2$ by energy transfer from triplet photosensitiser to molecular oxygen. However, only photosensitiser ion radicals are produced during irradiations in the absence of oxygen. Since the formation of $^1O_2$ is critical to the effectiveness of PDT agents, it is important to note that DPC is a very effective $^1O_2$ sensitiser. DPC and DPBC are capable of producing superoxide anion radical under aerobic conditions and photosensitiser anion radicals under anaerobic conditions. Under aerobic conditions the formed photosensitiser anion radicals react rapidly with molecular oxygen to produce $O_2^{\bullet-}$.

The results of this study also indicate that DPP-based photosensitisers can exhibit photodynamic activity via type I and II mechanisms. This is evident from observations showing that DPBC is less effective than DPC in the production of singlet oxygen but more efficient in the production of superoxide anion radicals and photosensitiser anion radicals.

It is clear from our results that DPP-based photosensitisers have potential utility as PDT agents and merit further investigation.

Acknowledgements

The NSFC (39830090, 20172055), 973 Project (2000078100) and the National Key Laboratory for Structural Chemistry of Unstable and Stable Species supported this work.

References

[1] Macdonald IJ, Dougherty TJ. J Porphyrins Phthalocyanines 2001;5:105.
[2] Henderson BW, Dougherty TJ. Photodynamic therapy, basic principles and clinical applications. New York: Marcell Decker, 1992.
[3] Ochsner MJ. Photochem Photobiol B Biol 1997;39:1.
[4] Bonnett R. Chem Soc Rev 1995;24:19.
[5] Henderson BW, Dougherty TJ. Photochem Photobiol 1992;55:145.
[6] Sternberg ED, Dolphin D. Tetrahedron 1998;54:4151.
[7] Bonnett R. Rev Contemp Pharmacother 1999;10:1.
[8] Kessel D, Dougherty TJ. Rev Contemp Pharmacother 1999;10:19.
[9] Brückner C, Posakony JJ, Johnson CK, Boyle RW, James BR, Dolphin D. J Porphyrins Phthalocyanines 1998;2:455.
[10] Boyle RW, Brückner C, Posakony J, James BR, Dolphin D. Organic Syntheses 1999;76:287.
[11] Brückner C, Dolphin D. Tetrahedron Letters 1995; 36:3295.
[12] Boyle RW, Dolphin D. J Chem Soc Chem Commun 1995: 527.

[13] Seybold PG, Gouterman M. J Mol Spectrosc 1969;31:1.
[14] Diwu ZJ, Lown JW. J Photochem Photobiol A: Chem 1992;64:273.
[15] Whitlock HW, Hanauer R, Oester MY, Bower BK. J Am Chem Soc 1969;91:7485.
[16] Lindsey JS, Schreiman IC, Hsu HC, Kearney PC, Marguerettaz AM. J Org Chem 1987;52:827.
[17] Eisner U, Linstead RP. J Chem Soc 1955:3749.
[18] Lang K, Wagnerova M, Stopka P, Dameran W. J Photochem Photobiol A: Chem 1992;67:187.
[19] Manring LE, Kramer MK, Foote CS. Tetrahedron Letters 1984;25:2523.
[20] Moan J. Acta Chem Scand B 1980;34:519.
[21] Moan J, Wold E. Nature 1979;279:450.
[22] Turro, NJ. Modern molecular photochemistry. Benjamin/Cummings Publishing Co., Inc. 1978. p. 579.