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Photogeneration of Singlet Oxygen ($^1O_2$) and Free Radicals ($Sen^{\cdot -}$, $O_2^{\cdot -}$) by *tetra*-Brominated Hypocrellin B Derivative

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To improve photodynamic activity of the parent hypocrellin B (HB), a *tetra*-brominated HB derivative (compound **1**) was synthesized in high yield. Compared with HB, compound **1** has enhanced red absorption and high molar extinction coefficients. The photodynamic action of compound **1**, especially the generation mechanism and efficiencies of active species ($Sens^{\cdot -}$, $O_2^{\cdot -}$ and $^1O_2$) were studied using electron paramagnetic resonance (EPR) and spectrophotometric methods. In the deoxygenated DMSO solution of compound **1**, the semiquinone anion radical of compound **1** is photogenerated *via* the self-electron transfer between the excited and ground state species. The presence of electron donor significantly promotes the reduction of compound **1**. When oxygen is present, superoxide anion radical ($O_2^{\cdot -}$) is formed *via* the electron transfer from $Sens^{\cdot -}$ to the ground state molecular oxygen. The efficiencies of $Sens^{\cdot -}$ and $O_2^{\cdot -}$ generation by compound **1** are about three and two times as much as that of HB, respectively. Singlet oxygen ($^1O_2$) can be produced *via* the energy transfer from triplet compound **1** to ground state oxygen molecules. The quantum yield of singlet oxygen ($^1O_2$) is 0.54 in $CHCl_3$ similar to that of HB. Furthermore, it was found that the accumulation of $Sens^{\cdot -}$ would replace that of $O_2^{\cdot -}$ or $^1O_2$ with the depletion of oxygen in the sealed system.

Keywords: *tetra*-Brominated hypocrellin B; Semiquinone anion radical; Superoxide anion radical; Singlet oxygen; Electron paramagnetic resonance

INTRODUCTION

Photodynamic therapy (PDT) has received much attention in recent years as a novel anti-tumor therapy.[1–3] Selective photosensitizer and strong light source that matches with the photosensitizer are two essential factors; especially the photosensitizer is the key for PDT study. So far the only photosensitizer approved by the United States FDA is

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Photofrin II®. However, it has several important disadvantages, such as its complicated composition, poor red absorption in tissue and prolonged cutaneous phototoxicity.[4,5] The limitations have led to the investigation of other photosensitizers.

Hypocrellins, which grow abundantly in China, have been selected as potential photosensitizers for PDT due to their high quantum yields of singlet oxygen, fast clearance from normal tissue, strong photogeneration of anion radicals in deoxygenated media, availability in pure monomeric form and facility for site-directed chemical modifications.[6–8] To improve further the photodynamic activity of hypocrellin parents, we have synthesized some hypocrellin derivatives and studied their photophysical, photochemical and photobiological properties.[9–18]

Among these derivatives, brominated hypocrellins have enhanced red absorption and high molar extinction coefficients compared with HB.[14] The efficiencies of singlet oxygen ($^1O_2$) and hydroxyl radical ($^{\cdot}OH$) generation by brominated hypocrellins were enhanced under heterogeneous systems.[19] It was also found that brominated hypocrellins could kill Hela cells[20] and do damage to calf thymus DNA[21] photodynamically more efficiently than their parent compounds.

Although brominated hypocrellins demonstrated promising phototherapeutic potential, the low yield and complicated by-products have been troubling the investigators.[15] Here, we report the synthesis of a *tetra*-brominated hypocrellin B derivative (**1**) in high yield. As compared with its parent HB, the compound **1** showed enhanced red absorption and high molar extinction coefficients. In addition, we investigated the generation of active species during the photosensitization of compound **1**, i.e. the generation of semiquinone anion radical, superoxide anion radical and singlet oxygen by electronic paramagnetic resonance (EPR) and spectrophotometric methods.

MATERIALS AND METHODS

Reagents

HB was prepared as described previously.[22] 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 9,10-diphenyl-anthracene (DPA), 2,2,6,6-tetramethyl-4-piperidone (TEMP) and 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical (TEMPO) were purchased from Aldrich Chemical Company. Catalase and superoxide dismutase (SOD) were purchased from Sigma Chemical Company. Cysteine, reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide (NADH) were obtained from Biochem. Technology Corporation, the Chinese Academy of Sciences. 1,4-Diazabicyclo[2.2.2]octane (DABCO) and dimethylsulfoxide (DMSO) were purchased from Merck Chemical Company. Sodium azide (NaN_3), bromine, deuterated solvents and other agents of analytical grades were purchased from Beijing Chemical Plant. Water was freshly distilled before use. pH values of the irradiated solutions were adjusted by using KH_2PO_4–Na_2HPO_4 buffers (pH 7.4, 8.0). The working stock solutions were prepared immediately before use.

Synthesis of The *tetra*-Brominated Hypocrellin B Derivative (compound 1)

HB (130 mg, 0.25 mmol) was dissolved in chloroform (10 ml), followed by the dropwise addition of a solution of bromine (0.13 ml, 1 mmol) in chloroform (5 ml) with vigorous stirring. The resulting solution was stirred for further 60 min at room temperature (20°C). The reaction mixture was washed with water, dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to get a violet solid. The solid was separated by TLC on a 1% citric acid–silica gel plate, using 12:6:0.1 (v/v/v) petroleum ether–ethyl acetate–ethanol as developing agent, and one major product ($R_f = 0.44$) was obtained. The compound was further purified

with TLC and the desired product (**1**) was obtained in *ca.* 45% yield (95 mg).

Compound **1**: UV–Vis (λ_{max}, $\log \varepsilon$): 502 nm (4.47), 596 nm (4.09); IR (KBr disk) (ν_{max}): 3447 cm^{-1} (phenolic OH), 1724 cm^{-1} (acetyl C=O), 1603 cm^{-1} (quinonoid C=O); H-NMR (δ): 15.64 (s, 1H, exchangeable with D_2O, 4 (9)-OH), 15.57 (s, 1H, exchangeable with D_2O, 9 (4)-OH), 6.03, 6.09 (dd, 2H, 16-H_2, J_{AB} = 1.5 Hz), 5.55 (s, 1H, 13-H), 4.20 (s, 3H, 2-OCH_3), 4.10 (s, 3H, 11-OCH_3), 3.96 (s, 3H, 7-OCH_3), 3.93 (s, 3H, 6-OCH_3), 2.46 (s, 3H, 18-CH_3); MS (FAB): 843 (M−H).

Characterization Measurements on Photosensitizers

The UV–Vis absorption spectra were recorded on a Shimadzu UV-1601 spectrophotometer. IR spectra were measured on a BIO-RAD FTS 165 grating spectrophotometer. ^{1}H-NMR spectra were run on a Varian XL-400 (300 MHz) spectrophotometer in deuterated chloroform with tetramethylsilane as the internal standard. MS spectra were performed on a MALDI-TOF BIFLEX III Bruker spectrometer.

EPR Measurements

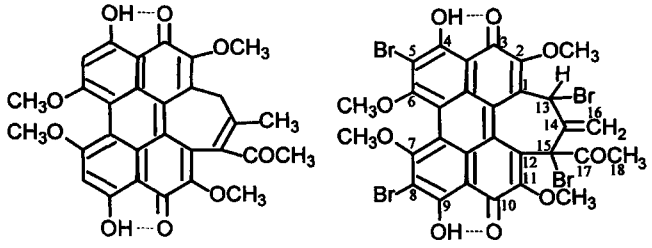
The EPR measurements were performed at room temperature using a Bruker ESP 300E spectrometer. A 532 nm Quanta-Ray Nd:YAG laser was used as the light source. Unless otherwise indicated, the following instrumental settings were used: microwave power, 8.08 mW, modulation amplitude, 1 G; sweep width, 100 G; receiver gain, 1.25×10^4. Samples (40 μl) were injected quantitatively into quartz capillaries designed specially for EPR analysis. Anaerobic samples were prepared in cuvettes that allowed purging the reactive volume with argon for 30 min in the dark. To compare the photodynamic activity of compound **1** with that of HB, the concentrations of them were adjusted to remain the same optical density (OD) at 532 nm.

Determination of the Quantum Yield of $^1O_2$ Generation

The quantum yields of $^1O_2$ generation by HB and compound **1** were determined using the DPA-bleaching method established by Diwu *et al.*[23] The photo-oxidation of DPA sensitized by compound **1** or HB was carried out on a "merry-go-round" apparatus, using 578 nm light as light source obtained from the combination of a high-pressure mercury lamp (500 W) with a 578 nm monochromatic filter. The reactions were followed spectrophotometrically by observing the decrease of absorption peak at 374 nm of DPA (where the sensitizer used has the lowest absorption) as a function of irradiation time.

RESULTS AND DISCUSSION

tetra-Brominated HB derivative (**1**) was synthesized in high yield under mild conditions. The chemical structures of compound **1** and



HB compound **1**

FIGURE 1 The chemical structures of HB and *tetra*-brominated HB derivative (compound **1**).

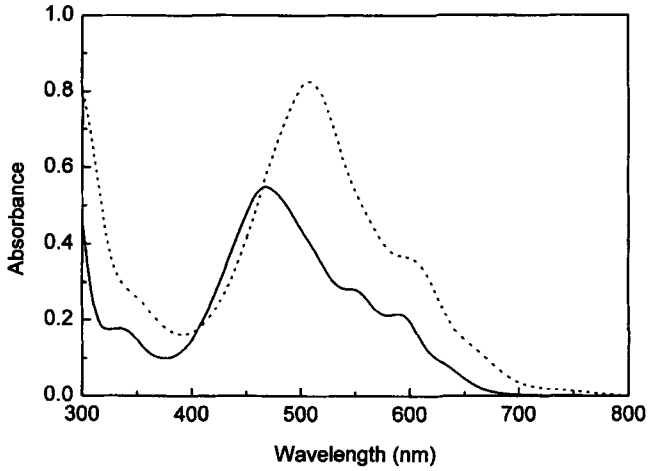


FIGURE 2 Absorption spectra of HB and compound **1** in chloroform. (—) HB, 20 μM; (· · ·) compound **1**, 20 μM.

HB were shown in Fig. 1. The absorption peaks of compound **1** shifted red to 502 (ε 29,512 $M^{-1}cm^{-1}$) and 596 nm (ε 13,302 $M^{-1}cm^{-1}$) with enhanced molar extinction coefficients compared with HB (Fig. 2). Investigations demonstrated that the generation of semiquinone anion radical in the absence of oxygen and that of reactive oxygen species (ROS) in the presence of oxygen, such as superoxide anion radical ($O_2^{\cdot-}$) and singlet oxygen ($^1O_2$) play an important role in the photoinduced damage of malignant tumors.[24] The chromophore of compound **1** is modified against HB due to the bromination on the skeleton of perylenequinone of HB. Therefore, it is necessary to survey the photosensitizing property of compound **1**.

Generation of Semiquinone Anion Radical

Illumination of compound **1** (1 mM) in an argon-saturated DMSO solution for 2 min led to the generation of a strong EPR signal (g = 2.0023) as shown in Fig. 3A. The signal intensity depended on the concentration of compound **1**, oxygen, irradiation time and intensity. The concentration of compound **1** exerted such a strong effect on the generation of this radical that the signal could be observed even in air-saturated DMSO solution at high concentration (5 mM). In order to identify the EPR signal shown in Fig. 3A, a typical electron donor, NADH (2.5 mM), was added to the deoxygenated DMSO solution of compound **1** and irradiated for 30 s. The EPR signal obtained (Fig. 3B) was similar to that in Fig. 3A, but the signal intensity was enhanced significantly. When other electron donors (cysteine or GSH) were used instead of NADH, similar results were observed. This indicated the anionic nature of compound **1** radical. No EPR signal could be detected in the absence of compound **1** or illumination (Fig. 3C), which implied that the EPR signal was generated *via* photosensitization of compound **1**. Moreover, the EPR signal was scavenged (Fig. 3D) by the bubbling of oxygen.

In accordance with these observations, the EPR signal shown in Fig. 3A could be safely assigned to the semiquinone anion radical of compound **1**. In the absence of electron donors, the semiquinone anion radical might be formed *via* self-electron transfer between the triplet and ground state of compound **1** (Eqs. (1) and (2)). In the presence of electron donors, the electron transfer from electron donor (D) to triplet compound **1**

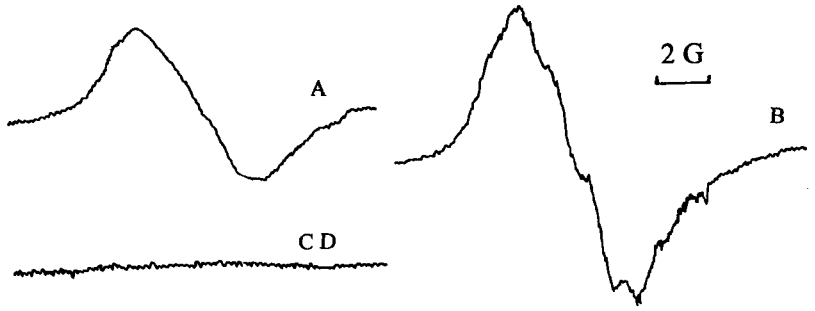


FIGURE 3 (A) Photoinduced EPR spectrum from a deoxygenated DMSO solution of compound **1** (1 mM) on illumination for 2 min. (B) Same as (A) except that NADH (2.5 mM) was added. (C) Same as (A) except that compound or illumination was omitted. (D) Same as (A) except that oxygen was bubbled through the solution after illumination. Instrumental setting: microwave power, 1 mW; modulation amplitude, 0.1 G; scan width, 20 G; receiver gain, 5×10^4.

also generated the semiquinone anion radical of compound **1** (Eq. (3) where Sens denotes compound **1**).

$$\text{Sens} \xrightarrow{h\nu} {}^1\text{Sens} \xrightarrow{\text{isc}} {}^3\text{Sens} \quad (1)$$

$${}^3\text{Sens} + \text{Sens} \rightarrow \text{Sens}^{\cdot -} + \text{Sens}^{\cdot +} \quad (2)$$

$${}^3\text{Sens} + \text{D} \rightarrow \text{Sens}^{\cdot -} + \text{D}^{\cdot +} \quad (3)$$

In addition, we surveyed the generation efficiencies of the semiquinone anion radical of compound **1** and HB (Fig. 4). The result showed that the generation efficiency of the semiquinone anion radical of compound **1** is about three times as much as that of HB. It is noteworthy that no hyperfine splitting was observed for compound **1**. This may be due to the bromination of the two aromatic hydrogen atoms (5 and 8 sites) in compound **1**, which contributes to the hyperfine structures of EPR spectrum in HB.[25]

Generation of Superoxide Anion Radical ($O_2^{\cdot -}$)

It was mentioned above that the photo-induced EPR spectrum of compound **1** shown in Fig. 3A disappeared when oxygen was bubbled through

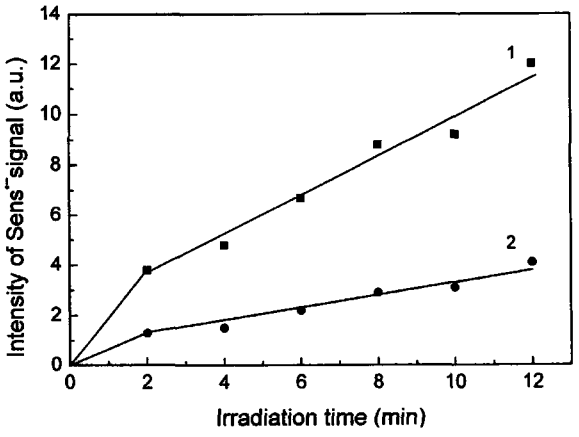


FIGURE 4 Dependence of the EPR signal intensity of semiquinone anion radical on irradiation time by photolysis of deoxygenated DMSO solution containing compound **1** (1 mM) (line 1). Line 2: Same as line 1 but compound **1** was replaced by HB (2.3 mM).

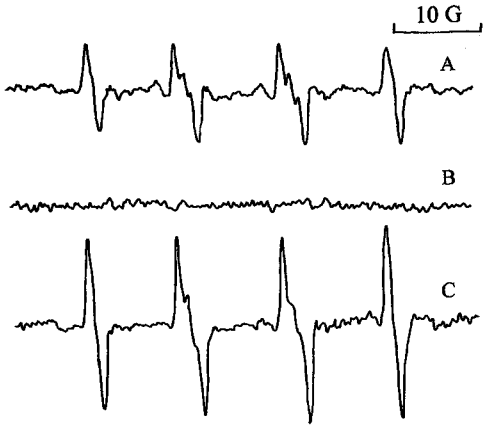


FIGURE 5 (A) EPR spectrum of the DMPO–$O_2^{\cdot -}$ adduct produced on irradiation of an air-saturated DMSO solution of compound **1** (0.1 mM) and DMPO (50 mM). (B) Same as (A) except that SOD (30 $\mu g\,ml^{-1}$) was added. (D) Same as (A) except that NADH (2.5 mM) was added.

the deoxygenated compound **1** solution. When DMPO and oxygen were introduced into the reaction system, a new EPR signal appeared. This suggested the oxidation of the semiquinone anion radical of compound **1** by dissolved oxygen and the formation of another radical that could be trapped by DMPO. When the air-saturated DMSO solution of compound **1** (0.1 mM) and DMPO (50 mM) was irradiated, an EPR signal appeared immediately (Fig. 5A). This EPR spectrum were characterized by three coupling constants, which are due to the nitrogen and two hydrogen atoms at β and γ positions. The g factor and determined constants ($g = 2.0055$, $\alpha^N = 12.8\,G$, $\alpha_\beta^H = 10.5\,G$ and $\alpha_\gamma^H = 1.3\,G$) were in good agreement with the literature for DMPO–superoxide radical adduct.[26] The addition of SOD ($30\,\mu ml^{-1}$) prior to illumination inhibited the EPR signal intensity (Fig. 5B). These observations suggested the correct assignment of the EPR spectrum shown in Fig. 5A to the DMPO–$O_2^{\cdot-}$ adduct.

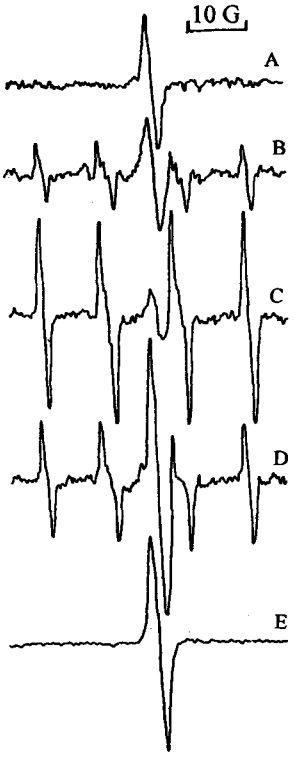


FIGURE 6 The EPR spectra produced from the sealed air-saturated DMSO solution containing compound **1** (5 mM) and DMPO (100 mM) with the different irradiation times: (A) 1 min; (B) 2 min; (C) 8 min; (D) 12 min; (E) 15 min.

To provide further evidence on the formation mechanism of superoxide anion radical during compound **1**—mediated photosensitization, the following experiments were carried out. The addition of electron donor such as NADH (2.5 mM) greatly enhanced the EPR intensity of DMPO–$O_2^{\cdot-}$ (Fig. 5C). Alternatively, it is possible for NADH to react directly with $^1O_2$ to produce $O_2^{\cdot-}$.[27] Hence the possible contribution of $^1O_2$ to the formation of $O_2^{\cdot-}$ during the compound **1**—mediated photosensitization has been investigated. The addition of singlet oxygen quencher DABCO (10 mM) did not decrease the signal intensity of DMPO–$O_2^{\cdot-}$ adduct,[28] indicating that the reduction of $^1O_2$ to $O_2^{\cdot-}$ could be ignored. Moreover, catalase ($50\,\mu g\,ml^{-1}$) and hydrogen peroxide (10 mM) had a negligible effect, thus excluding a role of H_2O_2 in the formation of $O_2^{\cdot-}$ photosensitized by compound **1**. From these results, the formation of $O_2^{\cdot-}$ that occurred in the absence (Eq. (4)) or presence of electron donors (Eqs. (5) and (6)) may be rationalized by the following processes:

$$\text{Sens}^{\cdot-} + O_2 \rightarrow O_2^{\cdot-} + \text{Sens} \qquad (4)$$

$$^3\text{Sens} + \text{NADH} \rightarrow \text{Sens}^{\cdot-} + \text{NAD}^{\cdot} + H^+ \qquad (5)$$

$$\text{NAD}^{\cdot} + O_2 \rightarrow O_2^{\cdot-} + \text{NAD}^+ \qquad (6)$$

The consistent enhancement effect of electron donors on the formation of $O_2^{\cdot-}$ and that of $\text{Sens}^{\cdot-}$ suggested that $\text{Sens}^{\cdot-}$ might be the precursor for the formation of $O_2^{\cdot-}$ (Eq. (4)). In addition, $O_2^{\cdot-}$ could also be produced by processes (5) and (6) in which the radicals formed in electron transfer reaction between NADH and triplet compound **1** could reduce O_2 to $O_2^{\cdot-}$. Indeed, at high concentrations of NADH (2.5 mM), we postulated that the majority of $O_2^{\cdot-}$ produced probably arose from the chemistry of $NAD^{\cdot}$.[29]

To clearly demonstrate the transformation between the semiquinone anion radical ($Sens^{\cdot -}$) and superoxide anion radical ($O_2^{\cdot -}$), the sealed air-saturated DMSO solution of compound **1** (5 mM, at high concentration) and DMPO (100 mM) was investigated. At the beginning of irradiation (1 min), a strong semiquinone anion radical ($Sens^{\cdot -}$) signal appeared (Fig. 6A), which was in good agreement with the above observation. When irradiation time reached 2 min, the DMPO–$O_2^{\cdot -}$ adduct signal appeared accompanied by the decrease of $Sens^{\cdot -}$ signal intensity (Fig. 6B). The signal intensity of DMPO–$O_2^{\cdot -}$ adduct increased to the maximum within 8 min (Fig. 6C). On further irradiation, the DMPO–$O_2^{\cdot -}$ signal decreased to disappearance, while the $Sens^{\cdot -}$ signal increased subsequently (Fig. 6D,E). This confirmed that oxygen played a key role in the competition and transformation between $Sens^{\cdot -}$ and $O_2^{\cdot -}$ in the photosensitization process.

The decreasing process of DMPO–$O_2^{\cdot -}$ adduct signal intensity may be due to its short half-life. With the depletion of oxygen, the above-mentioned reaction (eqn. 4) couldn't occur, *i.e.* $Sens^{\cdot -}$ couldn't be transformed to $O_2^{\cdot -}$. Thus the $Sens^{\cdot -}$ signal increased with prolonging the irradiation time. This further supported that $Sens^{\cdot -}$ is the precursor for the formation of $O_2^{\cdot -}$.

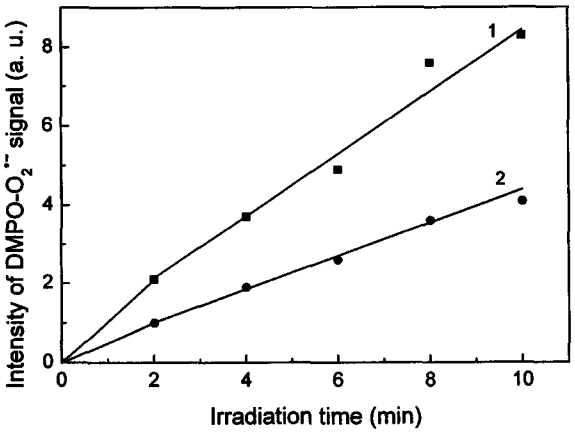


FIGURE 7 Dependence of the EPR signal intensity of DMPO–$_2^{\cdot -}$ adduct on irradiation time derived from an air-saturated DMSO solution containing compound **1** (0.1 mM) and DMPO (50 mM) (line 1). Line 2: Same as line 1 but compound **1** was replaced by HB (0.23 mM).

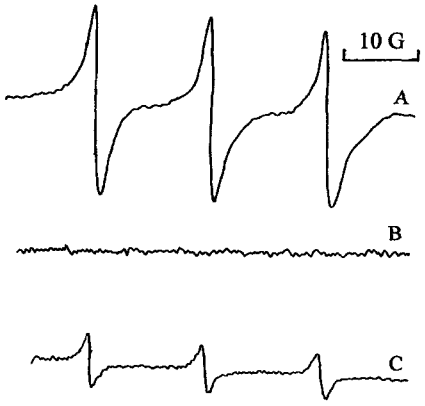


FIGURE 8 (A) EPR spectrum produced by irradiation of an air-saturated $CHCl_3$ solution containing compound **1** (40 μM) and TEMP (30 mM). (B) Same as (A) except that compound **1**, oxygen or irradiation was omitted. (C) Same as (A) except that DABCO (1 mM) was added.

Attempts were also made to compare the abilities of $O_2^{\cdot -}$ generation by compound **1** and HB on photosensitization. The results were shown in Figure 7. It showed that compound **1** can also efficiently photosensitize the generation of $O_2^{\cdot -}$ and it is about two times as effective as HB.

Generation of Singlet Oxygen ($^1O_2$)

EPR Detection

When an air-saturated DMSO solution of compound **1** (40 μM) and TEMP (30 mM) was irradiated at room temperature, an EPR spectrum of triplet peaks with equal intensity, characteristic of a nitroxide radical, was observed (Fig. 8A). The hyperfine splitting constant and g factor of the photosensitized oxidation product of TEMP by compound **1** were identical to those of commercial TEMPO (α^N = 16.2 G, g = 2.0056). In the absence of compound **1**, oxygen or irradiation, no EPR signal could be

detected (Fig. 8B). These data demonstrated that the formation of the nitroxide radical was a photodynamic process.

It has previously been reported that TEMPO might be generated from TEMP and singlet oxygen.[30] To provide further evidence to support the involvement of $^1O_2$ in compound **1** photosensitizing process, the following experiments were carried out. In the presence of $^1O_2$ scavenger (DABCO or NaN_3), the EPR signal was suppressed (Fig. 8C). The effect of deuterium solution on the yield of TEMPO was also studied. It was found that the intensity of the EPR signal increased by approximately three times when $CHCl_3$ was replaced by $CDCl_3$ as solvent (data not shown). These two powerful tools, both of which are diagnostics for $^1O_2$, suggest that TEMPO is derived from the reaction of TEMP with $^1O_2$. The compound **1** photosensitization generation of $^1O_2$ is considered to proceed *via* energy transfer from triplet compound **1** to ground state oxygen (Eq. (7)).

$$^3\text{Sens} + O_2 \rightarrow {}^1O_2 + \text{Sens} \qquad (7)$$

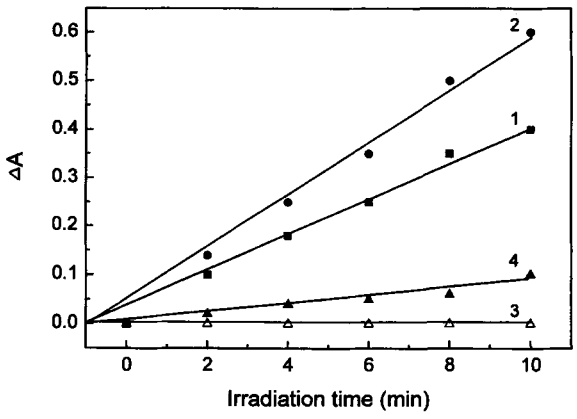
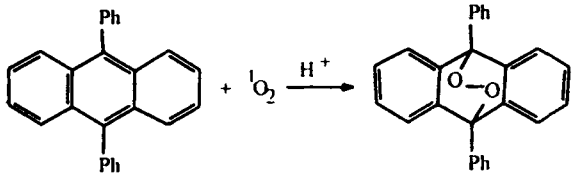


FIGURE 9 Photosensitized DPA-bleaching by measuring the absorbance decrease (ΔA) at 374 nm as a function of irradiation time in oxygen-saturated $CHCl_3$ solution containing compound **1** (40 μM) and DPA (0.3 mM) (line 1). Line 2: Same as line 1 but compound **1** was replaced by HB (92 μM). Line 3: Same as line 1 except that compound 1, oxygen or illumination was omitted. Line 4: Same as line 1 except that NaN_3 (5 mM) was added)

Determination of $^1O_2$ Quantum Yield Generated by Compound 1

The DPA-bleaching method has been confirmed to be an efficient measurement for the quantum yield of $^1O_2$ generated during photosensitization (Eq. (8)).[23]



(8)

In order to determine the quantum yield of $^1O_2$ generated by compound **1**, we adopted the 9,10-DPA bleaching method using HB as reference. During the measurements, the optical densities at 578 nm of the two samples were adjusted to be the same. Figure 9 shows the rates of DPA-bleaching photosensitized by compound **1** (line 1) and HB (line 2) as a function of irradiation time in $CHCl_3$. Control experiments indicated that no DPA-bleaching occurred when photosensitizers, oxygen or irradiation were absent (line 3, Fig. 9). The addition of DABCO or NaN_3 (5 mM) almost completely inhibited DPA-bleaching (line 4, Fig. 9). These confirmed that the bleaching of DPA resulted from the reaction of DPA with $^1O_2$ formed by compound **1** photosensitization. The $^1O_2$-generating quantum yield for compound **1** in $CHCl_3$ was estimated to be 0.54, relative to HB (0.76).

Transformation Between Singlet Oxygen and the Semiquinone Anion Radical of Compound 1

TEMPO could also be used to detect $Sens^{\cdot -}$ *via* Eq. (9). This unique and dual role of TEMPO could be conveniently used to detect the transformation between $^1O_2$ and $Sens^{\cdot -}$ as the

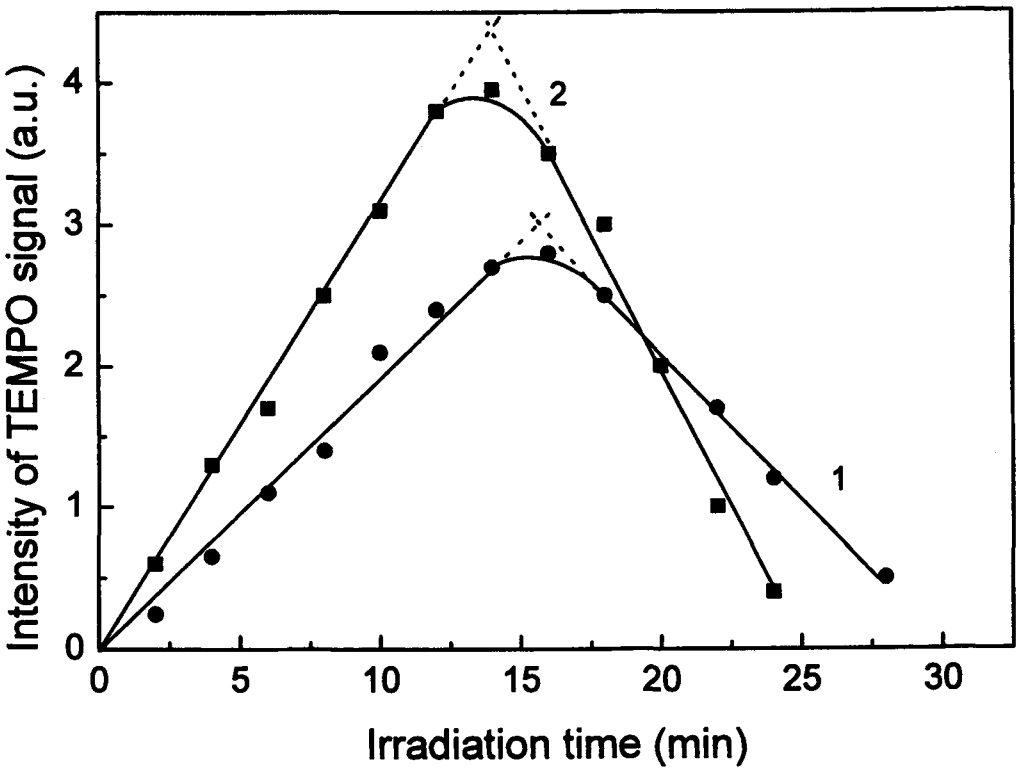


FIGURE 10 Dependence of the TEMPO signal intensity on the irradiation time when a sealed system of compound **1** (40 μM) and TEMP (30 mM) was irradiated (line 1). Line 2: Same as line 1 but compound **1** was replaced by HB (92 μM).

oxygen concentration changed.

$$Sens^{\cdot -} + TEMPO \rightarrow TEMPOH + Sens^{2-} \quad (9)$$

When the sealed air-saturated $CHCl_3$ solution of compound **1** (40 μM) and TEMP (30 mM) was irradiated, the ESR signal intensity of TEMPO increased to the maximum within about 6 min and then decreased with further irradiation (Fig. 10). The increasing process of TEMPO signal intensity was caused by the reaction of TEMP with $^1O_2$, and the decreasing process resulted from the spin destruction of TEMPO by compound **1** semiquinone radical as shown in Eq. (9). The decrease of oxygen concentration in the sealed system suppressed the formation of $^1O_2$, but promoted the generation of $Sens^{\cdot -}$. The turning point in Fig. 10 indicated that the accumulation of $^1O_2$ was replaced by that of $Sens^{\cdot -}$ and that oxygen was depleted. Figure 10 indicated that the formation of $HB^{\cdot -}$ was faster than that of $Sens^{\cdot -}$ under comparable conditions. The accumulation of $Sens^{\cdot -}$ may replace that of singlet oxygen ($^1O_2$) as oxygen was depleted.

CONCLUSIONS

In this paper, we reported the synthesis of a *tetra*-brominated hypocrellin B derivative (compound **1**) in high yield. The absorption bands of compound **1** shift bathochromically and molar extinction coefficients were improved compared with HB. The results of this work provide EPR and spectrophotometric evidence for the generation of $Sens^{\cdot -}$, $O_2^{\cdot -}$ and $^1O_2$ active species during compound **1** photosensitization process. The

efficiencies of $Sens^{\cdot -}$ and $O_2^{\cdot -}$ generation by compound **1** are about three and two times as much as that of HB, respectively, and moreover, the $^1O_2$ generation efficiency is similar to that of HB. The competition and transformation between active oxygen species and semiquinone anion radical in the compound **1** photosensitization process depends on the content of oxygen.

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