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Spectroscopic characterization of dipicolinic acid and its photoproducts as thymine photosensitizers

Giacomo Nardi, Mauricio Lineros-Rosa, Fabrizio Palumbo, Miguel A. Miranda *, Virginie Lhiaubet-Vallet *

Instituto Universitario Mixto de Tecnología Química, Universitat Politècnica de València, Consejo Superior de Investigaciones Científicas, Avenida de los Naranjos s/n, 46022, Valencia, Spain

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ABSTRACT

Dipicolinic acid (DPA), present in large amount in bacterial spores, has been proposed to act as an endogenous photosensitizer in spore photoproduct formation. The proposed mechanism involves a triplet-triplet energy transfer from DPA to thymine. However, up to now, no spectroscopic studies have been performed to determine the interaction between the endogenous compound and the nucleobase, probably due to its photolability in aqueous solutions. Here, triplet excited state properties of DPA are reported together with its bimolecular quenching rate constant by thymidine, k_q of ca. 5.3×10^9 M^{-1} s^{-1}. To run more reliable studies, a stable methyl ester derivative of DPA, which exhibits the same spectroscopic properties as the parent compound, is also described. Finally, DPA photoproducts are characterized. Studies of their triplet excited state properties have demonstrated that, interestingly, one of them is able to photosensitize thymidine triplet excited state formation.

1. Introduction

DNA damage has attracted a huge interest over the years in connection with its harmful deleterious consequences linked to mutation and cancer [1–3]. It is now well-established that in eukaryotic and vegetative prokaryotic cells, the main damages observed under direct UV radiation are the bipyrimidinic lesions (Chart 1), i.e. cyclobutane pyrimidine dimers (CPD) and (6–4) photoproducts (6-4PP). However, bacterial spores have a singular response to UV with the sole formation of a photoproduct, evidenced more than 50 years ago and tagged spore photoproduct (SP) [4]. This lesion contains a covalent methylene link between two adjacent thymines and has been fully characterized by NMR as 5-(*R*-thyminyl)-5,6-dihydrothymine (Chart 1) [5].

Despite the remarkable results reported over half a century, the molecular mechanisms underlying the formation of SP are still not fully understood. Dehydration, complexation of DNA by α/β-type small, acid-soluble spore proteins (SASP), and the large amount (~10–15% dry weight) of dipicolinic acid (DPA, Chart 2) have been shown to be key factors in the photochemistry of bacterial spore DNA [6,7]. From a chemical point of view, a mechanism of SP formation has been proposed by Li using a deuterium labeling strategy, which points toward an intramolecular H-atom transfer from the methyl group of one thymine to the C6 carbon of the other one [8].

Spore photoproduct not only differs from the other dimers in its formation but also in its repair process, because it cannot be reversed through the action of photolyases as CPD and 6-4PP do. Indeed, this photoproduct is eliminated from DNA by a dark-repair mechanism that involves the specific SP lyase enzyme, which belongs to the radical S-adenosylmethionine (SAM) enzyme family [9,10,11]. Thus, bacterial spore resistance to UVC radiation has been associated with the unique photochemical behavior of its nucleic acid, which leads to exclusive formation of SP, and with the ability of SP to undergo subsequently an efficient repair.

The possible role of DPA as an endogenous photosensitizer has been addressed over the years, and its influence on SP or CPD formation has been reported in the literature [6,7,12,13]. Addition of DPA to dry films of isolated DNA or frozen solutions of thymidine increases the yield of SP and the ratio of CPD to 6-4PP. In liquid solution, the presence of DPA also improves significantly the yield of CPD in thymidine exposed to UVC [6,7,12]. Altogether, the results available until now strongly point toward a triplet-triplet energy transfer (TTET) between DPA and thymine residues [12,14]. The involvement of the thymine triplet excited state in SP formation has been further supported by the experiments run on dry film photoreaction of thymidine in the presence of pyridopsoralen derivatives or benzophenone under UVA irradiation [15,16]. However, full characterization of the DPA photophysical properties has not been achieved yet, probably due to its photolability in solution [14,17]. Specifically, a time-resolved study on the kinetics of the interaction between DPA triplet excited state and thymidine would provide highly valuable information on the occurrence of TTET. Moreover, in spite of its efficient photodegradation in solution, the structures and

* Corresponding authors.
E-mail addresses: mmiranda@qim.upv.es (M.A. Miranda), lvirgini@itq.upv.es (V. Lhiaubet-Vallet).

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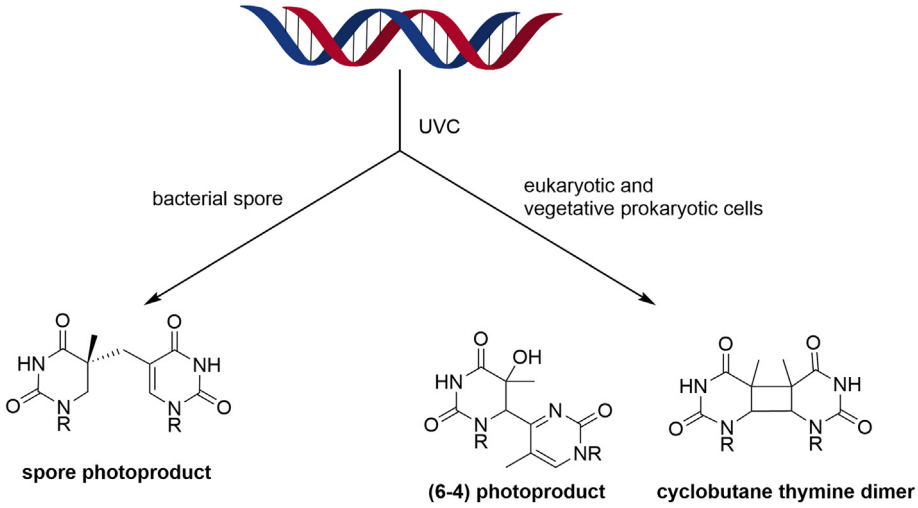

Chart 1. Structures of spore photoproduct (**SP**), cyclobutane thymine dimers and (6–4) photoproduct (**6-4PP**).

photosensitizing potential of DPA photoproducts have not been elucidated. All these aspects are investigated in the present article, paying a particular attention to the DPA triplet excited state behavior, in order to shed some light on the peculiar photoreactivity of bacterial spore DNA.

2. Material and methods

2.1. Chemicals

2,6-Pyridinedicarboxylic acid (dipicolinic acid, DPA), 5- and 6-bromo-2-picoline, n-butyllithium, tributyltin chloride, β-carotene, xanthone, thymidine (Thd), sulfuric acid (98%) and chromium (VI) oxide (CrO_3) were from Sigma Aldrich; 6,6′-dimethyl-2,2′-bipyridine (**1a**) was from TCI Europe. All compounds were used without further purification. The reagent grade solvents were obtained from Scharlau and used without further purification. Phosphate buffered saline (PBS) solutions of 0.01 M (pH = 7.4) were prepared by dissolving tablets (Sigma-Aldrich) in Milli-Q water. The model compounds Thy-Thy and Thy<>Thy were synthesized as previously described in the literature [18].

2.2. Instruments

2.2.1. NMR spectroscopy

^{1}H NMR and ^{13}C NMR were recorded on a 300 MHz spectrometer (AV300, Bruker) operating in the Fourier transform mode. The chemical shifts are reported in ppm (parts per million).

2.2.2. UPLC-MS/MS

Chromatography was performed on an ACQUITY UPLC system (Waters Corp.) with a conditioned autosampler at 4 °C. The separation was carried out on an ACQUITY UPLC HSS T3 C18 column (150 mm × 2.1 mm i.d., 1.8 μm). The column temperature was maintained at 40 °C. The analysis was achieved using methanol:water (containing 0.01% formic acid) 20:80 v:v as the mobile phase with a flow rate of 0.2 mL/min. The injection volume was 1 μL. The Waters ACQUITY™ XevoQToF Spectrometer (Waters Corp.) was connected to the UPLC system via an electrospray ionization (ESI) interface. The ESI source was operated in positive ionization mode with the capillary voltage at 3.0 kV. The temperature of the source and desolvation was set at 100 °C and 400 °C, respectively. The cone and desolvation gas flows were 100 L h^{-1} and 800 L h^{-1}, respectively. All data collected in Centroid mode were acquired using Masslynx™ software (Waters Corp.). Leucine-enkephalin was used as the lock mass generating an $[M + H]^+$ ion (m/z 556.2771) at a concentration of 250 pg/mL and flow rate of 50 μL/min to ensure accuracy during the MS analysis.

2.2.3. Microwaves reactor

Reactions promoted by microwaves were performed in a MARS (microwave accelerated reaction system) model unit (CEM Corporation, 2450 MHz) with Microwave Teflon liner (EasyPrep Temperature Control Vessel Assembly, CEM Corporation).

2.2.4. Photoreactors

Irradiation in the UVC was performed by means of a multilamp photoreactor equipped with fluorescent tubes of maximal output at ca. 254 nm (Philips - TUV15W G15T8). UVB irradiation was performed in the same photoreactor using lamps with a maximum output at 300 nm (from Luzchem).

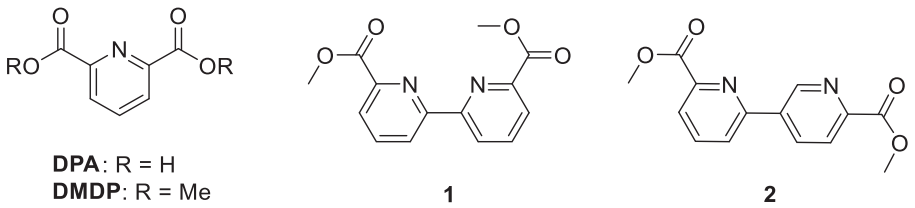

Chart 2. Chemical structures of dipicolinic acid (**DPA**), its methyl ester derivative (**DMDP**), photoproducts **1** and **2**.

2.2.5. UV–Vis absorption spectroscopy

Absorption spectra of the samples were measured with a single beam Varian UV–Vis model Cary 50 Scan spectrophotometer, using 1 cm pathway quartz cuvettes.

2.2.6. Laser flash photolysis (LFP)

Two laser flash photolysis systems were employed for the studies. For 266 and 355 nm excitation, experiments were carried out using the fourth and third harmonic, respectively, of a pulsed Nd:YAG L52137 V LOTIS. The laser flash photolysis system consisted of the pulsed laser, a 77250 Oriel monochromator, and a photomultiplier (PMT) system made up of side-on PMT, PMT housing, and a PMT power supply. The output signal from the Tektronix oscilloscope was transferred to a personal computer for study. The single pulses were ca. 10 ns duration, and the energy was ca. 15 mJ/pulse. All transient spectra were recorded using 1×1 cm^2 quartz cells with 4 mL capacity, and solutions were bubbled for 10 min with N_2, air, or O_2, before acquisition. The absorbance of the samples was kept in the range 0.30–0.40 at the laser excitation wavelength.

For quenching experiments, stock solutions of quenchers were prepared so that it was only necessary to add microliter volumes to the sample cell to obtain appropriate concentrations. A linear quenching plot was obtained, and the resulting rate constant (k_q) was calculated from the slope of the Stern-Volmer plot: $\tau_0/\tau = 1 + k_q\tau_0[Q]$ where τ_0 (in s) is the triplet lifetime in the absence of quencher, τ is the triplet lifetime (in s) in the presence of the quencher, and [Q] is the quencher concentration in mol·L^{-1}.

2.2.7. Phosphorescence emission

Phosphorescence spectrum was obtained from a Photon Technology International (PTI, TimeMaster TM-2/2003) spectrofluorometer equipped with a pulsed Xe lamp. The apparatus was operated in time-resolved mode, with a delay time of 0.5 μs. Compounds were dissolved in ethanol, put in a quartz tube (5 mm of diameter) and cooled at 77 K. These experiments were run under air atmosphere.

2.3. Synthesis

2.3.1. Dimethyl dipicolinate ester (DMDP)

Dipicolinic acid (250 mg, 1.50 mmol) was dissolved in MeOH (250 mL), and concentrated H_2SO_4 (125 μL) was added dropwise to the solution. After stirring under reflux for 24 h at 70 °C, the reaction mixture was basified to a pH 7.8 using Na_2CO_3 and 250 mL of brine was added. Methanol was evaporated under vacuum, and the obtained aqueous solution was extracted with ethyl acetate (3×). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to afford DMDP as a white crystalline solid (280 mg, 1.43 mmol, 95%). No further purification was needed. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.32$ (d, $J = 7.8$, 2H), 8.02 (t, $J = 7.8$ Hz, 1H), 4.02 (s, 6H). NMR data coincide with those previously described in literature [19].

2.3.2. Synthesis of 6,6′-dimethyl-2,2′-bipyridinedicarboxylate (1)

6,6′-Dimethyl-2,2′-bipyridine (**1a**, 50 mg, 0.3 mmol) was placed into a 25 mL round-bottom flask cooled with an ice bath. Concentrated sulfuric acid (3 mL) was added dropwise under stirring, followed by small portions of the chromium (VI) oxide (170 mg, 1.7 mmol). The mixture was stirred overnight at 70 °C. Afterwards, the solution was cooled to room temperature and was slowly poured to 100 mL of methanol. After stirring under reflux for 24 h at 70 °C, the reaction mixture was basified to a pH 7.8 using Na_2CO_3 and 100 mL of brine was added. Methanol was evaporated under vacuum, and the obtained aqueous solution was extracted with ethyl acetate (3×). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to afford **1** (61 mg, 0.23 mmol, 75%). No further purification was needed. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.73$ (dd, $J_1 = 7.9$ Hz, $J_2 = 1.1$ Hz, 2H), 8.15 (dd, $J_1 = 7.9$ Hz, $J_2 = 1.1$ Hz, 2H), 7.99 (t, $J = 7.9$ Hz, 2H), 4.0 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.8, 155.6, 147.7, 138.3, 125.6, 125, 53.1$. HRMS (ESI$^+$): Calculated for $C_{14}H_{13}N_2O_4$ $(M + H)^+$: 273.0875; Found: 273.0873.

2.3.3. Synthesis of 6,6′-dimethyl-2,3-bipyridine (2a)

6,6′-Dimethyl-2,3′-bipyridine was synthesized from the Stille coupling reaction between 5-bromo-2-methylpyridine and 6-tributylstannyl-2-methylpyridine, which was obtained from 6-bromo-2-picoline following the procedure described in the literature [20]. 5-Bromo-2-methylpyridine (170 mg, 1 mmol) and $Pd(PPh_3)_4$ (41 mg, 3.5% mol) were dissolved in dry toluene (4 mL) and 6-tributylstannyl-2-methylpyridine (420 mg, 1.1 mmol) was added. The mixture was stirred for 24 h under reflux. After evaporation of toluene under vacuum, the residue was dissolved in dichloromethane and the palladium black was removed by filtration through Celite. The filtrate was washed with aqueous HCl (1 M) and transferred dropwise to an aqueous saturated solution of Na_2CO_3. The resulting oil was extracted with CH_2Cl_2. The organic phase was washed with brine, and the solvent was removed. The residue was purified by silica gel chromatography using hexane:ethyl acetate (1:1, v:v) as eluent to afford **2a** (110 mg, 0.6 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.04$ (d, $J = 2$ Hz, 1H), 8.23 (dd, $J_1 = 7.7$ Hz, $J_2 = 2$ Hz, 1H), 7.65 (t, $J = 7.7$ Hz, 1H), 7.52 (d, $J = 7.7$ Hz, 1H), 7.26 (d, $J = 7.7$, 1H), 7.1 (d, $J = 7.7$ Hz, 1H), 2.62 (s, 3H), 2.61 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 158.8, 158.6, 154.4, 147.5, 137.1, 134.8, 132.4, 123.1, 122.0, 117.3, 24.7, 24.2$. HRMS (ESI+): Calculated for $C_{12}H_{13}N_2$ $(M + H)^+$: 185.1079; Found: 185.1084.

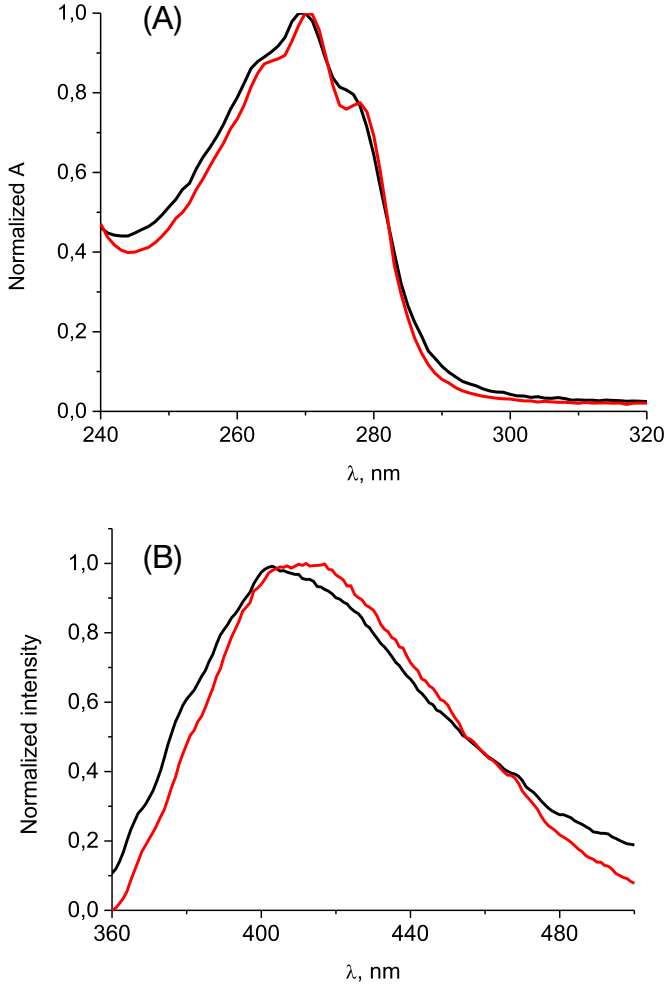

Fig. 1. (A) Normalized UV absorption spectra of DPA (black line) in PBS and DMDP (red line) in H_2O:MeCN (9:1, v:v), (B) Phosphorescence spectrum ($\lambda_{exc} = 266$ nm) of DPA (black line) and DMDP (red line) in EtOH at 77 K.

2.3.4. Synthesis of 6,6′-dimethyl-2,3′-bipyridinedicarboxylate (2)

6,6′-dimethyl-2,3′-bipyridine (**2a**) (50 mg, 0.3 mmol) was placed into a round-bottom flask cooled with an ice bath. Concentrated sulfuric acid (3 mL) was added dropwise under stirring, followed by small portions of the chromium (VI) oxide (170 mg, 1.7 mmol). The mixture was stirred overnight at 70 °C. Afterwards, the solution was cooled to room temperature and was slowly poured to 100 mL of methanol in a teflon vessel for microwave apparatus, which was then fixed to the rotor mechanism of the microwave cavity. The most appropriate reaction conditions were found for a temperature of heating of 80 °C (5 min) and, then the sample was held for 10 min applying a radiation of 175/125 W. The sample was cooled to room temperature and the vessel was removed from the microwave cavity. The reaction mixture was basified to a pH 7.8 using Na_2CO_3 and 250 mL brine was added. Methanol was evaporated under vacuum, and the obtained aqueous solution was extracted with ethyl acetate 3 times. The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The resulting residue was subjected to flash chromatography using ethyl acetate:hexane:dichloromethane (1:1:4, v:v:v) as eluent, compound **2** was then crystallized from ethanol solution (Yield:70%).

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.30$ (dd, $J_1 = 2.2$ Hz, $J_2 = 0.8$ Hz, 1H), 8.61 (dd, $J_1 = 8.2$ Hz, $J_2 = 2.2$ Hz, 1H), 8.28 (dd, $J_1 = 8.2$ Hz, $J_2 = 0.8$ Hz, 1H), 8.19–8.14 (m, 1H), 8.02–7.96 (m, 2H), 4.04 (s, 3H), 4.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 165.6$, 154.1, 148.9, 148.3, 138.4, 137.1, 136.1, 125.4, 124.8, 124.3, 53.2. HRMS (ESI+): Calculated for $C_{14}H_{13}N_2O_4$ $(M + H)^+$: 273.0875; Found: 273.0873.

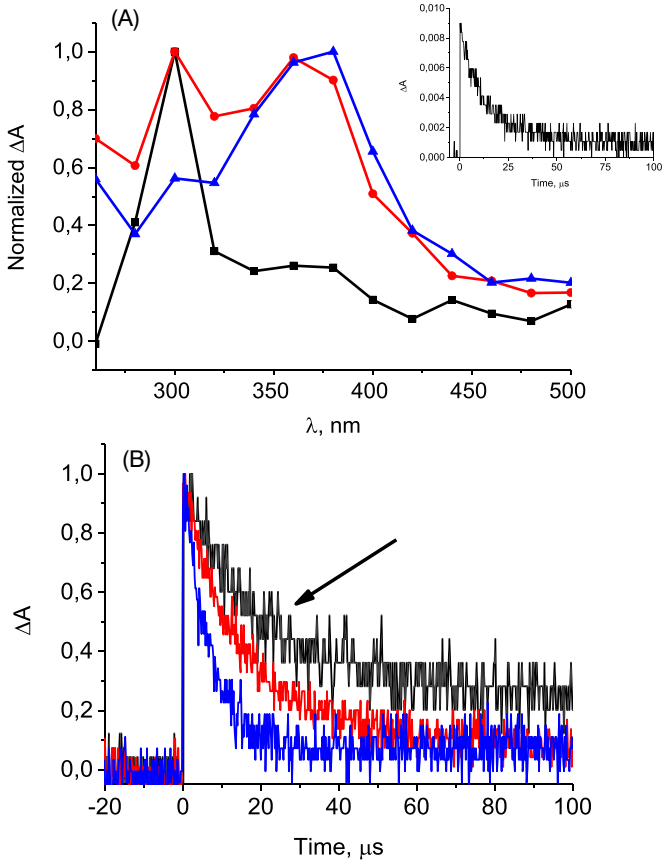

Fig. 2. (A) Transient absorption spectra of N_2 degassed PBS solution of DPA obtained changing the sample after each pulse/wavelength record, using fresh (■), one pulse (●) or two pulses (▲) pre-irradiated solution. Inset: corresponding decay of DPA monitored at 300 nm. (B) Decays monitored at 300 nm for deaerated PBS solution of DPA in the presence of increasing amounts of Thd (0, 6.2 and 20 μM).

2.4. Photolysis of DPA

Irradiation of DPA (3 mg/mL) was performed in PBS solution, under N_2 atmosphere, by means of a multilamp photoreactor equipped with 8 UVC tubes for 5 min. After removal of water under vacuum, the residue was dissolved with 20 mL of methanol in a teflon vessel for microwave apparatus. The esterification was performed as abovementioned (see synthesis of **2**). The photoreaction was monitored by high-performance liquid chromatography using a reverse phase column (C18 Mediterranea sea, 25 × 1.0 cm, acetonitrile:water, 60:40, v:v, flow 0.7 mL/min) coupled with UV detection (λ = 254 nm).

2.5. Thymine cyclobutane dimers photosensitization by DMDP and photoproduct 1

Nitrogen-purged solutions of Thy-Thy (0.5 mM) in acetonitrile: water mixture (1:1, v:v) were irradiated with UVB light up to 60 min in the presence or absence of DMDP or compound **1** (1.5 mM). Aliquots were collected at different irradiation times and were analyzed by HPLC (Agilent) equipped with a diode array detector (DAD), for all chromatograms the detection wavelength was 240 nm. Samples were analyzed using a reverse phase Mediterranea Sea C18 (25 cm × 0.46 cm, 5 μm) column. Chromatographic conditions for the analysis of the irradiations of Thy-Thy alone and in the presence of DMDP were an isocratic mixture of H_2O (at pH 3, adjusted with trifluoroacetic acid): acetonitrile (80:20, v:v) at a flow rate of 1 mL/min. The injection volume was of 10 μL. Irradiation of Thy-Thy with photoproduct **1** was analyzed using a gradient elution. The column was equilibrated in H_2O (at pH 3, adjusted with trifluoroacetic acid): acetonitrile (80:20, v:v) at a flow rate of 1 mL/min. The amount of acetonitrile was maintained for 20 min, then increased from 20% to 100% in 5 min, kept at 100% for 5 min, restored to the initial composition in 5 min, and finally maintained at this composition for 20 min. The injection volume was of 10 μL.

3. Results

3.1. Photophysics of DPA

The absorption spectrum of dipicolinic acid (DPA) in phosphate buffer saline solution (PBS, pH 7.4) was centered at 270 nm (Fig. 1A, black line) and extended up to ca. 295–300 nm. This low capability to absorb in the UVB region would make DPA a poor sensitizer under the conditions present at earth surface. Concerning fluorescence emission, DPA only showed a very weak signal (data not shown), which could not be accurately characterized due to the changes occurring during measurements, associated with DPA photolability [14,17].

By contrast, phosphorescence emission upon excitation of DPA at 266 nm in EtOH glass at 77 K consisted of a band with maximum centered at ca. 410 nm (Fig. 1B). As this emission did not exhibit fine structure, the triplet excited state energy, E_T, was estimated from the wavelength corresponding to 20% of the highest intensity, obtaining a value of ca. 328 kJ mol^{-1} [18,21]. This E_T is clearly higher than that of the triplet excited state of thymine in DNA with a value of ca. 267 kJ mol^{-1} [21,22], thus confirming the ability of DPA to photosensitize the formation of cyclobutane thymine dimers by means of a triplet-triplet energy transfer (TTET) mechanism [12,23].

Next, laser flash photolysis experiments were performed to obtain key information on the DPA triplet excited state (^{3}DPA*) at room temperature in aqueous media. As mentioned above for the fluorescence experiments, photodegradation was an important drawback to record the transient absorption spectrum. Therefore, a point by point experiment was run, using a fresh sample for each laser pulse (266 nm) *i.e.* each recorded wavelength. Fig. 2A shows the spectra obtained in this way for DPA in deaerated PBS, using fresh (black squares), one pulse (red circles) or two pulses pre-irradiated solutions (blue triangles).

Table 1
Triplet excited state lifetime (τ in μs), transient absorption band ($\lambda_{T\text{-}T}$ in nm), energy (E_T in kJ mol^{-1}) and bimolecular quenching rate constants (k_q in M^{-1} s^{-1}).

	τ	$\lambda_{T\text{-}T}$	E_T	$k_q(O_2)$	k_q(β-car)	k_q(Thd)
DPA	19.4 (±0.9)	300	328		–	5.3 (±0.6) × 10^9
DMDP	2.75 (±0.03)	300	323	1.6 (±0.1) × 10^9	–	1.9 (±0.1) × 10^9
1	21.6 (±0.2)	380	286	5.2 (±0.5) × 10^8	1.6 (±0.2) × 10^{10}	5.0 (±0.1) × 10^8
2	29.9 (±0.4)	400	266	6.6 (±0.2) × 10^8	1.8 (±0.1) 10^{10}	<10^7

An oxygen-quenchable absorption transient, with maximum at 300 nm and a lifetime of ca. 20 μs, was observed for the fresh DPA sample. This signal was assigned to a triplet excited state (^{3}DPA*) on the basis of previous literature data [14]. Then, a step further was made to investigate the possibility of a triplet-triplet energy transfer between ^{3}DPA* and thymidine (Thd) by analyzing the changes in ^{3}DPA* kinetics in the presence of the nucleoside. In this context, quenching experiments were performed by monitoring at 300 nm the shortening of ^{3}DPA* decay in the presence of increasing amounts of Thd (Fig. 2B). A Stern-Volmer representation of the data gave a bimolecular rate constant of 5.3 × 10^9 M^{-1} s^{-1} (Table 1). This value is in the range expected for TTET from a high triplet excited state photosensitizer to afford formation of thymidine triplet excited state (^{3}Thd*) [23,24].

Interestingly, the spectra obtained with 1 or 2 pulses pre-irradiated solutions (Fig. 2A, red circle and blue triangles, respectively) showed the decrease of ^{3}DPA* transient absorption, together with the appearance of a new peak at 380–400 nm, which might correspond to the transient absorption band of photoproduct(s).

3.2. Characterization of DPA photoproducts

The structures of these photoproducts were determined upon isolation and characterization of the compounds obtained after UVC-irradiation of N_2-degassed PBS solutions of DPA. For convenience, in order to facilitate the chromatographic separation and to follow the course of the reaction by HPLC, the photoreaction mixtures were esterified before analysis (see SI for more details). The chromatogram obtained after 6 min of irradiation is shown on Fig. 3 (line D); it reveals how DPA, appearing as its dimethyl ester DMDP and eluting at 12 min (see line A for DMDP authentic sample), is transformed into two main photoproducts eluting, after esterification, at 14 min and 20 min.

In a first step, these compounds were isolated by preparative HPLC, and tentatively characterized by NMR (see SI) and HRMS as compounds 1 and 2 (Chart 2). Accordingly, HRMS revealed that the methyl esters of both photoproducts are isomeric and correspond to the formula $C_{14}H_{12}N_2O_4$. The assignments were further confirmed by comparison with authentic samples obtained by alternative synthesis (Fig. 3, lines B and C, for synthesis details, see Sections 2.3.2 to 2.3.4 and for NMR spectra see SI).

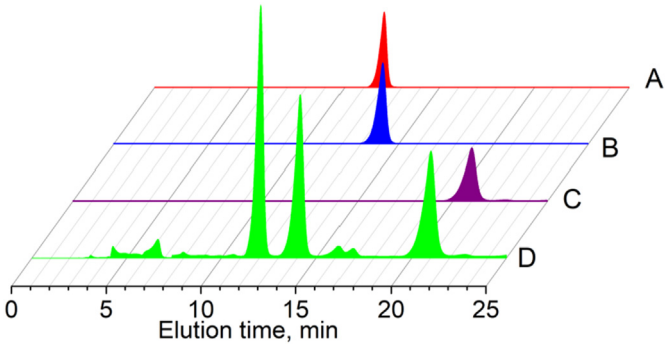

Fig. 3. HPLC chromatograms obtained for A) DMDP, B) photoproduct **2**, C) photoproduct **1**, D) UVC irradiated sample (6 min at 254 nm, PBS/N_2, 5 mg/mL). The obtained samples were esterified before analysis (see Section 2.4).

These photoproducts would arise from decarboxylative photodimerization processes, as already described for similar compounds such as picolinic acid [25]. Indeed, under the photolysis conditions (pH 7.4), the DPA is mainly under its dianionic form (DPA^{2-}, Scheme S1 in SI) with a low concentration of the monoanion (DPA^-) [26]. As proposed for picolinic acid, the excited triplet state of DPA^{2-} can interact with DPA^- to generate the pyridyl radical (I) together with radical II. Radical I can then dimerize to form the 2,2′-bipyridine photoproduct, or react with DPA^{2-} in the ground state to finally yield the 2,3′-bipyridine derivative (see Scheme S1).

Having established the nature of the photoproducts, it was anticipated that upon esterification of DPA to DMDP photodecarboxylation should be blocked and a consequent photodegradation should be prevented (Chart 2). This prompted us to investigate the photophysical properties of DMDP in more detail and to compare them with those of the parent carboxylic acid DPA.

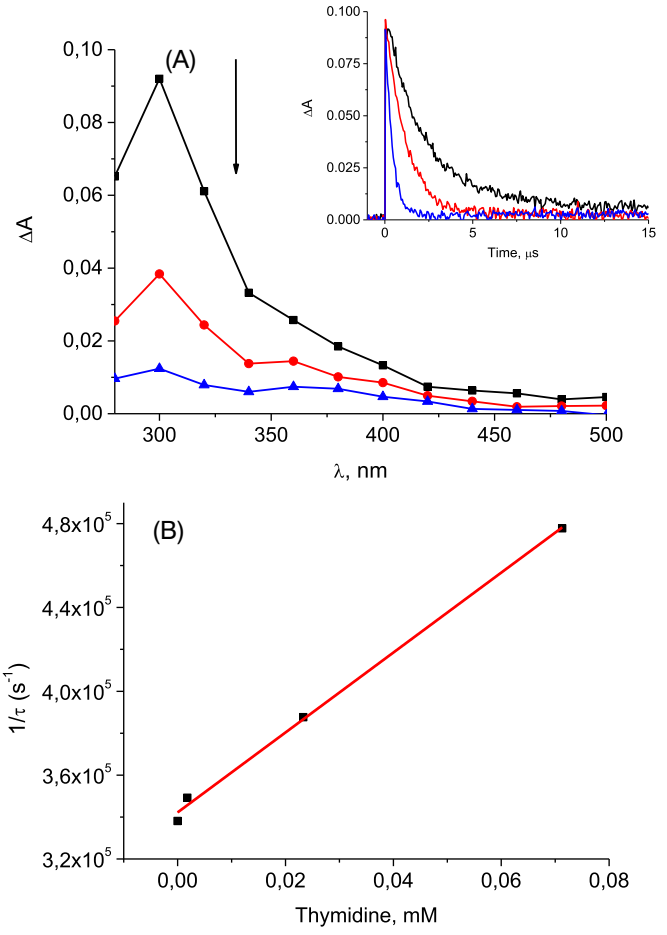

Fig. 4. (A) Transient absorption spectra of DMDP in deareated MeCN:H_2O (1:9, v:v) from 0.4 to 9 μs after the 266 laser pulse. Inset: decays monitored at 300 nm under N_2 (black), air (red) or O_2 (blue) atmosphere. (B) Stern-Volmer representation for the quenching of ^{3}DMDP* by Thd.

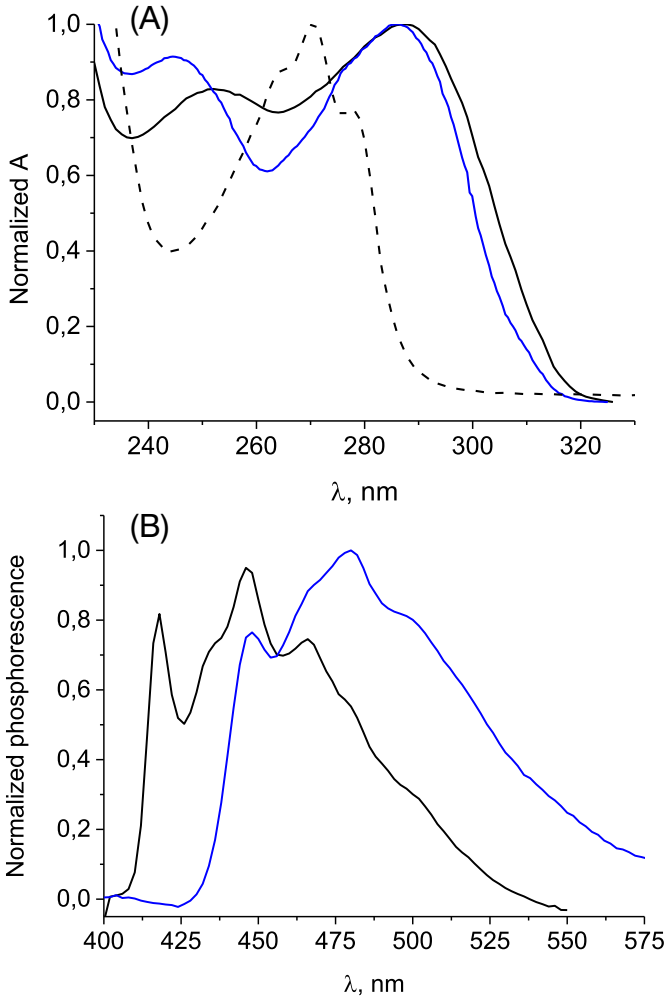

Fig. 5. A) Normalized absorption spectra of photoproducts **1** (black line) and **2** (blue line) in PBS, DMDP spectrum (dash line) is given for comparison B) Normalized phosphorescence emission spectra (λ_{exc} = 300 nm) of photoproducts **1** (black line) and **2** (blue line) in EtOH.

3.3. Photophysics of DMDP, a stable DPA derivative

As stated above, DMDP in aqueous solution (H_2O:MeCN, 9:1, v:v) exhibits an absorption spectrum very similar to that of DPA, with a maximum peaking at 270 nm (Fig. 1A, red line). The same is true for low temperature emission in EtOH at 77 K (Fig. 1B, red line). For DMDP, a E_T of ca. 323 kJ mol^{-1} was estimated from the wavelength corresponding to the 20% of maximum emission intensity. Moreover, steady-state photolysis performed at 254 nm confirmed the photostability of this DPA derivative (Fig. S1).

Laser flash photolysis of DMDP was performed in H_2O:MeCN (9:1, v:v). The photostability of this dimethylated derivative allows running the experiment without changing the solution after each 266 nm pulse. A transient absorption band with a maximum similar to that of ^{3}DPA*, centered at 300 nm was detected (Fig. 4A). This signal decays with a lifetime of 2.75 μs, which was quenched in the presence of oxygen with a bimolecular rate constant of 1.6×10^9 M^{-1} s^{-1} (Table 1). This signal was assigned to the triplet-triplet transition of DMDP by comparison with the data obtained above for DPA. Finally, the potential of DMDP to photosensitize thymidine triplet excited state formation was investigated and a bimolecular quenching rate constant of ca. 1.9×10^9 M^{-1} s^{-1} was determined (Fig. 4B, Table 1).

Overall, DMDP and DPA exhibit similar absorption and triplet excited state properties, the dimethyl ester derivative can thus be considered as a useful photostable DPA model.

3.4. Photophysics of DPA photoproducts

Next, the attention was focused on the two DPA photoproducts in order to investigate whether they can also act as cyclobutane thymine dimer photosensitizers. The UV-absorption spectra of photoproducts **1** and **2** showed a common band peaking at 287 nm that extended up to 315–320 nm (Fig. 5A). They also exhibited an absorption at lower wavelength, with a λ_{abs} of 253 and 245 nm for **1** and **2**, respectively. Phosphorescence was registered in EtOH glass at 77 K using 300 nm as excitation wavelength (Fig. 5B). Compound **1** showed a blue shifted emission of ca. 30 nm by respect to that of compound **2**, pointing to a higher triplet state energy (E_T) for the former compound. Indeed, these energies were determined from the wavelength of the first peak at ca. 418 and 449 nm, which corresponds to E_T of 286 and 266 kJ mol^{-1} for **1** and **2**, respectively.

Triplet excited state behavior in solution, at room temperature, was investigated by laser flash photolysis. As shown in Fig. 6, compounds **1** and **2** in deaerated PBS exhibited a transient absorption peaking at 380 and 400 nm, respectively. Interestingly, these bands appear in the same region as the spectrum of photolyzed DPA shown in Fig. 2A (blue and red traces), especially for 380 nm band of photoproduct **1**. Decays were fitted with a monoexponential function, and values of 21.6 and 29.9 μs were determined for **1** and **2**, respectively. These species were quenched by oxygen, with k_q of ca. 5.2–6.6 × 10^8 M^{-1} s^{-1} (Table 1). Moreover, experiments in the presence of β-carotene showed the typical behavior of triplet-triplet energy transfer process with a decrease of the photoproducts **1** and **2** bands concomitantly with the growth of the β-carotene triplet absorption at ca. 510 nm (Fig. 7A and S2). Diffusion controlled k_q (Table 1) were determined for this quenching in acetonitrile. Further evidence supporting the triplet nature of these species was obtained from xanthone quenching by **1** and **2**, where the photoproducts act as energy acceptors. After xanthone

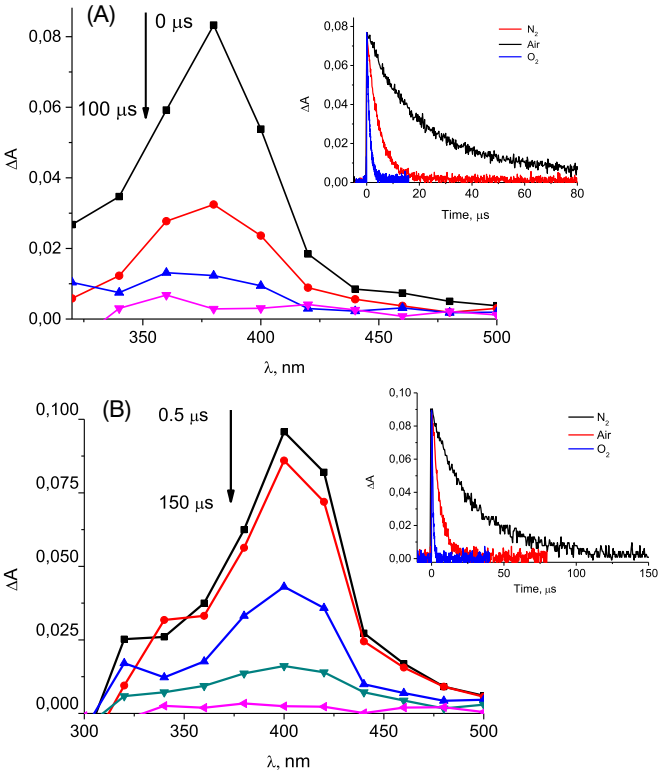

Fig. 6. Transient absorption spectra of compound **1** (A) and compound **2** (B) in PBS solution using a 266 nm laser pulse. Insets: Decays monitored under nitrogen, air or oxygen atmosphere.

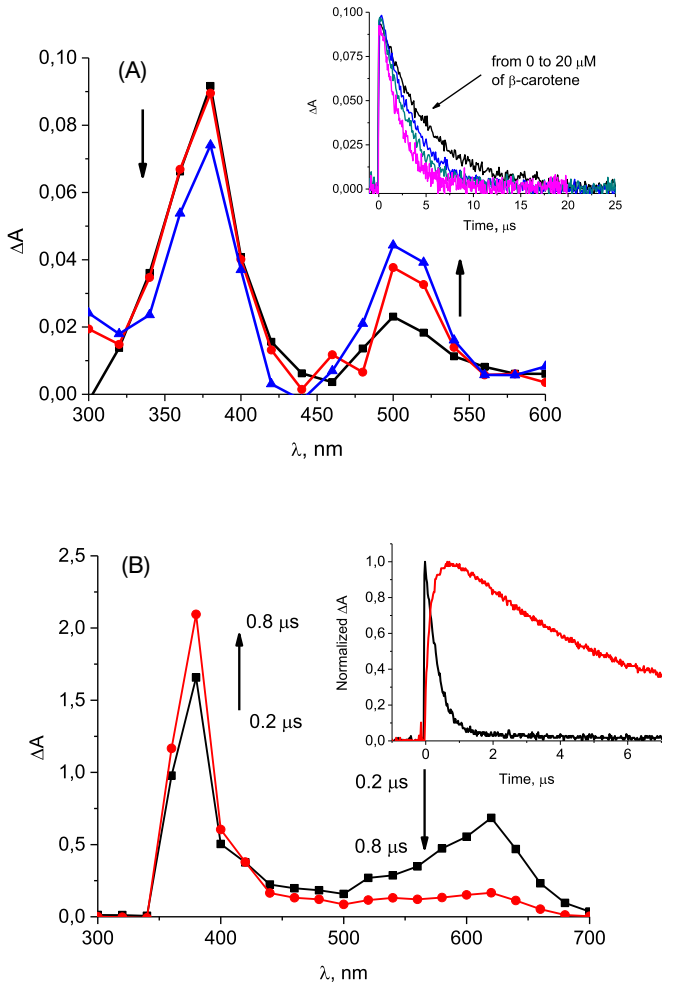

Fig. 7. (A) Transient absorption spectra of photoproduct **1** in deaerated acetonitrile in the presence of β-carotene after 266 nm laser pulse. Insets: decays in the presence of increasing amounts of β-carotene; (B) Transient absorption spectra of xanthone in the presence of 238 μM of **1** after 355 nm laser pulse excitation. Inset: kinetic traces observed at 380 nm (red line) and 620 nm (black line).

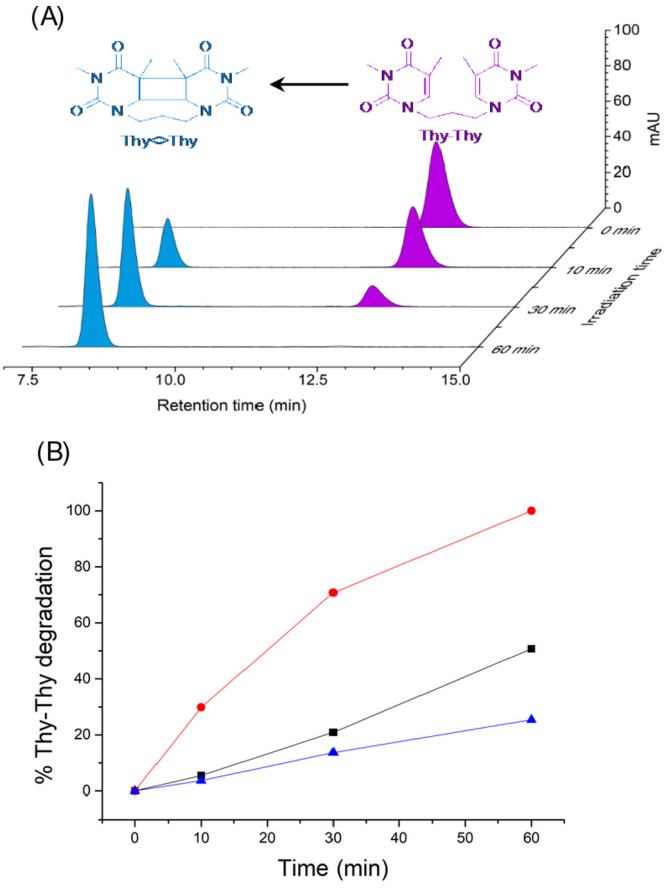

Fig. 8. A) HPLC chromatograms recorded at 240 nm for a solution of Thy-Thy: **1** (0.5 mM:1.5 mM) in H_2O:MeCN (1:1, v:v) irradiated at 300 nm. B) Time-dependent photodegradation of Thy-Thy UVB-irradiated in the presence of DMDP (black), **1** (red) and alone (blue).

excitation at 355 nm in the presence of **1**, the typical triplet-triplet energy transfer behavior was registered with the decrease of xanthone triplet transient absorption at 620 nm together with the concomitant growth of the 380 nm band of **1** (Fig. 7B). Similar results were obtained for photoproduct **2** (Fig. S3).

In connection with cyclobutane thymine dimer formation, the E_T value of photoproduct **1** is higher than that of thymine in DNA, and thus this compound should behave as a photosensitizing agent able to transfer its energy to thymine, affording the nucleobase triplet excited state. In this context, laser flash photolysis of **1** in the presence of thymidine showed that the photoproduct triplet was indeed quenched with a k_q of ca. 5.0×10^8 M^{-1} s^{-1} (Fig. S4). By contrast, a similar experiment performed with **2** gave a $k_q < 10^7$ M^{-1} s^{-1}. These results showed the capability of **1**, but not **2**, to populate ^{3}Thd* by TTET.

Finally, steady-state photolysis was performed to follow the formation of cyclobutane thymine dimers (Thy<>Thy) photosensitized by **1**. For this purpose, a model system consisting in two covalently linked thymine units was used (Thy-Thy, Fig. 8). A mixture of **1** (1.5 mM) and Thy-Thy (0.5 mM) was irradiated with UVB lamps, and the course of the reaction was analyzed by HPLC. As shown in Fig. 8A, a clean reaction occurred with formation of only one new compound, which was assigned to *cis-syn* Thy<>Thy by comparison with a standard sample [18].

Parallel experiments performed with deaerated solutions of Thy-Thy alone (Fig. S5) and in the presence of **1** revealed that, although under the employed irradiation conditions some Thy<>Thy was directly formed due to direct light absorption by the Thy chromophore, the photodimerization process was clearly higher when **1** was present in the solution (Fig. 8B). This indicates that, as anticipated, the DPA photoproduct acts as a sensitizer for Thy<>Thy formation. Interestingly, the results obtained in the presence of 1.5 mM DMDP (Fig. 8B and Fig. S6) showed that in spite of a higher E_T, the photostable derivative of DPA produces a lower yield of cyclobutane dimers under the used conditions due to its lower absorption in the UVB range used for the experiment (Fig. 5A).

4. Conclusion

A spectroscopic study of dipicolinic acid (DPA) has been performed to bring more information on its potential photosensitizing properties. In this context, a particular attention has been paid on its triplet excited state and its interaction with thymidine. To avoid artifacts due to DPA photolability, its dimethyl ester derivative has been considered, and the triplet state properties of DPA (i.e. an energy E_T of ca. 328 kJ mol^{-1}, a transient absorption band centered at 300 nm, a bimolecular rate constant for quenching by thymidine k_q of ca. 10^9 M^{-1} s^{-1}) have been confirmed. Moreover, DPA photoproducts have been associated with a decarboxylative photodimerization, giving rise to 2,2′- and 2,3′-bipyridine derivatives. The former exhibits the properties of an efficient thymidine photosensitizer, as observed by laser flash photolysis and HPLC experiments. Altogether, these results establish the capacity of DPA (and of one of its photoproducts) triplet excited state to transfer its energy to thymidine, photosensitizing the formation of cyclobutane pyrimidine dimers or spore photoproducts under dry conditions.

CRediT authorship contribution statement

Giacomo Nardi: Investigation, Data curation. **Mauricio Lineros-Rosa:** Investigation, Validation, Data curation. **Fabrizio Palumbo:** Investigation, Validation, Data curation. **Miguel A. Miranda:** Conceptualization, Writing - review & editing, Funding acquisition. **Virginie Lhiaubet-Vallet:** Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of competing interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2020.118898.

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