

Photophysics and photodimerization of 6,5'-dimethylangelicin in different solvents

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Abstract

The photophysical and photochemical behaviour of 6,8-dimethyl-2*H*-furo[2,3-*h*]chromen-2-one (6,5'-dimethylangelicin, 6,5'-DMA) was studied by steady state and pulsed techniques and by semiempirical calculations. The fluorescence characteristics and the triplet state properties were determined in solvents of different polarity/proticity. The obtained values of fluorescence quantum yields and lifetimes together with the triplet formation quantum yield indicate that the energy gap between the two lowest excited states controls the decay pathways of the singlet. In non-polar and non-protic solvents the internal conversion is almost the sole decay process of the singlet, while in protic solvents the intersystem crossing reaches substantial quantum yields. The effect of ground state concentration on the fluorescence lifetime suggested the occurrence of aggregation while the triplet lifetime was quenched and the rate constant of self-quenching was determined.

The 6,5'-DMA photodimerization was investigated in different solvents and in different experimental conditions. Two pyrone-pyrone and one furan-pyrone photodimers of 6,5'-DMA were the main photoproducts detected in the irradiated solution. The effects of the 6,5'-DMA concentration and of oxygen on the amounts of the dimers obtained together with the photophysical behaviour gave indications on the formation mechanism of different dimers.

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1. Introduction

Naturally occurring furocoumarins (psoralens and angelicins) and some of their synthetic derivatives are members of a class of photobiologically active compounds used as light-activated drugs in several skin diseases [1–3]. The photochemotherapeutic activity of furocoumarins may have antiproliferative or stimulating effects on skin cells (treatment of psoriasis and vitiligo, respectively) [4] and effects against immunologic disorders [3]. The antiproliferative activity of furocoumarins has been mainly attributed to their ability to covalently photobind DNA forming mono- and di-adducts

[5], while the pigmentogenic effect seems to be connected with their ability to induce photolesions in lipids of the cell membrane or to stimulate enzymatic repair of the DNA damage [2,6,7].

The ability of linear furocoumarins (psoralens) in forming mono- (mainly) and di-adducts with the pyrimidine bases gives rise to crosslinking between DNA chains [5]. For this reason the photochemotherapy with psoralens has some side effects such as skin phototoxicity and risk of skin cancer [1,8–10]. Angelicins (furocoumarins with angular structure) which can only form monoadducts, generally show lower mutagenic activity [11,12] and phototoxic effects on the skin [13–15]. Therefore, detailed studies on several angelicin derivatives were undertaken to correlate the various aspects of their photobiological activity with their photophysical and photochemical properties [16–19].

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Furocoumarins, besides the photocycloaddition to DNA and fatty acids, can undergo photodimerization. This process appears to compete with the phototherapeutic activity, since no biological activity has been reported for these dimers. This photoreaction has been investigated mainly for psoralens under different experimental conditions and scarcely for angelicin (for a review, see [20]). Particular attention was reserved to the number and structure of the dimers obtained and to the nature of the excited states involved in the photoprocess. The irradiation of 8-methoxy- and 8-acetoxypsoralen [21,22] in dichloromethane produced only the *trans-syn* pyrone–pyrone (p–p) dimer where the two furocoumarin moiety are both bound in the 3,4-positions of the pyrone rings. The corresponding 4',5'-dihydro homologues gave, besides the mentioned photoproduct, the *cis-anti* p–p dimer. The results gave evidence that the formation of the former and the latter involves the triplet and singlet excited state of the parent molecule, respectively [21,22].

As concerns angelicins, dimerization was studied with two methyl derivatives [23]. In particular, 6,5'-dimethylangelicin (6,5'-DMA) gave a pyrone–pyrone dimer, of unidentified regio- and stereochemistry, and a pyrone–furan dimer with *cis-syn* configuration [23].

In the present paper further photochemical and photophysical studies on 6,5'-DMA are reported. They are aimed at the understanding of the photoreaction mechanism leading to the dimers and the excited states involved. The photodimerization of 6,5'-DMA was studied in different solvents, in the presence and in the absence of oxygen, and the relative yields for the formation of the dimers were evaluated at different concentrations. A detailed characterization of the lowest excited singlet and triplet states was also carried out. The fluorescence quantum yield and lifetime were measured in different solvents and the triplet state was investigated by laser flash photolysis determining the spectroscopic and kinetic properties and the effect of 6,5'-DMA concentration on the triplet lifetime in different solvents.

2. Experimental

2.1. Materials and experimental measurements

6,8-Dimethyl-2H-furo[2,3-h]chromen-2-one, (6,5'-dimethylangelicin, see Scheme 1) was synthesized as in a previous work [24]. The solvents, cyclohexane (Cx, Baker UV grade), dioxane (Dx, Fluka, UV grade), acetonitrile (MeCN, Carlo Erba RPE grade), ethanol (EtOH, Carlo Erba, RS grade), trifluoroethanol (TFE, Fluka, puriss. grade) and water milliQ (Waters) were used.

2.2. Semiempirical calculations

The absorption spectra were predicted by use of the semiempirical ZINDO/S-CI method (Hyperchem 7.0 package), after geometrical optimization with the PM3 model.

The configuration interaction (CI) calculations included the singly excited configurations built from the 11 highest occupied (HOMO) and 11 virtual (LUMO) molecular orbitals [25,26].

In order to get information on the triplet energy of 6,5'-DMA, the heat of formation of the ground state and of the lowest excited triplet state (T_1) were predicted by the PM3 and PM3-CISDT models, respectively, of MOPAC 2002 (CACH 6.1 package) [27]. The five highest occupied (HOMO) and two virtual (LUMO) molecular orbitals were used to originate 475 singly, doubly and triply excited configurations included in the CISDT calculation. The reliability of this method was tested by calculating a value of $63.5 \text{ kcal mol}^{-1}$ for the triplet energy of the parent compound angelicin, to be compared with the experimental $63.2 \text{ kcal mol}^{-1}$ [28].

2.3. Techniques

Absorption spectra were recorded on a Perkin-Elmer (Lambda 800) spectrophotometer.

A Philips HPW 125 lamp (emitting over 95% at 365 nm) was used for irradiation of 6,5'-DMA solutions in pyrex test tubes, cooled by water circulation. The light dose on the tubes was $0.55 \text{ J cm}^{-2} \text{ min}$, as measured by a Cole-Palmer Instrument radiometer (Niles, IL, equipped with a 365-CX sensor). When required, solutions were saturated by bubbling with oxygen or nitrogen.

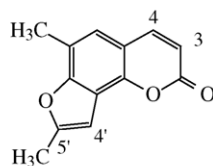
A Perkin-Elmer Series 200 instrument, equipped with a Diode Array detector was used for high performance liquid chromatography (HPLC) measurements. The column was a reverse-phase Water Spherisorb S5 ODS2 ($5 \mu\text{m}$, $250 \text{ mm} \times 4.6 \text{ mm}$) eluted with MeCN–H₂O mixtures (20:80 for 5 min, then linearly increased to 80:20 within 20 min and maintained at 80:20 for 20 min).

To isolate photoproducts, the irradiated solutions were dried under reduced pressure and submitted to thin-layer chromatography (TLC) using silica gel plates (F254, Merck). A rough separation was achieved eluting the plates with chloroform, while eluents for purification are indicated in the Results section.

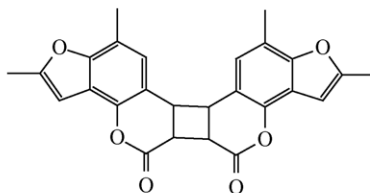
The nuclear magnetic resonance spectra (¹H-NMR) were acquired on a Bruker AMX spectrometer operating at 300 MHz. Samples were dissolved in CDCl₃ or CD₃COCD₃ containing 0.1% TMS as the internal standard.

Mass spectra were obtained by means of a Mariner API-TOF spectrometer (PerSeptive Biosystems), by direct injection of the samples dissolved in methanol-formic acid (99:1).

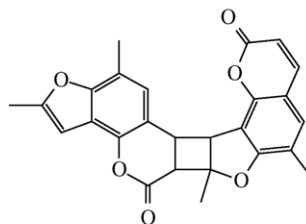
Fluorescence spectra and quantum yields were measured with a Fluorolog-2 (Spex, F112AI) spectrophotofluorometer (mean deviation of three independent measurements, ~5% in deaerated solutions, absorbance <0.1 at the excitation wavelength) using anthracene in ethanol as standard, $\phi_F = 0.27$ [28]. The fluorescence lifetimes, τ_F (mean deviation of three independent measurements, ~10%) were measured by a Spex Fluorolog- τ_2 system, which uses the phase modulation tech-



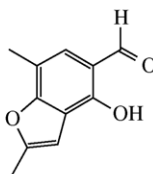
6,8 dimethyl-2H-furo[2,3-h]chromen-2-one
(6,5'-Dimethylangelicin, 6,5'-DMA)



2,9,11,14-tetramethyl-5a,5b,12b,12c-tetrahydro-5H,6H-
furo[2''3''-7',8']chromeno[4',3':3,4]cyclobuta[1,2-c]furo[2,3-h]chromene-5,6-dione
(Pyrone-pyrone-6,5'-DMA dimer)



2,5b,7,14-tetramethyl-5a,5b,12c,12d-tetrahydro-5H,11H-
chromeno[8'',7'':4',5']furo[3',2':3,4]cyclobuta[1,2-c]furo[2,3-h]chromene-5,11-dione
(Pyrone-furan-6,5'-DMA dimer)



4-Hydroxy-2,7-dimethyl-benzofuran-5-carbaldehyde

Scheme 1. Molecular structure of 6,5'-dimethylangelicin (6,5'-DMA), of its pyrone–pyrone (p–p) and pyrone–furan (p–f) dimers and of photoproduct 1 (4-hydroxy-2,7-dimethyl-benzofuran-5-carbaldehyde).

nique (excitation wavelength modulated in the 1–300 MHz range; time resolution ~ 10 ps). The frequency-domain intensity decay (phase angle and modulation versus frequency) was analyzed with the Global UnlimitedTM (rev. 3) global analysis software [29].

The experimental set-up used for determining the triplet spectra and yields has been described elsewhere [30,31] The excitation wavelength of 355 nm from the third harmonics of a Nd:YAG laser (Continuum, SureLiteII, pulse width ~ 7 ns and energy < 3 mJ pulse⁻¹) was used. Absorption spectra were constructed based on measurements every 10 nm over the 300–800 nm spectral range, averaging at least 10 shots per wavelength recorded. The triplet lifetimes were measured with laser fluence ≤ 1 mJ pulse⁻¹.

The product $\Delta\epsilon_T \times \phi_T$ for 6,5'-DMA in various solvents was obtained by the laser energy effect on the change of absorbance (ΔA) measured at λ_{\max} and using an optically matched solution ($A_{355} \approx 0.2$) of benzophenone in acetonitrile ($\epsilon_{520} \times \phi_T = 6500 \text{ M}^{-1} \text{ cm}^{-1}$ at 520 nm) [32] to calibrate the instrumental response. Plots of ΔA versus laser dose were linear and passed through zero, thus indicating that only one-photon processes were occurring. The triplet quantum yield (ϕ_T) of 6,5'-DMA was evaluated by determining the absorption coefficient change ($\Delta\epsilon_T$) of 6,5'-DMA using benzophenone as a donor in acetonitrile ($\Delta\epsilon_T = 6500 \text{ M}^{-1} \text{ cm}^{-1}$ at 520 nm) [32] or anthracene as an energy acceptor in ethanol ($\Delta\epsilon_T = 52000 \text{ M}^{-1} \text{ cm}^{-1}$ at ~ 430 nm) [32]; experimental error $\pm 15\%$.

The singlet oxygen yields (ϕ_{Δ}) of DMA were determined by measuring the phosphorescence intensity of $O_2(^1\Delta_g)$ with a germanium diode detector in air-equilibrated solutions. The amplified signal extrapolated at zero time (in mV) was plotted as a function of laser dose obtaining a good linear relationships for 6,5'-DMA and for phenalenone used as a standard ($\phi_{\Delta} = 0.97 \pm 0.03$) [33]. The ϕ_{Δ} values of 6,5'-DMA were then obtained from the ratio of the related slope with that of phenalenone multiplied by the known ϕ_{Δ} of the standard. At least 50 kinetic measurements were averaged for each 6,5'-DMA solution.

All measurements were carried out at 22 ± 2 °C; the solutions were saturated by bubbling with argon, unless otherwise indicated.

3. Results and discussion

3.1. Ground state absorption and semiempirical calculations

The absorption spectrum of 6,5'-DMA is located in the 220–380 nm range, the position of its maximum (λ_{\max}) being solvent dependent. Spectra recorded in five solvents are reported in Fig. 1. In the non-polar cyclohexane, Cx (where a slight vibrational structure is evidenced) and in the polar/protic trifluoroethanol (TFE) λ_{\max} values are at 297 and 310 nm, respectively. This red-shift, that is well related with the parameter $E_T(30)$ of the solvent (see below), is characteristic of other methylangelicins and indicates that the excited π , π^* state is more polar than the ground state [34]. The absorption coefficients at the maxima of the spectra (ϵ_{\max}) are of the order of $10^4 \text{ M}^{-1} \text{ cm}^{-1}$ (in EtOH the ϵ_{\max} value is $1.05 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), in agreement with a partially allowed $\pi \rightarrow \pi^*$ transition.

To get better insight into the transitions involved in the $S_0 \rightarrow S_n$ absorption of DMA and the nature and state order,

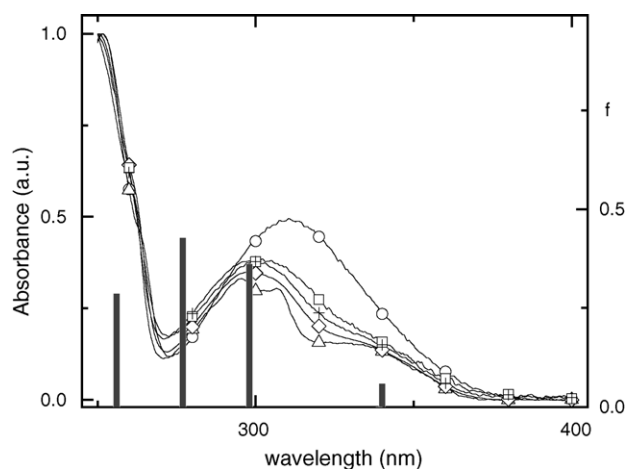


Fig. 1. Absorption spectra of 6,5'-DMA in TFE (○), MeCN (+), EtOH (□), Dx (◇) and Cx (△). The vertical lines represent the oscillator strengths calculated by ZINDO.

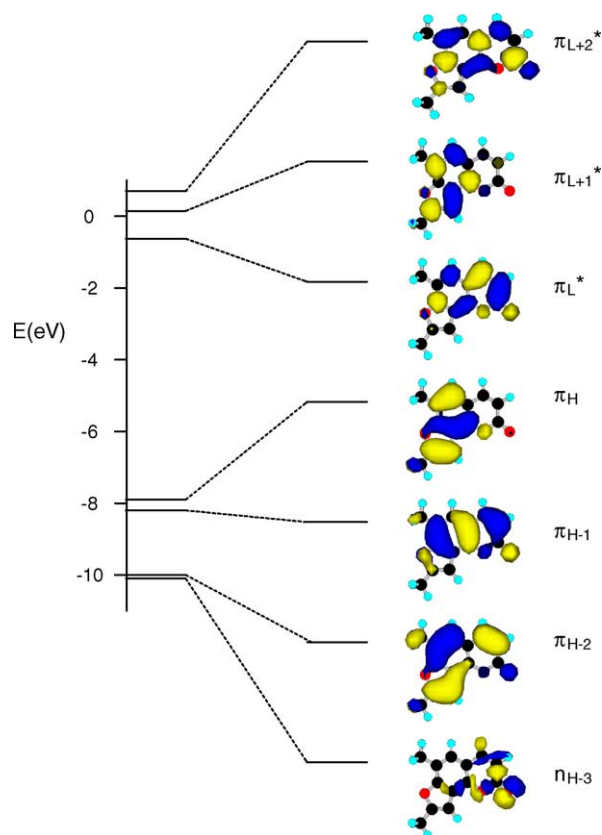


Fig. 2. Frontier molecular orbitals of 6,5'-DMA calculated by ZINDO/S.

semiempirical calculations were performed by the ZINDO/S-CI method [25,26]. The configuration interaction (CI) calculations included singly excited configurations built from the 11 highest occupied (HOMO) and 11 virtual (LUMO) molecular orbitals. The derived HOMO and LUMO are shown in Fig. 2. The HOMO is mainly localized on the furan moiety with the involvement of the central ring, while the LUMO is characterized by a displacement of the charge density towards the pyrone moiety. Moreover, in the LUMO + 1 orbital there is an antibonding charge density in the 4',5' position of the furan ring. Fig. 2 also shows that the bond order of the 3,4 and 4',5' positions decreases on going from HOMO to LUMO and LUMO + 1, respectively; in fact, in the last orbitals nodal planes appear. Even though other nodal positions are present in the molecular orbitals, those in 3,4 and 4',5' are the most important ones because they are involved in the photocycloaddition process of 6,5'-DMA. In particular, they are involved in the formation of pyrone–pyrone and pyrone–furan dimers described in this paper.

The semiempirical ZINDO/S-CI calculations predict for 6,5'-DMA five transitions in the 250–350 nm range: $S_0 \rightarrow S_1$ is $n \rightarrow \pi^*$ in nature while the other four ($S_0 \rightarrow S_n$) are of $\pi \rightarrow \pi^*$ character. The calculated λ_{\max} values, the oscillator strengths (f) and the orbitals involved in the transitions are reported in Table 1. The values of the oscillator strengths for the various transitions can be compared with the experimental absorption spectra of Fig. 1. The calculated and experimental

Table 1

Electronic transitions of 6,5'-DMA calculated by the ZINDO/S-CI semiempirical method after geometrical optimization with the PM3 model

Transition	λ_{\max} (nm)	f	Orbitals	%
$S_0 \rightarrow S_1$	340	0.001	$n_{H-3} \rightarrow \pi_L^*$	50
			$n_{H-3} \rightarrow \pi_{L+2}^*$	29
$S_0 \rightarrow S_2$	339	0.061	$\pi_H \rightarrow \pi_L^*$	52
$S_0 \rightarrow S_3$	300	0.37	$\pi_{H-1} \rightarrow \pi_L^*$	75
$S_0 \rightarrow S_4$	277	0.41	$\pi_H \rightarrow \pi_{L+1}^*$	44
			$\pi_{H-1} \rightarrow \pi_{L+1}^*$	22
$S_0 \rightarrow S_5$	256	0.29	$\pi_H \rightarrow \pi_{L+1}^*$	40
			$\pi_H \rightarrow \pi_{L+1}^*$	26

values are in good agreement, thus indicating that the calculation method used is useful to describe the ground and excited states of 6,5'-DMA. Its spectral feature is exclusively determined by the four $\pi \rightarrow \pi^*$ transitions because the f value of the forbidden $n \rightarrow \pi^*$ one (not reported in Fig. 1) is, as expected, very low. It has to be noted that the two lowest excited singlet states are predicted to be in practice at the same energy. As it will be shown from the fluorescence data, the closeness of these two states of different nature is important in determining the decay pathways of 6,5'-DMA singlet state ("proximity effect", see below).

3.2. Fluorescence

The spectrofluorimetric measurements showed that the wavelength of the emission maximum and the fluorescence quantum yield of 6,5'-DMA is strongly dependent on the solvent polarity/proticity (Table 2). The emission spectra (Fig. 3) shifted to the red by increasing the polarity/proticity of the solvent and become broader and structureless; a red-shift of about 7000 cm^{-1} is observed on going from Cx to TFE, indicating that stabilization of the $\pi \rightarrow \pi^*$ emitting state occurs in the presence of solvents which are polar and/or able to form hydrogen bonds with 6,5'-DMA. The red-shift of the fluorescence maximum is well correlated with the solvent parameter $E_T(30)$ proposed by Reichardt [35], as indicated by the linear plot (correlation coefficient 0.98) of the energy of the emitting state obtained from the fluorescence maximum (in cm^{-1}) versus $E_T(30)$ (Fig. 4). This indicates that $E_T(30)$ well describes the mixed effect of polarity and hydrogen bond formation on the fluorescent state of 6,5'-DMA.

The fluorescence quantum yield (ϕ_F) of 6,5'-DMA in Cx and Dx is particularly low and was estimated to be

Table 2

Absorption and emission properties of 6,5'-DMA in different solvents

Solvent	λ_{abs} (nm)	λ_{em} (nm)	ϕ_F	τ_F (ns)	k_F (s^{-1})
Cyclohexane	295; 305; 330sh	360	<0.0001	<0.02	
Dioxane	300; 315sh	380	<0.0001	<0.02	
Acetonitrile	300; 315sh	435	0.001	<0.02	$>5.0 \times 10^7$
Ethanol	305	460	0.007	2.3 ^a	0.3×10^7
TFE	310	485	0.030	2.8	1.1×10^7

^a Measured at 6,5'-DMA concentration $\leq 10^{-5}$ M.

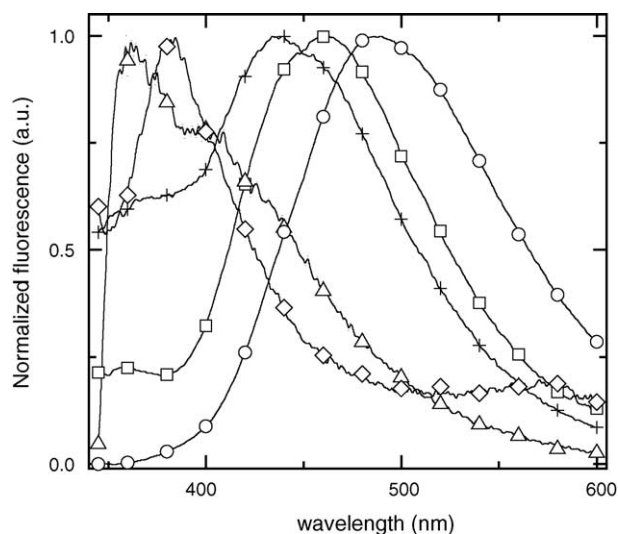


Fig. 3. Normalized emission spectra of 6,5'-DMA in TFE (○), MeCN (+), EtOH (□), Dx (◇) and Cx (△).

lower than 10^{-4} . In polar/protic solvents the emission efficiency increases (Table 2) and ϕ_F reaches the value of 0.03 in TFE. Even though a marked increase (more than thirty times) was observed on going from Cx to TFE, the fluorescence quantum yield remains very small in all solvents and therefore the radiative decay has to be considered a negligible process in the deactivation of 6,5'-DMA singlet excited state.

The fluorescence lifetimes (τ_F) were measured only in EtOH and TFE (Table 2) due to the low ϕ_F values and likely short τ_F values in less polar solvents. Either in EtOH and TFE the fluorescence decay of 6,5'-DMA was measured using low angelicin concentrations ($[6,5'\text{-DMA}] \leq 10^{-5}$ M) and was well fitted by a monoexponential function; fluorescence lifetimes of few ns were obtained. These τ_F values are in agreement with the values previously measured for other angelicines [34]. Considering the measured ϕ_F and τ_F in EtOH and TFE and the estimated values in MeCN, the k_F values

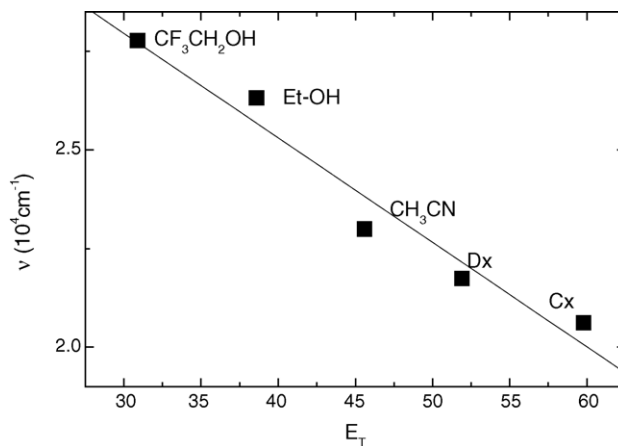


Fig. 4. Dependence of the fluorescence maximum energy of 6,5'-DMA on the $E_T(30)$ solvent parameter.

were determined (Table 2); in these solvents k_F values are in agreement with a partially allowed $\pi \rightarrow \pi^*$ transition.

In EtOH solutions the effect of the ground state concentration on the fluorescence decay was investigated. At concentrations higher than 10^{-5} M the fluorescence decay could be satisfactorily fitted by a bi-exponential function whose parameters (lifetimes and pre-exponential factors) changed with the concentration. In general, the long-lived component was about 3.0 ns while the lifetime of the shorter one was always below 0.9 ns and its contribution increased to up 80% increasing the ground state concentration. This behaviour could be due to the formation of aggregates of 6,5'-DMA (see below).

3.3. Laser flash photolysis

Upon laser excitation of 6,5'-DMA at 355 nm in Cx, MeCN, EtOH and TFE a transient absorption was detected that was assigned to the lowest triplet state of 6,5'-DMA since in all solvents it appeared within the laser pulse, was quenched by oxygen with an almost diffusional rate constant, decayed with lifetime in the μs range, and its decay followed a first-order kinetics at low pulse intensity and low ground state concentration ($<10^{-5}$ M, see below). The time resolved absorption spectra of the transient triplet in MeCN, EtOH and TFE are reported in Fig. 5. The spectra have similar features in the three polar solvents and are characterized by a broad absorption band between 400 and 500 nm (Table 3); the relative intensity at different wavelengths was slightly affected by the solvent. The triplet lifetimes, reported in Table 3, show a moderate dependence on the solvent being longer in TFE.

The product between the triplet quantum yield and the triplet absorption coefficient ($\phi_T \times \Delta\varepsilon_T$) measured in all solvents, increased substantially with the solvent polarity (Table 3). Although an effect of the solvent on $\Delta\varepsilon_T$ cannot be excluded, these findings suggested that ϕ_T is influenced by the solvent polarity/proticity similarly to what observed for 6,5'-DMA ϕ_F values and for ϕ_T values of other methylangelicins [34]. The triplet absorption coefficient of 6,5'-DMA at the maximum of absorption (500 nm) was determined in MeCN and EtOH by use of the energy transfer methods as described in Section 2. $\Delta\varepsilon_T$ values of 3600 and 2070 $\text{M}^{-1} \text{cm}^{-1}$ were obtained for MeCN and EtOH, respectively. Using the absorption coefficient and the $\phi_T \times \Delta\varepsilon_T$ values the quantum yields for the triplet production (ϕ_T) were found to be 0.14

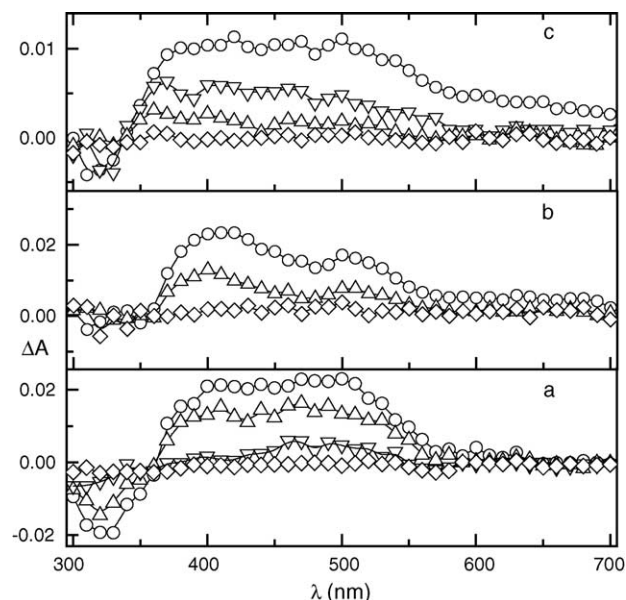


Fig. 5. Time resolved spectra of 6,5'-DMA in EtOH (a) recorded 0.56 (○), 1.5 (▽), 5.0 (Δ) and 15 (◇) μs ; in MeCN (b) recorded 0.30 (○), 1.4 (Δ) and 8.0 (◇) μs ; in TFE (c) recorded 0.20 (○), 0.56 (▽), 1.3 (Δ), and 15 (◇) μs after the laser pulse ($\lambda_{\text{exc}} = 355$ nm).

and 0.40 for MeCN and EtOH, respectively and they are reported in Table 3 together with intersystem crossing rate constants k_{ISC} . Assuming that the absorption coefficient of 6,5'-DMA triplet in TFE is the same as that measured in EtOH, ϕ_T in TFE was estimated to be 0.44.

In both cases, the measured ϕ_T values are substantial and indicate that the intersystem crossing, in polar/protic solvents, is an important decay pathway of 6,5'-DMA. The marked increase of ϕ_T with the solvent proticity confirms that the deactivation paths of 6,5'-DMA singlet state can be rationalized in terms of "proximity effect" [36,37].

The 6,5'-DMA triplet lifetime was dependent on the ground state concentration indicating that self-quenching took place; the measured self-quenching rate constant was close to the diffusional limit (ca. $3 \times 10^9 \text{M}^{-1} \text{s}^{-1}$).

The 6,5'-DMA triplet was quenched by molecular oxygen with a rate constant (k_{ox} , Table 3) close to the diffusional limit (k_{diff}). In TFE, where k_{diff} determined from the viscosity coefficient was $3.7 \times 10^9 \text{M}^{-1} \text{s}^{-1}$, k_{ox} value is particularly high ($2.0 \times 10^9 \text{M}^{-1} \text{s}^{-1}$). Only in MeCN, the quenching of 6,5'-DMA triplet was accompanied by the formation

Table 3
Triplet properties of 6,5'-DMA in different solvents

Solvent	λ_{max} (nm)	τ_T (μs)	$\phi_T \times \Delta\varepsilon_T$ [$\Delta\varepsilon_T$ ($\text{M}^{-1} \text{cm}^{-1}$)]	ϕ_T	k_{ox} ($\text{M}^{-1} \text{s}^{-1}$)	k_{ISC} ($\times 10^8 \text{s}^{-1}$)	k_{IC} ($\times 10^8 \text{s}^{-1}$)
Cyclohexane	420; 500	1.3	200				
Acetonitrile	400; 500	1.4	490 [3600]	0.14	3.7×10^9	>70	>430
Ethanol	400; 500	1.6	820 [2070]	0.40	1.3×10^9	1.7	2.6
TFE	400; 500	3.0	920	(0.44) ^a	2.0×10^9	1.6	1.9

^a Calculated assuming the same $\Delta\varepsilon_T$ value measured in EtOH.

of singlet oxygen, $O_2(^1\Delta_g)$ since only in this solvent the IR-luminescence of singlet oxygen could be detected upon excitation of 6,5'-DMA in air-equilibrated solution. No luminescence was observed in the absence of substrate or by bubbling argon. The luminescence decay was described by a first order kinetics with a lifetime very close to that reported in the literature. The quantum yield for singlet oxygen production (ϕ_Δ) in MeCN was determined by use of phenalene as reference. A ϕ_Δ value of 0.03 was obtained. This value is low with respect to the triplet quantum yield and shows that only 18% of triplet are able to produce $O_2(^1\Delta_g)$; it is also low with respect to the ϕ_Δ values of other angelicins previously investigated [34].

The ϕ_F and ϕ_T values reported in Tables 2 and 3 allow the internal conversion quantum yield (ϕ_{IC}) together with the corresponding rate constants k_{IC} to be determined in acetonitrile, ethanol and TFE (Table 3). In MeCN k_{IC} value (Table 3) is very high compared to k_F and k_{ISC} indicating that internal conversion is the most important decay process of the singlet excited state in this solvent. Increasing the proticity of the medium (ethanol and then TFE) k_{IC} decreases becoming comparable with k_{ISC} (Table 3). This photophysical behaviour can be explained in terms of nature, energy gap and interactions between the lowest excited singlets of different nature. The effects of these parameters on the singlet decay was described in details by Lim et al. for psoralens [36,37] using the term "proximity effect". This effect is based on vibronic interactions between the close-lying $^1(n,\pi^*)$ and $^1(\pi,\pi^*)$ states which lead to an efficient $S_1 \rightarrow S_0$ internal conversion (IC) compare to the other decay processes [36,37]. The closer the two states are, the higher the vibronic interaction is; this is the case of non-polar solvents where for 6,5'-DMA ϕ_F is negligible and the triplet undetectable and then S_1 decays, mainly, through internal conversion. Increasing the proticity of the solvent, the $^1(n,\pi^*)$ state is shifted to higher energy so the gap between the singlet states of different nature increases and therefore the vibronic interaction and the rate constant for IC decrease. These changes allow decay pathways competitive with IC to take place (Tables 2 and 3).

3.4. Mechanism of photodimerization of 6,5'-DMA

Steady state irradiation of 6,5'-DMA was carried out in different solvents. In cyclohexane 6,5'-DMA was stable confirming the hypothesis that the main decay pathway of S_1 is the internal conversion. In more polar/protic solvents, the photolysis rate, measured as disappearance of 6,5'-DMA depended on the medium and increased in the order chloroform < acetonitrile < ethanol < methanol < water.

Irradiation of a nitrogen-purged methanol solution of 6,5'-DMA (10^{-5} M) gave rise to extensive photolysis of the compound with formation of several photoproducts, as shown in Fig. 6. Unreacted 6,5'-DMA gave the peak at 19.3 min, while the peaks at 20.3 and 28.2 min were due to the pyrone–furan (compound 2) and pyrone–pyrone (3) cyclodimers already described [23], respectively. Further peaks appeared at 18.7 (compound 1) and 30.2 min (compound 4). When the solution was not deaerated, the peak of compound 1 appeared more evident whereas that of compound 3 was less intense. The new compounds 1 and 4 were produced in higher amount by irradiating more concentrated solutions, followed by TLC separation using chloroform as the eluent.

The chromatographic and NMR data (TLC: $R_f=0.45$ (as a yellow-pink fluorescent band, purified using hexane-THF, 80:20, $R_f=0.44$). MS: m/z 291 (M+H)⁺. ¹H-NMR (300 MHz, CDCl₃): δ 10.28 (s, 1H, CHO), 10.1 (broad, 1H, OH), 8.50 (s, 1H, H-5), 6.77 (unresolved q, 1H, H-4'), 2.55 (broad, 6H, CH₃-6 and CH₃-5') allowed compound 1 to be identified as the *o*-hydroxyaldehyde deriving from the oxidative fission of the pyrone ring of 6,5'-DMA, as depicted in Scheme 1. A compound with similar structure has been described as coming from photolysis of 8-MOP [38]. The formation of this photoproduct probably accounts for the lack or inefficient singlet oxygen sensitization.

Irradiation at 254 nm led to the photoreversion of 4–6,5'-DMA indicating, together with the chromatographic and NMR data (TLC: $R_f=0.18$ (as a non-fluorescent band, purified using ethyl acetate/ethanol, 97:3, $R_f=0.53$). MS: m/z 393 (M+H)⁺. ¹H-NMR (300 MHz, acetone-d₆): δ 7.01 (unresolved q, 2H, H-5), 6.45 (q, 2H, H-4', $J=1$ Hz), 3.78 (d,

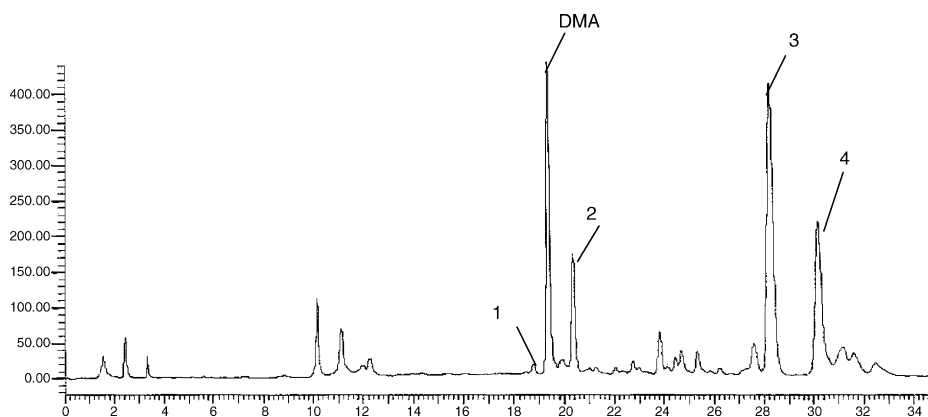


Fig. 6. HPLC profile of a de-aerated ethanol solution of 6,5'-DMA irradiated at 365 nm light for 180 min (detection wavelength 254 nm).

Table 4

Areas of chromatographic peaks (detected at 254 nm) of 6,5'-DMA (unreacted), **2**, **3** and **4** dimers (formed) after irradiation for 90 min in nitrogen (N₂), air (A) and oxygen (O₂) equilibrated ethanol solutions of 6,5'-DMA

Compound	Chromatographic peak areas		
	N ₂	A	O ₂
6,5'-DMA	6.5	15.0	24.6
Pyrone-furan dimer (2)	1.4	1.7 ^a	1.5
Pyrone-pyrone dimer (3)	14.0	9.0	6.1
Pyrone-pyrone dimer (4)	1.6	2.9 ^a	1.6

^a Slightly over-estimated for overlapping of the previous peak.

2H, H-4, $J=7.7$ Hz), 3.51 (*d*, 2H, H-3, $J=7.7$ Hz), 2.33 (*d*, 6H, CH₃-5', $J=1.0$ Hz), 2.29 (*d*, 6H, CH₃-6, $J=0.8$ Hz), that compound **4** is a pyrone-pyrone dimer of the angelicin, like **3**. As for **3**, the symmetry of the molecule did not allow its regio- and stereo-chemistry to be determined.

Oxygen reduced the rate of photodimerization of 6,5'-DMA in all solvents; Table 4 refers to a typical experiment carried out in ethanol. In particular, the amount of unreacted 6,5'-DMA increased with the concentration of oxygen and the yield of pyrone-pyrone **3** was reduced by oxygen while those of **2** and **4** were practically unaffected. To be noted that, due to the lack of a suitable calibration curve for the dimers, the numbers in Table 4 do not indicate the absolute amounts, but only represent the area of the chromatographic peaks, with respect to that of unirradiated 6,5'-DMA taken as 100%.

As concerns cyclodimers, **3** (a pyrone-pyrone dimer) formed in all solvents tested (MeOH, EtOH, ethyl acetate, dioxane, chloroform), whereas **2** (the pyrone-furan dimer) and **4** (the second pyrone-pyrone dimer) only formed in the more polar solvents like alcohols.

The effect of 6,5'-DMA concentration on the formation yields of the two photodimers (**2** and **3**) was also investigated in ethanol. In Fig. 7 the percentages (with respect to the starting 6,5'-DMA) of these photodimers formed, after 90 min of irradiation, are reported as a function of concentration in the range $0.2\text{--}8 \times 10^{-4}$ M. The formation to the f-p dimer is, in practice, independent of 6,5'-DMA concentration while that of p-p dimer increased proportionally with the ground state concentration reaching a constant value for [6,5'-DMA] higher than 6×10^{-4} M.

In order to rationalize the photodimerization mechanism, the photochemical behaviour of 6,5'-DMA has to be analyzed together with its photophysical properties. The formation of cyclobutane ring involves the carbon atoms 3 and 4 (pyrone moiety) or 4' and 5' (furan moiety); this is in agreement with the results of semiempirical calculations which indicate the decrease of the bond order in the C₃-C₄ and C₄'-C₅' bonds when electronically excited states are populated, as shown by the nodal pattern of the corresponding molecular orbitals (Fig. 2). In particular, the singlet excited state which presents a nodal plane only in C₃-C₄ bond can lead to the formation of both the p-p and f-p photodimers through interactions with another molecule in the ground state.

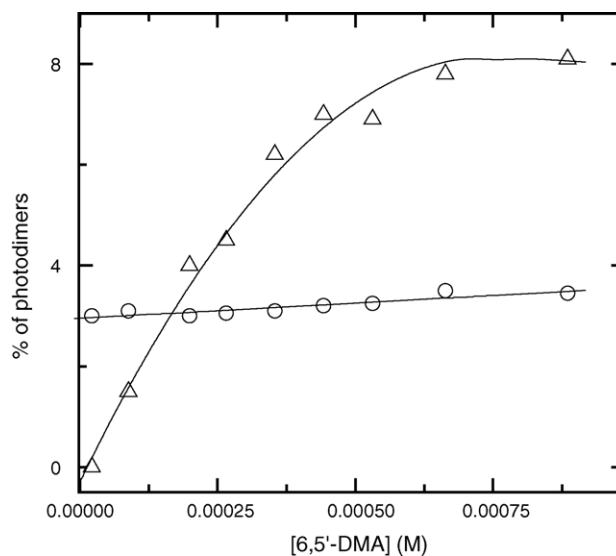


Fig. 7. Percentage of (○) f-p and (△) p-p dimers of 6,5'-DMA obtained after 90 min of irradiation time in ethanol (see Section 2).

On the other hand, the lowest triplet state is predicted to be located at 62.4 kcal mol⁻¹ above the ground state and to be described by molecular orbitals with nodal planes both in the C₄'-C₅' ($\pi_H \rightarrow \pi_L^*$, 40%) and C₃-C₄ ($\pi_H \rightarrow \pi_{L+1}^*$, 31%) positions, is able to form p-p, f-p and f-f dimers. Although the presence of the latter dimers cannot be excluded among the numerous photoproducts generated by UVA irradiation, their yield would anyway be low.

The main photoproducts experimentally found after the irradiation are the p-p and f-p dimers, whose relative yields are shown in Fig. 7, as a function of 6,5'-DMA concentration. The strong dependence on the concentration of p-p dimer recalls the involvement of a long-lived excited state such as the triplet. This is in agreement with the high rate constant for self-quenching (k_{sq}) determined for the triplet; in fact, at [6,5'-DMA] $\sim 10^{-4}$ the self-quenching ratio ($1 + k_{sq} \times \tau_T \times 10^{-4}$) is less than 1.5 while at the highest concentration used the ratio is about 8 and therefore the triplet is completely quenched, leading to a plateau in the formation yield of p-p. The second finding in favour of the involvement of the triplet in the formation of this dimer is the marked quenching effect of oxygen. Oxygen, in fact, quenches the 6,5'-DMA triplet with a rate constant close to that of self-quenching and therefore competes with 6,5'-DMA in the capture of the triplet. This quenching favours the decay of the triplet to the ground state and causes a decrease of the yield of cycloaddition. In de-aerated solutions, the amount of p-p dimer formed is three times higher than that found in the presence of O₂ (Table 4).

The yield of f-p dimer formation (Fig. 7) is practically independent of the 6,5'-DMA concentration and is not affected by oxygen (Table 4). The latter characteristic is typical for the involvement of a short-lived excited state, but the independence on the 6,5'-DMA concentration is not in agreement with a bimolecular photoreaction. Actually, the

yield of f–p dimer is higher than that of p–p even at very low concentration. Considering that for furocoumarins (in particular for angelicin) the aggregation in the ground state was demonstrated by absorption and emission spectroscopy [39] the hypothesis to correlate the experimental results, is that the f–p dimerization takes place through a ground state complex (where a pyrone ring is faced with the furan ring of the other molecule). In this case, the bimolecular photoreaction can occur without need of diffusion-controlled encounters and therefore can be efficient also in the short-lived singlet. A further support to this hypothesis comes from the comparison with literature data where the furan–pyrone dimer of psoralen was obtained upon irradiation of psoralen thin solid film [40], where the molecules are likely closely packed. The absorption and emission spectra of 6,5'-DMA did not clearly reveal association phenomena, but evidences for aggregation of this furocoumarin in ethanol came out from fluorescence lifetime measurements. In fact, the decay parameter was mono-exponential only for 6,5'-DMA concentrations $\leq 10^{-5}$ M, while for higher concentrations a decay curve well fitting a bi-exponential function was found. Complex fluorescence decays following bi- or tri-exponential functions with lifetimes and pre-exponential factors depending on the matrix and on the experimental conditions were measured for porphyrins [41,42] and in chlorophyll *a* [43] and were attributed to the formation of aggregated species.

A bi-exponential decay could be also due to the formation of excimers in the excited singlet, but the trend of the lifetime components with increasing 6,5'-DMA concentration is not in agreement with the formation of these species.

In this context also the formation of the second p–p dimer (4) (as shown by chromatographic analysis) is understandable. Since its formation is not affected by the oxygen, this photoproduct can be formed again in the singlet state (as the f–p dimer) but through a ground state complex in which the pyrone ring of the two monomers face each other. The low production efficiency of 4 did not allow to further investigation on the 6,5'-DMA concentration to confirm further the mechanism.

4. Conclusions

The absorption and emission spectra of 6,5'-DMA are red-shifted by increasing polarity/proticity of the solvent. Particularly large is the fluorescent maximum shift that is well correlated with the solvent parameter $E_T(30)$. Laser flash photolysis experiments showed that the sole transient observed by excitation of 6,5'-DMA in Cx, MeCN, EtOH and TFE is the triplet state whose formation quantum yield depended on the solvent.

The photophysical behaviour of 6,5'-DMA is determined by the vibronic interactions between the two close-lying lowest excited singlet states of $^1(n, \pi^*)$ and $^1(\pi, \pi^*)$ nature (proximity effect). In non-polar solvents the sole decay channel of the singlet is the internal conversion, but increasing solvent

polarity/proticity the fluorescence and intersystem crossing processes become important.

Upon UV irradiation 6,5'-DMA undergoes dimerization in polar/protic solvents to give mainly two pyrone–pyrone and one furan–pyrone photodimers. The effects of 6,5'-DMA concentration and of oxygen on the yields of the photodimers in ethanol and the high self-quenching rate constant of the 6,5'-DMA triplet allowed a mechanism for dimerization to be suggested: one p–p dimer is formed through the triplet state, and the f–p and the other p–p dimers are formed in the singlet state of micro-aggregates of 6,5'-DMA.

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