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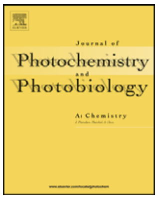

Photophysical properties of halo-derivatives of angelicins

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ABSTRACT

The properties of the lowest excited singlet and triplet state of four halo-angelicins (HA) have been investigated by steady-state and time-resolved spectrometric techniques. The study has been performed in solvents with different polarity and proticity: cyclohexane, dioxane, acetonitrile, ethanol and 2,2,2-trifluoroethanol. Absorption and emission spectra showed that the nature of lowest singlet state is mainly π,π^* and the transitions are allowed or partially allowed. Absorption coefficients, fluorescence quantum efficiency and lifetimes are also presented. Flash photolysis investigations have indicated the presence of a unique transient assignable to the lowest triplet state T_1. The triplet state has been characterized in terms of absorption spectra, decay kinetics, molar absorption coefficients and formation quantum yields. The racing of both intersystem crossing and fluorescence quantum yields going from a non-polar to a highly polar-protic solvent indicates the presence of a S_2 state, n,π^* in nature, close lying to the π,π^* one. The quantum yields of the singlet decay pathways (fluorescence, intersystem crossing and internal conversion) depends markedly on the energy gap between S_1 and S_2 states in agreement with the manifestation of the “proximity effect”. In fact in cyclohexane HA decay mainly through $S_1 \rightarrow S_0$ internal conversion, while in trifluoroethanol their fluorescence and intersystem crossing increase significantly.

Singlet-oxygen quantum yields have been also determined in order to understand the possible application of the investigated drugs in photodynamic therapy. The absence of photochemical pathways allowed the indirect evaluation of the internal conversion decay bringing to the achievement of a complete decay pathways picture.

1. Introduction

Naturally occurring furocoumarins 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 psoralens (linear furocoumarins) and angelicins (angular fourocoumarins) has been mainly attributed to their ability to form cycloadducts with DNA; the former forming mono- and di-adducts and the latter only monoadducts [5]. The pigmentogenic effect, on the other hand, is mainly connected to their ability to induce photodamages in lipids of the cell membrane or to stimulate enzymatic repair of the DNA damages [2,6,7].

The ability of psoralens in forming 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] which limit the possible applications of these furocoumarins. In fact there is some evidence that skin phototoxicity is related to the formation of DNA di-adducts [5–8], which are less efficiently repaired than monoadducts [11,12].

In contrast to psoralens, angelicins generally show lower mutagenic activity [13,14] and smaller phototoxic effects on the skin [15–17]. Therefore, detailed studies on several angelicin derivatives were undertaken to correlate the various aspects of their photobiological activity with their photophysical and photochemical properties [18–21]. In particular a characterization of the lowest excited states of methylangelicins and thioangelicins was performed [22,23].

In the present paper the investigation was extended to some chloro- and bromo-derivatives of angelicins (see Scheme 1) in order to evaluate the effect of heavy atoms on the rate parameters

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of the decay pathways and on singlet-oxygen production. For four halo-angelicins a detailed characterization of the lowest excited singlet and triplet states was carried out by determining the fluorescence quantum yields and lifetimes in different solvents and by determining the spectroscopic and kinetic properties of the triplet state by laser flash photolysis. The triplet quenching by molecular oxygen and the quantum yield for singlet-oxygen formation was also measured.

2. Experimental

2.1. Materials

The four compounds investigated (Scheme 1), which are halo-angelicine derivatives (abbreviation, HA), were synthesized as reported in the supplementary information. The purity of compounds (≥99%) has been checked by HPLC analysis. The solvents cyclohexane (Cx), dioxane (Dx), acetonitrile (MeCN), ethanol (EtOH) and 2,2,2-trifluoroethanol (TFE), of spectrophotometric grade, were all purchased from Fluka and used without any further purification. The chemicals anthracene, phenalenone and benzophenone (Aldrich, gold label) were also used without any further manipulation step.

2.2. Instrumentation

Absorption spectra were recorded with a PerkinElmer Lambda 800 spectrophotometer.

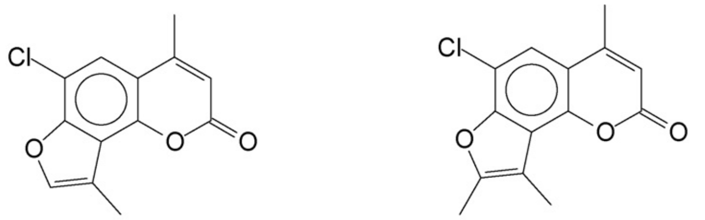
Emission and excitation spectra were measured with a Fluorolog-2 (Spex, F112AI) spectrophotofluorimeter. Quantum yields were determined comparing the emission spectra of the drugs, recorded on air-equilibrated solutions (absorbance <0.1 at the excitation wavelength to avoid auto-absorption), to that of anthracene in ethanol, $\phi_F = 0.27$ [24] used as standard solution. The fluorescence lifetimes, τ_F (mean deviation of three independent measurements, ca. 5%), were measured by a Spex Fluorolog-τ_2 system, which uses the phase-modulation technique (excitation wavelength modulated in the 1–300 MHz range; time resolution ca. 10 ps). The frequency-domain intensity decays (phase angle and modulation vs. frequency) were analyzed with the Globals Unlimited^{TM} (rev. 3) global analysis software [25].

Triplet formation quantum yields and lifetimes were measured with a nanosecond laser flash photolysis setup previously described (Nd:YAG Continuum, Surelite II, third harmonics, $\lambda_{exc} = 355$ nm, pulse width ca. 7 ns and energy ≤ 1 mJ pulse^{-1}) [26,27]. First-order kinetics were observed for the decay of the lowest triplet state (T–T annihilation was prevented by the low excitation energy). The triplet lifetimes were measured at an absorbance of ca. 0.2; the concentration effect on triplet lifetime was not investigated. The transient spectra were obtained by monitoring the change of absorbance each 10 nm over the 300–740 nm range and averaging at least 10 decays at each wavelength. Triplet–triplet molar extinction coefficients (ε_T) were determined by energy transfer. The calibration of the experimental setup was performed with an optically matched acetonitrile solution of benzophenone ($\phi_T = 1$ and $\Delta\varepsilon_T = 6500\ M^{-1}\ cm^{-1}$ at the corresponding absorption maximum) [28]. The $\Delta\varepsilon_T$ (and then ϕ_T) values, were determined by the energy transfer method by use of benzophenone as a donors in MeCN and anthracene as acceptor in the other solvents. The experimental errors on τ_T were estimated to be about ±10% and on $\Delta\varepsilon_T$ and ϕ_T of about ±15%. The quenching constants of the triplets by the molecular oxygen were evaluated by the slope of the linear plots of triplet lifetime vs. different oxygen concentration. The singlet-oxygen quantum yields (ϕ_Δ) were determined by recording the phosphorescence intensity of $O_2(^1\Delta_g)$ with a germanium diode detector in air-equilibrated solutions [22]. The amplified signal extrapolated at zero time was plotted as a function of the laser dose. Linear relationships were obtained for the four HA and for phenalenone used as standard ($\phi_\Delta = 0.97 \pm 0.03$) in organic solvents [29]. The ϕ_Δ values were then obtained from the ratio of the slopes of the substrate to that of phenalenone multiplied by the known ϕ_Δ of the standard. For each solution at least 200 kinetic decays were averaged. The experimental error on ϕ_Δ was estimated to be about ±15%.

All measurements were carried out at room temperature; the solutions were saturated with bubbling nitrogen.

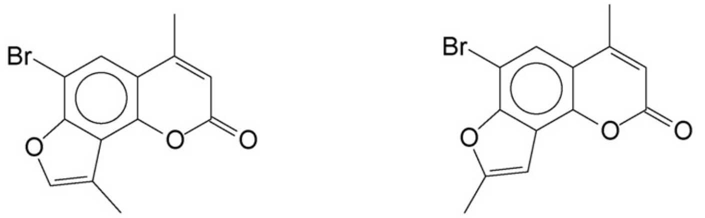
2.3. Semi-empirical calculations

The absorption spectra were predicted by use of the semi-empirical INDO1/CI method (MOPAC2002), after geometrical optimization with the PM3 model. The configuration interaction



6-chloro-4,9-dimethyl-2H-furo[2,3-h]chromen-2-one 6-chloro-4,8,9-trimethyl-2H-furo[2,3-h]chromen-2-one

ClA1 **ClA2**



6-bromo-4,9-dimethyl-2H-furo[2,3-h]chromen-2-one 6-bromo-4,8-dimethyl-2H-furo[2,3-h]chromen-2-one

BrA1 **BrA2**

Scheme 1.

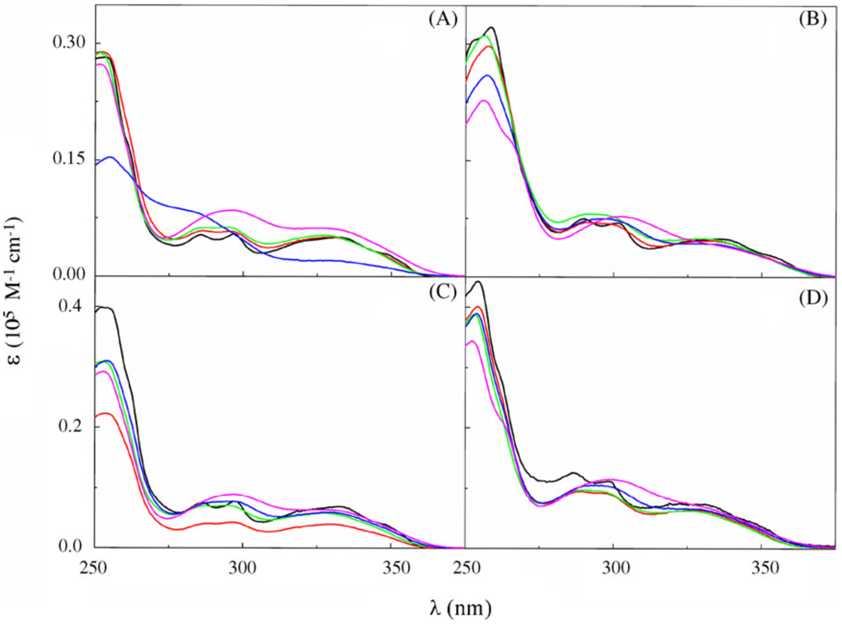


Fig. 1. Absorption spectra of ClA1 (panel A), ClA2 (panel B), BrA1 (panel C) and BrA2 (panel D) in solvent with different polarity: cyclohexane (black line), 1,4-dioxane (red line), acetonitrile (green line), ethanol (blue line), 2,2,2-trifluoroethanol (magenta line).

(CI) calculations included the singly excited configurations built from the 15 highest occupied (HOMO) and 15 virtual (LUMO) molecular orbitals [30,31].

3. Results and discussion

3.1. Absorption spectra

The ground-state absorption spectra of the four HA (Scheme 1) recorded in the 250–400 nm range, are reported in Fig. 1. The main absorption maxima and the extinction coefficients ($\varepsilon_{\max}$) in the different solvents are reported in Table 1. As for the angelicins and thioangelicins previously described [22,23] the spectra are characterized by three absorption bands appearing around 260, 285 and 335 nm.

Table 1
Absorption coefficients of HA in cyclohexane and ethanol

Compound	Solvent	$\lambda_{\max}$ (nm)	ε (M^{-1} cm^{-1})
ClA1	Cyclohexane	332	5,060
		286	5,340
		254	28,230
	Ethanol	333	2,010
		279	8,670
		255	15,350
ClA2	Cyclohexane	338	4,840
		301	6,930
		258	32,110
	Ethanol	335	4,220
		297	7,480
		257	25,990
BrA1	Cyclohexane	333	6,860
		297	7,700
		254	39,800
	Ethanol	330	5,900
		296	7,730
		254	30,990
BrA2	Cyclohexane	330	7,540
		299	11,380
		254	44,470
	Ethanol	330	6,490
		294	10,530
		254	38,930

To have more information about the nature of the $S_0 \to S_n$ transitions responsible for these absorption features, the absorption spectra in five solvents of different polarity and proticity, namely cyclohexane, 1,4-dioxane, acetonitrile, ethanol and 2,2,2-trifluoroethanol, were recorded (Fig. 1). The maximum positions of the HA are slightly affected by the polarity/proticity of the solvents (only for the 285 nm bands a clear red-shift, typical of the $\pi \to \pi^*$ transitions, was observed for ClA1, ClA2 and BrA1). Pronounced differences were detected in the spectral profiles. In particular, in cyclohexane the spectra present a vibrational structure ($\Delta\nu \approx 1300\,cm^{-1}$, which is assignable to the stretching of the aromatic C-C bonds) that decreases and disappears going to solvents of higher polarity. The ε values are of the order of $10^4\,M^{-1}\,cm^{-1}$ at 285 and 330 nm and $10^5\,M^{-1}\,cm^{-1}$ for the highest energy transition (~260 nm). These values suggest that all the bands are π,π^* in

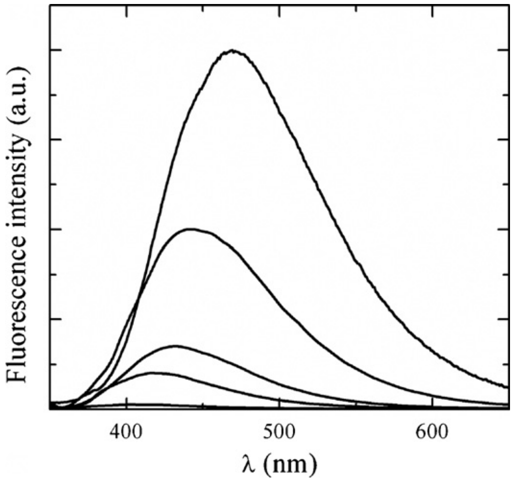


Fig. 2. Emission spectra of ClA2 in solvent with different polarity (from the bottom to the top: cyclohexane, 1,4-dioxane, acetonitrile, ethanol, 2,2,2-trifluoroethanol) normalized by fluorescence quantum yield.

Table 2
Fluorescence properties of HA in solvents with different polarity

Compound	Solvent	λ_{max} (nm)	ϕ_F	τ_F (ps)
ClA1	Cyclohexane	–[a]	≤0.0001[a]	–
	1,4-Dioxane	408	0.001	70
	Acetonitrile	414	0.001	270
	Ethanol	418	0.006	210
	2,2,2-Trifluoroethanol	428	0.02	720
ClA2	Cyclohexane	403	0.0005	<20[b]
	1,4-Dioxane	416	0.003	80
	Acetonitrile	431	0.007	150
	Ethanol	440	0.02	790
	2,2,2-Trifluoroethanol	469	0.04	2400
BrA1	Cyclohexane	400	0.0006	<20[b]
	1,4-Dioxane	407	0.001	23
	Acetonitrile	413	0.002	44
	Ethanol	416	0.004	80
	2,2,2-Trifluoroethanol	426	0.004	130
BrA2	Cyclohexane	408	0.0004	<20[b]
	1,4-Dioxane	418	0.002	160
	Acetonitrile	435	0.0014	30
	Ethanol	439	0.005	90
	2,2,2-Trifluoroethanol	436	0.005	130

[a] No fluorescence detected.

[b] Not measurable.

nature, moreover the one around 260 nm ($\varepsilon \sim 3–4 \times 10^5$ M^{-1} cm^{-1}) is fully allowed while the other two ($\varepsilon \sim 2–7 \times 10^4$ M^{-1} cm^{-1}) should be considered partially forbidden transitions.

As for methylangelicine [22] semi-empirical calculations predicted for all HA a $n \rightarrow \pi^*$ transition around 340 nm with an oscillator strength (f) of 0.0006 close to the lowest energy $\pi \rightarrow \pi^*$ transition (f = 0.044). Due to the difference of f values, $n \rightarrow \pi^*$ absorption does not affect the spectral features. Other $\pi \rightarrow \pi^*$ transitions reaching f values of 0.3–0.6 are predicted at 300–280 nm. Moreover, the n,π^* state is predicted to lie just 350 cm^{-1} below the π,π^* one. The proximity of a n,π^* state to the π,π^* state is crucial for the photophysical properties, particularly taking into account that the polarity/proticity effect changes the energy gap between the two π,π^* and n,π^* states and it may also vary the state order (see below).

3.2. Steady-state and time-resolved fluorescence

The properties of the lowest excited singlet states of HA were investigated by both steady-state and time-resolved fluorimetric techniques in several solvents. All HA exhibit, in all the solvents, a broad not resolved single emission band, that results red shifted with increasing the solvent polarity, from ~400 nm in cyclohexane to ~430 nm (and even to 460 nm for ClA2) in TFE. As an example, the emission spectra of ClA2 in the five solvents are reported in Fig. 2, where they have been normalized on the basis of the corresponding fluorescence quantum yield (ϕ_F). The fluorescence characteristics (emission maxima, quantum yields and lifetimes) of all HA at room temperature are summarized in Table 2. For ClA1 in Cx the fluorescence intensity was below the experimental sensitivity of the setup at disposal, so the quantum yield was only estimated and given as an upper limit. The red-shift of the HA fluorescence maximum (~3000 cm^{-1} for ClA2 and 1500 cm^{-1} for BrA1 on going from Cx to TFE) is consistent with a π,π^* nature of the emitting state, more polar than the ground state and stabilized by high polarity or hydrogen bonds donating solvents. This shift is well correlated with the solvent parameter $E_T(30)$ proposed by Reichardt [32], as suggested by the linear plots (correlation coefficient 0.999) of the fluorescence maximum energy (in cm^{-1}) vs. $E_T(30)$, reported in Fig. 3, where the

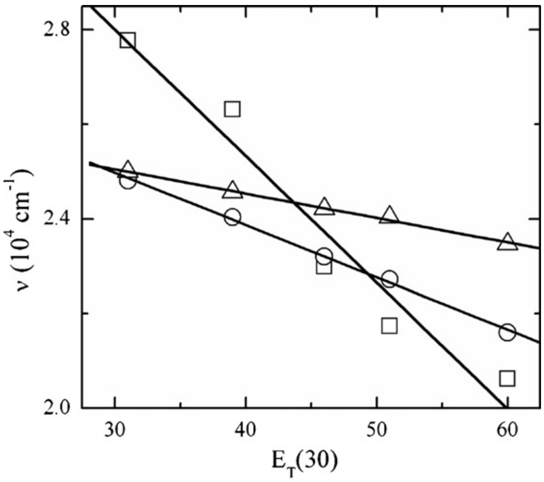


Fig. 3. Dependence of the fluorescence maximum energy of 6,5-DMA (□), ClA2 (○) and BrA1 (△) on the $E_T(30)$ solvent parameter.

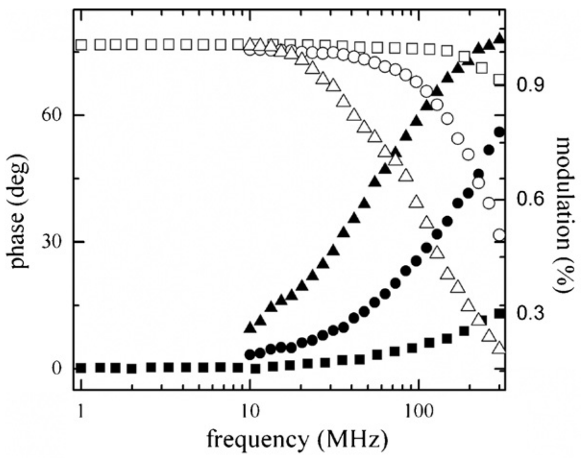


Fig. 4. Frequency responses (phase, full symbols; modulation, open symbols) of ClA2 in solvents with different polarity: acetonitrile (squares), ethanol (triangles), 2,2,2-trifluoroethanol (circles).

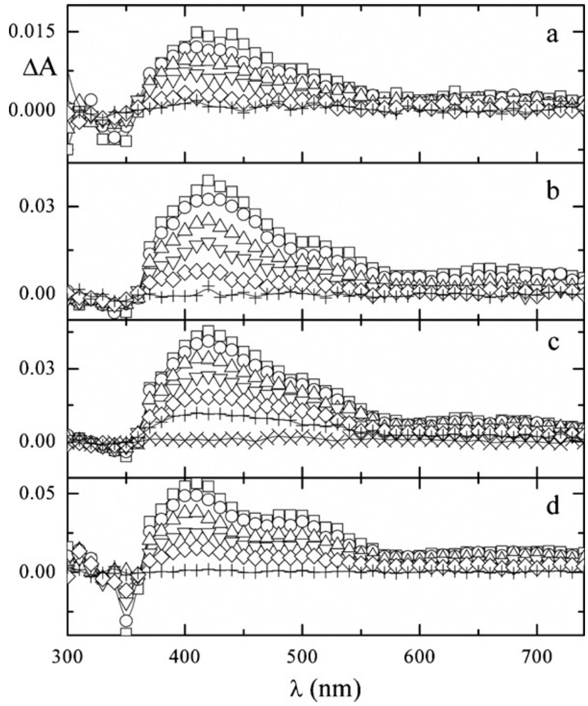


Fig. 5. Time-resolved triplet–triplet absorption spectra of ClA1 (a) recorded □ = 0.03 μs, ○ = 0.61 μs, △ = 0.99 μs, ▽ = 1.62 μs, ◇ = 3.30 μs, + = 6.42 μs after the laser pulse, ClA2 (b) recorded □ = 0.09 μs, ○ = 0.28 μs, △ = 0.52 μs, ▽ = 0.83 μs, + = 1.24 μs, × = 2.73 μs after the laser pulse, BrA1 (c) recorded □ = 0.03 μs, ○ = 0.08 μs, △ = 0.18 μs, ▽ = 0.27 μs, ◇ = 0.37 μs, + = 0.51 μs, × = 6.42 μs after the laser pulse, BrA2 (d) recorded □ = 0.04 μs, ○ = 0.07 μs, △ = 0.13 μs, ▽ = 0.21 μs, ◇ = 0.33 μs, + = 1.16 μs after the laser pulse in air-equilibrated ethanol solutions (λ_{exc} = 355 nm).

behavior of ClA2 and BrA1 is compared with that of dimethylangelicin, DMA [33]. It has to be noticed that the solvent effect on HA fluorescence maximum is much lower than that observed for DMA, probably due to a lower difference in the dipole moments between the ground and excited states of halo-derivatives. These behaviors indicate that $E_T(30)$ well describes the mixed effect of polarity and hydrogen bond formation on the fluorescent state of HA, as was found for dimethylangelicin [33].

The fluorescent quantum yields are very low, particularly in non-polar solvents. For ClA1 in Cx a ϕ_F value lower than 10^{-4} was estimated. In polar/protic solvents the emission efficiency increases (Table 2) and ϕ_F reaches the value of 0.04 for ClA2 in TFE. Even though a marked increase (even one hundred times) was observed on going from Cx to TFE, the ϕ_F values remain very low for all HA in all the solvents and therefore the radiative decay has to be considered a negligible process in the deactivation of these compounds. Excitation spectra (data not shown), recorded at the emission maximum, in all the cases fully overlapped the absorption ones.

The fluorescence lifetimes (τ_F) were measured by a frequency resolved technique. In Fig. 4, as an example, are reported the frequency responses (phase angle and modulation) for ClA2 in MeCN, EtOH and TFE. The fluorescence decay was well fitted by a monoexponential function. The τ_F values for all the systems investigated, obtained upon the best fittings of the experimental data, are reported in Table 2. It has to be pointed out how the lifetime changes dramatically with the solvent polarity (a rough idea can be found out from the curve intersections in Fig. 4, earlier the intersection longer the lifetime). The ϕ_F and τ_F values allow the fluorescence constant (k_F) to be determined; since both parameters increase in a similar way with the solvent, the k_F values do not change very much and are in the range $0.4–5.5 \times 10^7\ s^{-1}$ (see below) for the various compounds and solvents. These values are comparable with the results obtained on similar compounds [22,23] and their absolute values are in agreement with a partially allowed $\pi^* \rightarrow \pi$ transition.

3.3. ns-Laser flash photolysis

Upon 355 nm laser excitation of HA at room temperature, in Cx, Dx, MeCN, EtOH and TFE, transient absorptions that were assigned to the lowest triplet states (T_1) of HA were detected; in fact in all the solvents (i) they appeared within the laser pulse, (ii) were quenched by oxygen with an almost diffusional rate constant ($k_{ox} \sim 10^9\ M^{-1}\ s^{-1}$), (iii) decayed with a lifetime in the μs range following a first-order kinetics at low pulse intensity and low ground-state concentration, (iv) were sensitized by higher energy donor (benzophenone) and quenched lower energy acceptor (anthracene). The $T_1 \rightarrow T_n$ absorption shows one broad band around 410 nm, a shoulder at about 500 nm and a slightly absorbing band at 670 nm (see Figs. 5 and 6). The triplet lifetimes (τ_T) in the μs time scale (Table 3) were measured with laser energy $\leq$1 mJ pulse^{-1}. The τ_T values compiled in Table 3 show that the structure of the compound and the polarity of the medium have a negligible effect on the decay rate constants of T_1. Because of the triplet energy of angelicins is about 240 kJ mol^{-1} [22], energy transfer experiments involving these compounds were performed using benzophenone as an energy donor (in MeCN) and anthracene as an energy acceptor (in Dx and EtOH). In both cases, the quenching of the donor triplet was accompanied by the appearance of the sensitized $T_1 \rightarrow T_n$ absorption (of HA or anthracene, respectively). The extinction coefficients ($\Delta\varepsilon_T$) of triplet in MeCN were measured by the energy transfer method from triplet benzophenone ($^3B^*$) at λ_{exc} = 355 nm. For $^3B^*$ at 520 nm, $\Delta\varepsilon_T$ value of 6500 M^{-1} cm^{-1} in MeCN was used [28]. In EtOH and Dx, the $\Delta\varepsilon_T$ values of HA were determined by using anthracene as standard ($\Delta\varepsilon_T$ = 52,000 M^{-1} cm^{-1} at 430 nm and $\Delta\varepsilon_T$ = 50,000 M^{-1} cm^{-1} at 430 nm, respectively) [28].

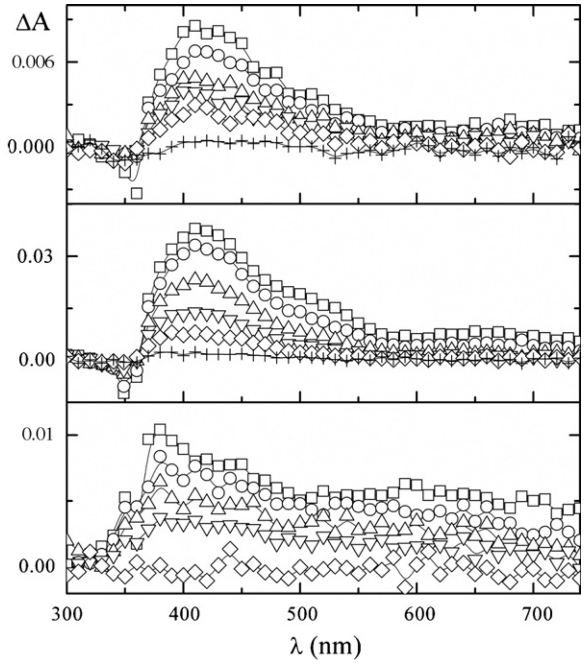


Fig. 6. Time-resolved triplet–triplet absorption spectra of ClA2 in 1,4-dioxane (top) recorded □ = 0.22 μs, ○ = 0.70 μs, △ = 1.28 μs, ▽ = 1.81 μs, ◇ = 2.53 μs, + = 6.37 μs after the laser pulse, acetonitrile (center) recorded □ = 0.09 μs, ○ = 0.28 μs, △ = 0.52 μs, ▽ = 0.83 μs, + = 1.24 μs, × = 2.73 μs after the laser pulse, 2,2,2-trifluoroethanol (bottom) recorded □ = 0.09 μs, ○ = 0.55 μs, △ = 0.75 μs, ▽ = 1.3 μs, ◇ = 2.2 μs after the laser pulse (λ_{exc} = 355 nm).

Table 3
Triplet properties of HA in solvents of different polarity at room temperature (λ_{exc} = 355 nm)

Compound	Solvent	λ_{max} (nm)	τ_T (μs)	ε_T (M^{-1} cm^{-1})[e]	Φ_T	k_{ox} (10^9 M^{-1} s^{-1})
ClA1	Cyclohexane	$\underline{410}$, 490, 670	1.2	(1)	0.18[a]	2.0
	1,4-Dioxane	$\underline{410}$, 500, 680	1.4	2440[b]	0.19	2.6
	Acetonitrile	$\underline{410}$, 510, 670	0.7	850[c]	0.47	2.8
	Ethanol	$\underline{410}$, 500, 670	1.1	2000[b]	0.85	1.9
	2,2,2-Trifluoroethanol	$\underline{410}$, 530, 670	2.0	(2)	0.97[d]	1.5
ClA2	Cyclohexane	$\underline{420}$, 510	1.4	(1)	0.07[a]	2.0
	1,4-Dioxane	$\underline{410}$, 510, 680	1.7	2800[b]	0.32	1.5
	Acetonitrile	$\underline{410}$, 500, 670	0.7	5300[c]	0.26	3.0
	Ethanol	$\underline{420}$, 510, 660	1.9	3100[b]	0.87	1.9
	2,2,2-Trifluoroethanol	$\underline{380}$, 450	1.6	(2)	0.93[d]	2.7
BrA1	Cyclohexane	$\underline{420}$, 510	0.7	(1)	0.21[a]	3.0
	1,4-Dioxane	$\underline{410}$, 490	1.2	2300[b]	0.74	2.3
	Acetonitrile	$\underline{420}$, 510, 660	0.7	5230[c]	0.48	2.7
	Ethanol	$\underline{420}$, 500, 670	1.6	5400[b]	0.63	2.7
	2,2,2-Trifluoroethanol	$\underline{410}$, 430, 680	1.4	(2)	0.75[d]	2.6
BrA2	Cyclohexane	$\underline{410}$, 490	1.25	(1)	0.12[a]	1.8
	1,4-Dioxane	$\underline{410}$, 490	1.2	4100[b]	0.36	2.0
	Acetonitrile	400, 480	0.45	4500[c]	0.45	2.6
	Ethanol	$\underline{410}$, 490, 670	1.3	3900[b]	0.83	2.3
	2,2,2-Trifluoroethanol	$\underline{410}$, 490, 680	1.5	(2)	0.99[d]	2.6

[1] Signal too weak in order to determine the absorption coefficient.
[2] No standard available.
[a] Assuming the same absorption coefficient of the dioxane.
[b] Evaluated using anthracene as an energy acceptor.
[c] Evaluated using benzophenone as an energy donor.
[d] Assuming the same absorption coefficient of the ethanol.
[e] Absorption coefficient measured at the underlined wavelength.

The ratio between the slopes of the linear plots of the absorbance change, ΔA, vs. laser dose of optically matched solutions of HA and benzophenone in MeCN allowed the $\Delta\varepsilon_T \times \phi_T$ products of HA in the five solvents to be obtained, using $\Delta\varepsilon_{520} \times \phi_T = 6500\,M^{-1}\,cm^{-1}$ for benzophenone. Considering the $\Delta\varepsilon_T$ and $\Delta\varepsilon_T \times \phi_T$ values, the triplet quantum yields, ϕ_T, were determined and are reported in Table 3. Due to the lack of suitable standards to obtain the $T_1 \rightarrow T_n$ extinction coefficients in Cx and TFE, it has been assumed that the $\Delta\varepsilon_T$ values in these solvents were equal to those measured in Dx and EtOH, respectively, because of the similar value of dielectric constants. The intersystem crossing quantum yield values are small in non-polar solvents but they increase with Dimroth's solvent parameter $E_T(30)$, reaching almost unity in TFE (0.99 for BrA2).

The intersystem crossing quantum yields of HA, compared with those reported for angelicins [22] and thioangelicins [23], show that the heavy atoms chlorine and bromine have similar effect in the intersystem crossing induction, particularly relevant in non-polar solvents, and that their effect is comparable with that of sulphur atom in thioangelicins [23].

3.4. Singlet-oxygen production

The T_1 state of HA was efficiently quenched by molecular oxygen with bimolecular rate constant ($k_{ox} = 1.5–3.0 \times 10^9\,M^{-1}\,s^{-1}$, see Table 3) close to the diffusional limit. The triplet quenching was accompanied by singlet oxygen, $O_2(^1\Delta_g)$, formation. Singlet-oxygen production was quantified measuring the phosphorence intensity at 1270 nm after excitation at 355 nm and standardizing the experimental setup with phenalenone. Laser intensities were kept low in order to avoid triplet–triplet annihilation. The luminescence detected by the Ge-photodiode was assigned to the $O_2(^1\Delta_g)$ phosphorescence for the following reasons: (1) it was produced within the instrumental time resolution, (2) it decayed by a first-order kinetic, (3) it was not observed in the absence of the substrate or by bubbling it with nitrogen. The singlet-oxygen decays with lifetimes depending on the solvent, in particular it decays with a lifetime of ~20 μs in Dx and EtOH and ~60 μs in acetonitrile. Typical singlet-oxygen decay kinetics are shown in Fig. 7 (right panel). In Fig. 7 (left panel) the different plot slopes for the four angelicins and the phenalenone (standard) in EtOH are shown; the slopes of the plots allowed ϕ_Δ values to be determined. The measurements were carried out on air-equilibrated solutions. The singlet-oxygen quantum yield was determined only in three of the solvents (Dx, MeCN and EtOH) used in the present work due to the weakness of the signal for the compounds in Cx ($\phi_\Delta < 0.01$) and the absence of an appropriate standard in TFE. The values founded span from 0.06

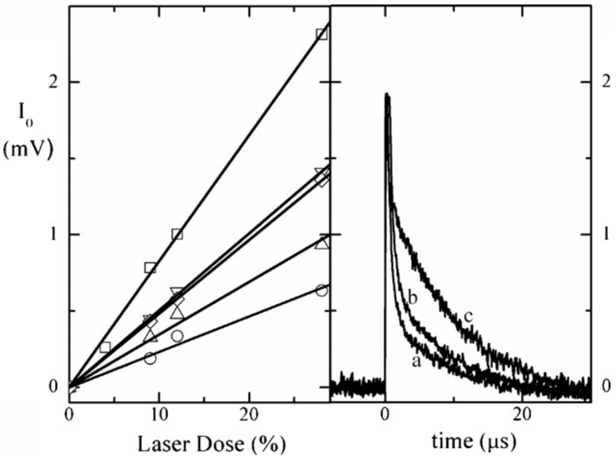


Fig. 7. Panel left: laser energy effect on the intensity of singlet-oxygen phosphorescence sensitized by phenalenone (□), ClA1 (○), ClA2 (△), BrA1 (▽) and BrA2 (◊) (λ_{exc} = 355 nm) in air-equilibrated ethanol solutions. Panel right: decay kinetics of singlet-oxygen phosphorescence sensitized by, ClA1 (a), ClA2 (b), and phenalenone (c) in air-equilibrated ethanol solutions (λ_{exc} = 355 nm).

Table 4
Quantum yields of singlet-oxygen formation sensitizer by HA in air-equilibrated solutions (λ_{exc} = 355 nm)

Compound	Solvent	Φ_Δ	S_Δ
ClA1	1,4-Dioxane	0.06	0.32
	Acetonitrile	0.06	0.13
	Ethanol	0.27	0.32
ClA2	1,4-Dioxane	0.13	0.41
	Acetonitrile	0.17	0.65
	Ethanol	0.40	0.46
BrA1	1,4-Dioxane	0.19	0.26
	Acetonitrile	0.20	0.42
	Ethanol	0.59	0.94
BrA2	1,4-Dioxane	0.19	0.53
	Acetonitrile	0.22	0.49
	Ethanol	0.57	0.69

in the less polar solvent (Dx) to 0.59 in the solvent with the highest polarity (EtOH). Generally, the HA have ϕ_Δ values higher than methylangelicins [22], as expected due to the presence of an heavy atom, and in some cases (Br-derivatives in ethanol) higher than thioangelicins [23]. Between HA the bromo-derivatives have ϕ_Δ higher than chlorine (see Table 4). Therefore, the bromo-HA seem to be, at least for the $O_2(^1\Delta_g)$ production, better than other angelicins for the photodynamic therapy. Considering that molecular oxygen does not quench S_1 state (too short τ_F values), the whole production of singlet oxygen has to be assigned as occurring exclusively via triplet state.

Calculated S_Δ values, representing the fraction of quenched triplet yielding singlet oxygen, are also reported in Table 4. S_Δ values range between 0.1 and 0.9 and again the higher values pertain to bromo-derivatives in polar solvents. These values are, in general, similar to those obtained for molecules like naphthalene [34] and biphenyl [35], and in the case of BrA1 S_Δ reaches the maximum value obtained for π,π^* aromatic triplets ($S_\Delta \sim 0.9$) [36]. This observation suggests that the efficient interactions of Br-derivatives in ethanol might be related to the nature of the lowest triplet state, whose investigation is in progress.

Table 5
Decay rate constants of the lowest excited singlet state of HA in solvents of different polarity

Compound	Solvent	k_F (10^7 s^{-1})	k_{ISC} (10^9 s^{-1})	k_{IC} (10^9 s^{-1})
ClA1	Cyclohexane	–	>9.00	>41.0
	1,4-Dioxane	1.43	2.71	11.6
	Acetonitrile	0.37	1.74	1.96
	Ethanol	2.86	4.05	0.69
	2,2,2-Trifluoroethanol	2.78	1.35	0.01
ClA2	Cyclohexane	>2.50	>3.50	>46.5
	1,4-Dioxane	3.75	4.00	8.46
	Acetonitrile	4.67	1.73	4.89
	Ethanol	2.53	1.10	0.14
	2,2,2-Trifluoroethanol	1.67	0.39	0.013
BrA1	Cyclohexane	>3.00	>10.5	>39.5
	1,4-Dioxane	4.35	32.2	11.3
	Acetonitrile	4.55	10.91	11.77
	Ethanol	4.55	7.88	4.58
	2,2,2-Trifluoroethanol	3.08	5.77	1.89
BrA2	Cyclohexane	>2.00	>6.00	>44.0
	1,4-Dioxane	1.25	2.25	3.99
	Acetonitrile	4.67	15.0	18.3
	Ethanol	5.56	9.22	1.83
	2,2,2-Trifluoroethanol	3.85	7.62	0.039

3.5. *Decay pathways*

The results reported in Tables 2 and 3 show that in cyclohexane the lowest excited singlet state (S_1) of halo-angelicins decays mainly through internal conversion (IC). In fact, the quantum yield of the IC process (ϕ_{IC}) can be equated to the difference $1 - \phi_F - \phi_{ISC}$ since no evidences of side-products were observed upon excitation of dilute solutions. Thus, in cyclohexane ϕ_{IC} is generally larger than 0.8. In solvents of higher polarity/proticity (MeCN, EtOH and TFE) the quantum yields of the competitive processes (fluorescence and intersystem crossing) increase markedly while ϕ_{IC} decreases and becomes 0.001 in TFE.

To better understand the solvent effect on the HA singlet decay, the rate constants of the various processes have to be taken into account. The values reported in Table 5 show that k_F is reasonably constant for the different angelicins in the considered solvents; k_{ISC} is slightly affected by the solvent and its changes cannot account for the changes of ϕ_T. On the contrary, k_{IC} values undergo a dramatic decrease, responsible of the increasing of ϕ_F and ϕ_{ISC}, being $>41.0 \times 10^9$ s^{-1} and 0.01×10^9 s^{-1} in Cx and TFE, respectively.

The absolute values of k_F indicate that the nature of the emitting state is the same in all the compounds and it does not change with the solvent. In general k_{ISC} values (Table 5) are quite high (in non-polar solvents in the range $2\text{–}32 \times 10^9$ s^{-1}) as expected for the presence of heavy atoms. These high k_{ISC} values originate, in cyclohexane and dioxane, intersystem crossing quantum yields one order of magnitude higher than those of methylangelicins [22] and comparable with those of thioangelicins [23].

On the basis of semi-empirical calculations, HA, as similar compounds previously studied [22,23], posses two very close singlet excited states of different in nature: $^1(n,\pi^*)$ and $^1(\pi,\pi^*)$. The solvent effect on quantum yields of the S_1 decays and fluorescence lifetimes gives strong evidence that the fluorescent state of HA is mainly controlled by the energy gap between these two singlet excited states. The relative importance of the S_1 decays of these compounds can be described in terms of “proximity effect”, that through a vibronic interaction between the close-lying $^1(n,\pi^*)$ and $^1(\pi,\pi^*)$ states leads to a huge value of k_{IC} and to an efficiency of the $S_1 \rightarrow S_0$ internal conversion near unity. This effect, which has been described in detail by Lim, was clearly underlined in the photophysical behavior of psoralen [37,38] and invoked for methyl- and thio-angelicins [22,23].

Halo-angelicins, in solvent of low/medium polarity, have ϕ_F values negligible (10^{-3}–10^{-5}); the close proximity of $^1(n,\pi^*)$ and $^1(\pi,\pi^*)$ states (expected by semi-empirical calculations) leads to a predominance of internal conversion with respect to the other singlet decay processes (k_{IC} is $\gg k_{ISC}$ in spite of heavy atom effect). In such solvents, the k_{CI}/k_F ratio is larger than 10^2. When the polarity and/or proticity of the solvent increases, the $^1(n,\pi^*)$ state energy increases more than that of $^1(\pi,\pi^*)$ and therefore the S_1–S_2 energy gap becomes higher. As a consequence, $^1(n,\pi^*)$–$^1(\pi,\pi^*)$ vibronic coupling decreases and the $S_1 \rightarrow S_0$ IC rate constant becomes lower. In trifluoroethanol k_{IC} is two orders of magnitude lower than in cyclohexane and lower than k_{ISC}. The τ_F values, which change from a ps to a ns time scale (Table 1) on going from Cx to TFE, reflect the solvent effect on k_{CI}, being k_F and k_{ISC} only slightly affected. Another important change on going from non-polar to polar solvents is the increased quantum yield of triplet formation (Table 2) mainly due to the decrease of k_{CI}. Since the decreasing of k_{IC} with increasing solvent polarity/proticity can be considered an indication of the “proximity effect” importance, the four HA reveal, in this respect, different behavior (Table 5). The decrease (as ratio of k_{IC} in Cx and TFE) is much higher for chloro- than for bromo-derivatives and it is particularly low for BrA1 that, in spite of the heavy atom and the decrease of the vibronic coupling, has a ϕ_T value reaching only 0.75 in TFE. This behavior found for HA can be related to the different values of their energy gap $^1(n,\pi^*)$–$^1(\pi,\pi^*)$ in the non-polar solvent Cx or to the different effect of the polar/protic solvents on the relative state energy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2008.02.022.

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