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Spectral features of substituted 9-(phenoxycarbonyl)-acridines and their protonated and methylated cation derivatives

Karol Krzymiński^{a,*}, Alexander D. Roshal^b, Agnieszka Niziołek^a

^a University of Gdańsk, Faculty of Chemistry, J. Sobieskiego 18, 80-952 Gdańsk, Poland
 ^b Kharkiv V.N. Karazin National University, Institute of Chemistry, Svoboda Square 4, 61 077 Kharkiv, Ukraine
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Abstract

The long-wavelength absorption of eight 9-(phenoxycarbonyl)-acridines and the 10-*H*-9-(phenoxycarbonyl)-acridinium and 10-methyl-9-(phenoxycarbonyl)-acridinium cations derived from them, substituted with an alkyl or trifluoroalkyl group at the benzene ring, occurs above 300 nm as the superposition of four bands. Three of these bands occupy comparable positions (expressed in nm) in all the compounds; the fourth one, however, changes position, appearing in neutral molecules as a long-wavelength shoulder below 400 nm, but in cations as an almost separate band above 400 nm. The weak fluorescence resulting from excitation within the long-wavelength absorption band is red-shifted relative to absorption, such that Stokes shifts are similar for both neutral molecules and cations. Stokes shifts tend to increase with the orientational polarisability of a medium. Computations predict that long-wavelength electronic transitions are accompanied by structural changes in molecules. They also indicate that such transitions are followed by roughly uniform electron density changes in whole molecules accompanied by small changes in their dipole moments, which accounts for the weak absorption in the long-wavelength region. The predicted radiative and non-radiative deactivation rate constants suggest the occurrence of efficient spin–orbital coupling in the molecules investigated, which is the cause of the relatively low fluorescence quantum yields. Apart from the cognitive significance of these investigations, the results demonstrate that absorption of radiation by 10-methyl-9-(phenoxycarbonyl)-acridinium cations above 400 nm may influence their chemiluminescence output.

Keywords: 9-(Phenoxycarbonyl)-acridines; 10-H-9-(phenoxycarbonyl)-acridinium cations; 10-Methyl-9-(phenoxycarbonyl)-acridinium cations; Electronic absorption and emission spectroscopy; Features of excited states

1. Introduction

Acridines have been a topic of interest for a long time owing to their interesting features and numerous applications. Their spectral properties have been much investigated, since these compounds absorb radiation in the near-UV region [1–5] and fluoresce [4–6] or phosphoresce [5,7–9] at the short-wave end of the visible region. Since both absorption and emission depend on the properties of the medium, acridines have been used to monitor the properties of liquid phases [10–12]. Further, the tautomeric phenomena taking place in some acridines have been investigated by electronic absorption and emission spectroscopy [13–15]. Lastly, numerous acridines exhibit biological activity: some of them have been used as antibacterial [1,16,17], anti-

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cancer [18,19], anti-amoebic [20], anti-inflammatory [21] or immuno-modulating drugs [22].

This publication focuses on derivatives of acridine-9carboxylic acid, i.e. derivatives of 9-(phenoxycarbonyl)acridines and their cationic forms protonated or methylated at the endocyclic N atom. These latter entities are chemiluminogenic in that after reaction with H₂O₂ in alkaline media, the product - electronically excited 10-methyl-9-acridinone - emits light [23–26]. The efficiency of chemiluminescence depends on the rate at which the original cations are converted to the latter molecules and the phenyl-containing entities are removed [27]. Substitution can affect the rate of this conversion, and hence the efficiency of chemiluminescence; several phenyl-substituted derivatives of 9-(phenoxycarbonyl)acridines were therefore synthesised in order to investigate their spectral properties. The chemiluminescence of 10-alkyl-9-(phenoxycarbonyl)-acridinium cations is affected by the presence of nucleophiles or anionic species, an effect that

^{*} Corresponding author. Tel.: +48 58 523 5467; fax: +48 58 523 5472. *E-mail address:* karolk@chem.univ.gda.pl (K. Krzymiński).

has been used to determine their content [28,29]. Alkylated 9-(phenoxycarbonyl)-acridines also occur as luminogenic fragments of chemiluminescent labels [25,30,31].

The aim of this investigation was to examine the spectral features of the title compounds and to discover the extent to which the absorption or emission of 10-methyl-9-(phenoxycarbonyl)-acridinium cations can affect originating from them chemiluminescence.

2. Materials and methods

2.1. Compounds

9-(Alkylphenoxycarbonyl)-acridines (1, Scheme 1) were synthesised as described in detail elsewhere [23,32]. In brief, 9-(chlorocarbonyl)-acridine (obtained by treating acridine-9-carboxylic acid with thionyl chloride) and stoichiometric amounts (3.6%, w/w each) of phenol or alkyl-substituted phenols (2-methylphenol, 3-methylphenol, 4-methylphenol, 2-ethylphenol, 2-isopropylphenol, 2-*tert*butylphenol, 2-trifluoromethylphenol) were mixed in anhydrous dichloromethane. The esterification was carried out in the presence of *N*,*N*-diethylethanamine (twofold molar excess) with continuous stirring and in the presence of catalytic amounts of *N*,*N*-dimethyl-4–pyridinamine (room temperature, 18–36 h). The crude esters were purified chromatographically (SiO₂ column; mobile phase: cyclohexane/ethyl acetate (3/2–2/1, v/v). Yields were 60–80%. The identity of **1b** and **1c** was confirmed by X-ray structure analysis [33,34]. Elemental analyses (EAGER 200 CHNS Analyser, Carlo Erba) and melting points (uncorrected) of 9-(alkylphenoxycarbonyl)-acridines (% found/calculated): **1a**: C 79.79/80.25, H 4.44/4.38, N 4.25/4.68, m.p. = 463–465 K; **1b**: C 80.68/80.49, H 4.81/4.82, N 4.45/4.47, m.p. = 411–412 K; **1c**: C 80.90/80.71, H 5.12/5.23, N 4.33/4.28, m.p. = 392–394 K; **1d**: C 80.85/80.92, H 5.52/5.61, N 4.08/4.10, m.p. = 395–397 K; **1e**: C 80.85/81.10; H 6.02/5.96, N 3.86/3.94, m.p. = 462–463 K; **1f**: C 80.61/80.49, H 4.84/4.82; N 4.41/4.47, m.p. = 428–429 K; **1g**: C 80.42/80.49, H 4.86/4.82, N 4.32/4.47, m.p. = 439–441 K; **1h**: C 68.57/68.67, H 3.37/3.29, N 3.77/3.81, m.p. = 408–409 K. The ¹H and ¹³C NMR spectra of the compounds will be presented and discussed elsewhere [35].

10-*H*-acridinium-9-(alkylphenoxycarbonyl) cations (**2a–2h**) appeared *in situ* in acetonitrile or dichloromethane when the esters (**1a–1h**), at concentrations of $1-5 \times 10^{-5}$ M, were treated with dilute hydrochloric acid (final concentration of HCl=1 × 10⁻⁴ M).

10-Methyl-9-(alkylphenoxycarbonyl)-acridinium trifluoromethanesulphonates (containing cations 3a-3h) were obtained by reaction of the above esters with a fivefold molar excess of methyl trifluoromethanesulphonate, both dissolved in anhydrous dichloromethane (Ar atmosphere, room temperature, 3–3.5 h). The crude products were dissolved in ethanol and the solutions filtered. An excess of diethyl ether was then added to each solution (25/1, v/v) and yellow precipitates were filtered off (yields 60–88%). The purity of the acridinium salts was checked



(f) $R_1 = 3$ -CH₃; (g) $R_1 = 4$ -CH₃; (h) $R_1 = 2$ -CF₃

Scheme 1. The entities investigated with the numbering of atoms indicated. (1) 9-(phenoxycarbonyl)-acridines: (1a) 9-(phenoxycarbonyl)-acridine, (1b) 9-(2methylphenoxycarbonyl)-acridine, (1c) 9-(2-ethylphenoxycarbonyl)-acridine, (1d) 9-(2-isopropylphenoxycarbonyl)-acridine, (1e) 9-(2-*tert*-butylphenoxycarbonyl)-acridine, (1f) 9-(3-methylphenoxycarbonyl)-acridine, (1g) 9-(4-methylphenoxycarbonyl)-acridine, (1h) 9-(2-trifluoromethylphenoxycarbonyl)-acridinie; (2) 10-H-9-(phenoxycarbonyl)-acridinium cation, (2b) 10-H-9-(2-methylphenoxycarbonyl)-acridinium cation, (2c) 10-H-9-(2-ethylphenoxycarbonyl)-acridinium cation, (2d) 10-H-9-(2-isopropylphenoxycarbonyl)-acridinium cation, (2e) 10-H-9-(2-tert-butylphenoxycarbonyl)-acridinium cation, (2f) 10-H-9-(3-methylphenoxycarbonyl)-acridinium cation, (2g) 10-H-9-(4-methylphenoxycarbonyl)-acridinium cation, (2h) 10-H-9-(2-tert-butylphenoxycarbonyl)-acridinium cation, (3c) 10-methyl-9-(2-tertylphenoxycarbonyl)-acridinium cation, (3d) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3d) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(4-methylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(3-methylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3g) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(3-methylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(3-methylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(3-methylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(2-tert)-butylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(3-methylphenoxycarbonyl)-acridinium cation, (3h) 10-methyl-9-(2by TLC (Silicagel 60; mobile phase: 0.1 M trifluoroacetic acid in chloroform); in each case a single signal was obtained (fluorescence detection, mid-pressure mercury UV lamp). The structure of compound **3b** was confirmed by X-ray structure analysis [36] and all compounds (**3a–3h**) by ¹H and ¹³C NMR spectrometry [35].

2.2. Spectral measurements

Absorption spectra were recorded on a Hitachi U3210 UV–vis spectrophotometer. The experimental absorption spectra were deconvoluted using Spectral Data Lab software [37]. Fluorescence spectra and quantum yields were measured with a Hitachi F4010 spectrofluorimeter with a solution of quinine sulphate in 0.05 M aq. sulphuric acid as the quantum yield reference standard (φ =0.56 [38]). The concentrations of the compounds investigated were 1–5 × 10⁻⁵ M. All the

solvents used for the spectral investigations – acetonitrile, dichloromethane, trichloromethane, acetone, butyl acetate and toluene – were of spectral grade and were selected so as to cover a broad range of orientational polarisability [39,40].

2.3. Calculations

The geometries of the entities (Scheme 1) in the vibrationally relaxed ground (S₀), singlet (S_i) and triplet (T_i) electronic states were obtained using the semi-empirical AM1 and AM1/CI methods [41] implemented in the MOPAC 2002 program [42]. The wavelengths and oscillator strengths of electronic absorption and emission transitions were calculated using the CNDO/S method implemented in HyperChem 7 with an 8×8 CI singly excited matrix [43]. The intersystem crossing rate constants (k_{ISC}) were estimated employing the CNDO/S/SOI method [44] with a 15×15 singly excited CI matrix. To obtain the matrix

Table 1

Absorption (above 300 nm) and fluorescence characteristics of 9-(phenoxycarbonyl)-acridines (**1a–1h**), 10-*H*-9-(phenoxycarbonyl)-acridinium cations (**2b–2h**) and 10-methyl-9-(phenoxycarbonyl)-acridinium cations (**3b–3h**)

Medium	Compound (Scheme 1)	λ_{abs}	λ_{fl}	Δv_{St}	ϕ	Compound (Scheme 1)	λ_{abs}	λ_{fl}	Δv_{St}	ϕ	Compound (Scheme 1)	λ_{abs}	λ_{fl}	Δv_{St}	ϕ
CH ₂ Cl ₂ CH ₃ CN	1a	382 363 381 361	455 454	5600 5700	1.3 1.6	2a	413 363 408 362	530	5700	0.3	3a	428 367 423 366	510 544	3720 5400	< 0.1
CH ₂ Cl ₂ CH ₃ CN	1b	383 362 381	456 453	5480 5620	0.8 1.6	2b	413 364 420	527	4860	0.2	3b	430 367 423	501 533	3300 5260	< 0.1 0.4
CH ₂ Cl ₂ CH ₃ CN	1c	361 383 362 381	454 455	5580 5560	0.6 1.8	2c	365 413 364 414	527	5200	0.2	3c	366 429 367 424	503 534	3400 4860	< 0.1 0.3
CH ₂ Cl ₂ CH ₃ CN	1d	361 382 363 381 361	455 454	5660 5640	0.6 1.6	2d	413 364 408 363	526	5510	<0.1	3d	428 367 423 366	506 532	3580 4820	< 0.1 0.4
CH ₂ Cl ₂ CH ₃ CN	1e	386 365 382 363	455 455	5460 5540	0.7 1.3	2e	412 365 410 365	529	5480	0.1	3e	431 369 426 368	502 538	3260 4880	0.2 0.5
CH ₂ Cl ₂ CH ₃ CN	1f	382 362 380 361	454 454	5600 5680	0.8 1.4	2f	413 363 411 363	532	5540	0.2	3f	430 368 423 366	523 512	4140 4320	< 0.1 < 0.1
CH ₂ Cl ₂ CH ₃ CN	1g	382 362 381 361	453 451	5560 5640	0.2 0.8	2g	413 363 411 362	535	5660	0.2	3g	428 367 423 366	520 513	4060 4120	< 0.1 < 0.1
CH ₂ Cl ₂ CH ₃ CN	1h	382 363 382 362	460 458	5760 5640	1.8 2.2	2h	415 365 408 364	524	5420	4.6	3h	432 370 419 364	527 525	4180 4820	6.6 12.1

 λ_{abs} , λ_{fl} : positions of band maximu in absorption and fluorescence spectra (in nm); $\Delta \nu_{St}$: Stokes shift (in cm⁻¹; $\Delta \nu_{St} = 1/\lambda_{exc} - 1/\lambda_{fl}$, where λ_{exc} : position of the long-wavelength band maximum in a fluorescence excitation spectrum (in nm); ϕ : fluorescence quantum yield (in %).

0.25

0.20

0.15 0.10 0.05 0.00

 Ψ_4

300

0.25

0.20

0.15

Ψ3 Ψ

350

Ψ

Ψ'1

450

400

Absorbance

elements of the spin-orbital coupling operator, single-, doubleand triple-centred integrals were taken into account. The intersystem crossing rate constants k_{ISC} (s⁻¹) were calculated from the equation [44]:

$$k_{\text{ISC}} = 3.95 \times 10^{11} \sum_{i} |\langle S_1 | \hat{\mathbf{H}} | T_i \rangle|^2 \times e^{-(E_{S_1} - E_{T_i} / 5 \times 10^3)}$$
$$= 3.95 \times 10^{11} \sum_{i} \beta_{1i}^2 F_{1i}$$

where $|\langle S_1|\mathbf{H}|T_i\rangle|$ and β_{1i} are the matrix elements of the spin-orbital coupling operator, E_{S_1} is the energy of state S_1 (in cm⁻¹), E_{T_i} represents the energies of all the triplet levels below S₁ (in cm⁻¹), and F_{1i} is the Franck-Condon factor, which includes the overlap between states S_1 and T_i for all of the above-mentioned intersystem crossing transitions. Fluorescence quantum yields were predicted on the assumption that intersystem crossing is the main pathway of radiationless deactivation. Rate constants of radiative deactivation (k_f) were further calculated by the CNDO/S/SOI method, starting from oscillator strength values [44,45].

The types of electronic transitions $(\pi \rightarrow \pi^* \text{ or } n \rightarrow \pi^*)$ involved were discovered by analysis of the components of configuration interaction matrices and excitation localisation numbers [46,47].

3. Results and discussion

3.1. Experimental absorption and fluorescence spectra

9-(Phenoxycarbonyl)-acridines (1), 10-H-9-(phenoxycarbonyl)-acridinium cations (2) and 10-methyl-9-(phenoxycarbonyl)-acridinium cations (3) exhibit long-wavelength absorption and fluorescence (Fig. 1 and Table 1); bathochromically shifted relative to that of acridine itself [48-50]. The long-wavelength absorption of these molecules occurs above 300 nm and is a superposition of four bands (Fig. 1). The positions of two of these bands and those of the fluorescence bands are given in Table 1. The band at the long-wavelength absorption shoulder is poorly resolved in the case of 9-(phenoxycarbonyl)acridines but quite well resolved for the cationic forms. The decadic molar absorption coefficients of these bands lie between $0.8-5 \times 10^4 \,\mathrm{M^{-1} \, cm^{-1}}$, which is characteristic of weak absorption. All the fluorescence bands are typically red-shifted in such a way that the Stokes shifts of the neutral and cationic entities are similar. Substitution at the phenyl ester ring only weakly influences the positions of the absorption and fluorescence bands within a given group of entities (1, 2, or 3). The Stokes shifts are similar for all the 9-(phenoxycarbonyl)-acridines. The differences in the Stokes shifts are the most pronounced in the case of 10-methyl-9-(phenoxycarbonyl)-acridinium cations.

3.2. Influence of the medium on spectral characteristics

The spectral features of each entity depend individually on the properties of the medium. Certain regularities are,



rescence (dotted line) spectra of **1a**, **2a** and **3a** in CH₃CN ($c = 1-5 \times 10^{-5}$ M; l=1 cm (upper graphs) together with positions of electronic absorption $(\Psi_1 = S_0 \rightarrow S_1, \Psi_2 = S_0 \rightarrow S_2, \Psi_3 = S_0 \rightarrow S_3, \Psi_4 = S_0 \rightarrow S_4)$ and fluorescence $(\Psi'_1 = S_1 \rightarrow S_0)$ transitions predicted at the CNDO/S level of theory (lower graphs).

however, discernible. In general, the absorption and fluorescence characteristics of neutral 9-(phenoxycarbonyl)-acridines are only weakly affected by solvent properties (Table 1). There are some similarities between the spectral characteristics of 10-H-9-(phenoxycarbonyl)-acridinium and 10-methyl-9-(phenoxycarbonyl)-acridinium cations, but the latter are the most susceptible to changes in the medium's properties. This is reflected not only in the positions of the absorption and fluorescence spectra, but also in the values of the Stokes shifts. In order to gain a broader insight into this problem, the Stokes shifts

Fluorescence intensity (arbitrary units)

1a

500 550600650

2a



Fig. 2. Plot of the Stokes shifts $(\Delta v_{\text{St}} = 1/\lambda_{\text{exc}} - 1/\lambda_{\text{fl}})$, where λ_{exc} , position of the long-wavelength band maximum in a fluorescence excitation spectrum) for **3a** dissolved in CH₂Cl₂ (1), toluene (2), chloroform (3), butyl acetate (4), acetone (5) and CH₃CN (6) against the orientational polarisability $(\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1))$, where ε , dielectric constant and *n*, refractive index.

corresponding to the long-wavelength band of **3a** were plotted against orientational polarisability (Fig. 2). This figure shows a certain trend: the higher the value of Δf , the greater the differences in electron density distribution in the ground and excited states of this entity. The value corresponding to dichloromethane does not follow this trend. There is no simple explanation of this finding.

3.3. Electronic transitions in the context of computational data

The computationally predicted positions of four longwavelength electronic absorption $(S_0 \rightarrow S_i)$ transitions are red-shifted relative to the maxima of the equivalent bands in the absorption spectra (Fig. 1). $S_0 \rightarrow S_1$ transitions are always of the $\pi \rightarrow \pi^*$ type, whereas $S_0 \rightarrow S_2$, $S_0 \rightarrow S_3$ and $S_0 \rightarrow S_4$ transitions are predominantly of the $\pi \rightarrow \pi^*$ type, but sometimes of the $n \rightarrow \pi^*$ type (Table 2); this is borne out by analysis of configuration interaction matrices, and is, moreover, in accordance what is known so far about acridine spectroscopy [5].

Computations predict rather poorly the position of fluorescence $(S_1 \rightarrow S_0)$ transitions, which are shifted bathochromically in cationic species and hypsochromically in neutral molecules relative to the band maxima in experimental spectra. The computational predictions are generally corroborated by the experimentally revealed spectral behaviour.

Electronic transitions bring about structural changes in neutral molecules and cationic entities that are reflected particularly in the mutual arrangement of the acridine, carboxyl and phenyl fragments (Table 3). Changes in the angles between the mean planes of these fragments apparently do not correlate with their structures or properties.

Electronic absorption and emission always accompany charge distribution changes; this always occurs during radiative transitions. Changes in relative Mulliken charges in selected

Table 2

Wavelengths (in nm) and oscillator strengths (in parentheses) of electronic absorption ($S_0 \rightarrow S_i$) and fluorescence ($S_1 \rightarrow S_0$) transitions, together with relevant excitation localisation numbers at acridinic (L_{Acr}), carboxylic (L_{COO}) and phenylic (L_{Ph}) sites (in %), predicted at the CNDO/S level for selected compounds

Compound	Absorption	Transition type	Fluorescence	L _{Acr}	L _{COO}	L_{Ph}
1a	379 (0.272)	$\pi \! ightarrow \! \pi^*$	401 (0.298)	100	0	0
	359 (0.030)	$n \! ightarrow \pi^*$		100	0	0
	351 (0.121)	$\pi ightarrow \pi^*$		100	0	0
	291 (0.012)	$\pi \! ightarrow \! \pi^*$		99	1	0
1e	378 (0.285)	$\pi\!\rightarrow\!\pi^*$	401 (0.283)	100	0	0
	359 (0.027)	$n \rightarrow \pi^*$		100	0	0
	358 (0.119)	$\pi ightarrow \pi^*$		100	0	0
	301 (0.017)	$\pi \! ightarrow \! \pi^*$		100	0	0
1h	379 (0.285)	$\pi \! ightarrow \! \pi^*$	400 (0.276)	100	0	0
	359 (0.022)	$n \rightarrow \pi^*$		100	0	0
	350(0.121)	$\pi ightarrow \pi^*$		100	0	0
	292 (0.012)	$\pi \! ightarrow \! \pi^*$		100	0	0
3a	437 (0.172)	$\pi \! ightarrow \! \pi^*$	593 (0.006)	100	0	0
	378 (0.009)	$\pi ightarrow \pi^*$		50	50	100
	375 (0.003)	$\pi ightarrow \pi^*$		50	50	100
	354 (0.154)	$\pi\!\rightarrow\pi^*$		100	0	0
3e	434 (0.138)	$\pi \! ightarrow \pi^{*}$	599 (0.002)	100	0	0
	408 (0.024)	$\pi ightarrow \pi^*$		50	50	100
	382 (0.015)	$\pi ightarrow \pi^*$		50	50	100
	372 (0.160)	$\pi\!\rightarrow\pi^*$		100	0	0
3h	432 (0.151)	$\pi \! ightarrow \pi^{*}$	490 (0.148)	100	0	0
	392 (0.229)	$\pi \! ightarrow \pi^*$		100	0	0
	368 (0.019)	$\pi ightarrow \pi^*$		50	50	100
	354 (0.223)	$\pi \! ightarrow \! \pi^*$		100	0	0









Fig. 3. Changes in relative Mulliken atomic charges accompanying electronic excitation (the difference between the relative atomic charges in the relaxed excited (S1; AM1/CI level of theory) and the relaxed ground (S0; AM1 level of theory) states). Empty circles indicate the decrease and filled circles the increase in electron density at a given atom; the area of the circles is proportional to the absolute values of the relative Mulliken charge change at a given atom.

entities demonstrate that charge distribution changes during $S_0 \rightarrow S_1$ transitions take place in whole molecules (Fig. 3). These changes are roughly uniform, which means that differences in dipole moments in states S_1 and S_0 are small in the case of neutral molecules (Table 3). This explains the relatively low absorption capacity of the compounds investigated.

Analysis of configuration interaction matrices provides excitation localisation numbers [45,46], which can be regarded as a measure of involvement of the various molecular sites in electronic transitions. It was predicted which of the acridinic (Acr), carboxylic (COO) or phenylic (Ph) sites in selected neutral molecules and cationic species contribute to the first four electronic transitions (Table 2). The excitation localisation numbers for $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$, $S_0 \rightarrow S_3$ and $S_0 \rightarrow S_4$ transitions in neutral molecules indicate that predominantly acridine systems should be involved in these transitions. The situation is similar in the case of $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_4$ transitions in cationic species. In $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_3$ transitions of cationic species all three of these sites should be involved. These findings do not confirm the predictions concerning electron distribution changes (Fig. 3), which suggest that excitation involves whole molecules. This is probable, since the measured and predicted positions of both Angle: C

Physicochemical and structural characteristics for selected compounds predicted at the AMI (S_0 state) and AMI/CI (S_1 state) levels of theory							
Characteristic	Compound						
	1a	1b	1e				
$\overline{\mu(\mathbf{S}_1)-\mu(\mathbf{S}_0)}$	0.3	-0.1	-0.2				
Angle: A	91 (81)	80 (81)	88 (73)				
Angle: B	21 (5)	4 (5)	12 (35)				
Angle: C	50 (92)	87 (65)	62 (73)				
	2a	2b	2e				
Angle: A	56 (79)	60 (85)	80 (86)				
Angle: B	16 (11)	9 (5)	62 (91)				
Angle: C	54 (54)	65 (67)	34 (31)				
	3 a	3b	3e				
Angle: A	95 (92)	96 (92)	75 (88)				
Angle: B	3 (5)	3 (5)	52 (94)				

Table 3 Physicochemical and structural characteristics for selected compounds predicted at the AM1 (S₀ state) and AM1/CI (S₁ state) levels of the

 μ : Dipole moments in the S₀ and S₁ states (in Debyes); A: between the mean plane of the acridine nucleus (delineated by C1–C9, N10, C11–C14) and the plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); B: between the mean plane of the acridine nucleus and the mean plane of the benzene ring (delineated by C18–C23) in the S₀ and S₁ states (in parentheses); C: between the mean plane of the benzene ring and plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); C: between the mean plane of the benzene ring and plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); C: between the mean plane of the benzene ring and plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); C: between the mean plane of the benzene ring and plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); C: between the mean plane of the benzene ring and plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); C: between the mean plane of the benzene ring and plane delineated by C15, O16, O17 in the S₀ and S₁ states (in parentheses); the values of angles A, B and C (in degrees) were obtained with the HyperChem program [43] using AM1 and AM1/CI optimised structures.

81 (92)

absorption and emission transitions are bathochromically shifted relative to those of native acridine [48–50], which would point to an extension of the conjugated system at the phenyl site via the carboxyl group. On the other hand, the pattern of the longwavelength absorption spectra of the compounds investigated is similar to that of the corresponding acridine [48–50]. This would indicate that the latter fragment predominantly affects the spectral behaviour of 9-(phenoxycarbonyl)-acridines and the protonated and methylated forms derived from them.

89 (93)

3.4. Fluorescence efficiency

The experimentally determined fluorescence quantum yields are generally low for both neutral molecules and cationic entities (Table 1); the fluorescence quantum yield of only one entity (**3h**) exceeds 10%. This means that non-radiative deactivation is the predominant process in the depopulation of electronically excited molecules. This is indeed confirmed by the results of our predictions (Table 4). They demonstrate that rates of intersystem crossing exceed rates of fluorescence decay by roughly two orders of magnitude. In such a situation, predicted fluorescence quantum yields are no greater than a few per cent. Rapid intersystem crossing is a consequence of the efficient spin–orbital coupling noted earlier in some acridine derivatives [5,51].

22 (30)

1h -0.6 86 (76) 8 (7) 78 (58)

2h 86 (81) 48 (42) 50 (38)

3h 84 (68)

43 (84)

44 (40)

3.5. Spectral features in relation to chemiluminescence phenomena

Oxidation of 10-methyl-9-(phenoxycarbonyl)-acridinium cations with H_2O_2 in alkaline media produces electronically

Table 4

The rate constants of radiative deactivation (k_f), the intersystem crossing rate constants (k_{ISC}) and fluorescence quantum yields (ϕ) predicted at the CNDO/S/S0I level of theory

Characteristic	Compound							
	1 a	1b	1e	1h				
$\overline{k_{\rm f}({\rm s}^{-1})}$	9.33×10^{6}	$7.18 imes 10^6$	8.00×10^{6}	1.25×10^{7}				
$k_{\rm ISC} ({\rm s}^{-1})$	8.11×10^{8}	9.02×10^{8}	1.33×10^{9}	7.76×10^{8}				
φ(%)	1.14	0.79	0.60	1.59				
	2a	2b	2e	2h				
$\overline{k_{\rm f}({ m s}^{-1})}$	5.69×10^{5}	6.60×10^{5}	3.13×10^{5}	9.27×10^{7}				
$k_{\rm ISC} ({\rm s}^{-1})$	2.02×10^{9}	1.17×10^{9}	3.68×10^{9}	2.61×10^{9}				
φ(%)	0.03	0.05	0.01	3.43				
	3 a	3b	3e	3h				
$\overline{k_{\rm f}({ m s}^{-1})}$	1.71×10^{6}	7.11×10^{5}	4.28×10^{5}	5.91×10^{7}				
$k_{\rm ISC} ({\rm s}^{-1})$	3.51×10^{9}	3.28×10^{9}	2.79×10^{9}	2.06×10^{9}				
φ(%)	0.04	0.02	0.02	2.79				

Fluorescence quantum yields were calculated on the assumption that intersystem crossing is the main pathway of non-radiative deactivation; $\phi = k_f/(k_f + k_{ISC})$.

excited molecules of 10-methyl-9-acridinone, which exhibit broad emission centred around 450 nm [52]. Such emission thus overlaps both the long-wavelength absorption and the fluorescence of the original cations. If the thus excited 10methyl-9-acridinone molecules emit radiation, this can be partially absorbed by native 10-methyl-9-(phenoxycarbonyl)cations and then emitted by them with a low quantum yield. Such processes will lower chemiluminescence efficiency, which is unwanted when one intends to investigate or apply this phenomenon analytically. It is difficult to assess to what extent the internal absorption and emission of the original acridinium cations actually affects chemiluminescence, since it is not known how long these entities remain in unchanged form following oxidation. Nevertheless, all oxidation steps proceed in a certain time [27] and some fraction of the original cations must always be present during the whole process. Internal absorption and emission should thus be taken into account as a factor determining the chemiluminogenic ability of 10-methyl-9-(phenoxycarbonyl)acridinium cations.

4. Concluding remarks

The results of these investigations indicate that substitution in the phenyl ester ring of 9-(phenoxycarbonyl)-acridines and their cationic forms obtained as a result of protonation or methylation at the endocyclic N atom does not substantially influence spectral characteristics within a given group of entities.

The lowest energy electronic transitions in all compounds are accompanied by electron density changes within the whole molecules. These changes are roughly uniform in all entities, which means that these transitions are relatively weak. The patterns of the long-wavelength absorption spectra of 9-(phenoxycarbonyl)-acridines, of the protonated and methylated forms derived from them, and of acridine are similar, which may indicate that it is predominantly the acridinic fragment that affects the spectral behaviour of the compounds investigated, as indicated by the predicted excitation localisation numbers.

The low quantum yields of fluorescence of the compounds studied are due to efficient spin–orbital coupling and relatively rapid non-radiative deactivation.

Absorption and fluorescence of 10-methyl-9-(phenoxycarbonyl)-acridinium cations can affect the chemiluminescence ensuing from their oxidation. This fact has to be considered when investigating or applying this phenomenon.

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