# Excited State Intramolecular Proton Transfer in 9-Oxo-9,10-dihydro-acridine-4-carboxylic Acid. Photophysical and Computational Studies[*]

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9-Oxo-9,10-dihydro-acridine-4-carboxylic acid in both methanol and acetonitrile possesses a dual fluorescence and complex fluorescence intensity decay, whereas 9-oxo-9,10-dihydro-acridine-2-carboxylic acid shows only one emission band and mono-exponential fluorescence intensity decay. Theoretical calculations, using a semiempirical PM3 method, confirm, in the case of 9-oxo-9,10-dihydro-acridine-4-carboxylic acid, the excited state intramolecular proton transfer process from nitrogen atom to the oxygen of carbonyl of the carboxylic group.

**Key words**: excited state intramolecular proton transfer, 9-oxo-9,10-dihydro-acridine-4-carboxylic acid, fluorescence spectroscopy, PM3 calculations

The excited state intramolecular proton transfer (ESIPT) is an intriguing phenomenon that attracted great interest in recent years, due to the advent of refined spectroscopic techniques like picosecond measurements or supersonic jet. The process usually occurs in heterocyclic molecules, which possess functional groups involved in an intramolecular hydrogen bond. An electronic excitation often leads to a dramatic change in acid-base properties of chemical species that brings about fast proton transfer in the excited state. In most cases of ESIPT discovered so far, the proton donor group is a hydroxyl one and the proton acceptor is either a carbonyl oxygen or a =N- atom. ESIPT has been observed in a wide class of molecules and found important both in UV photochemical reactions in plants and in tautomeric interconversion of nucleic acids [1–6].

While searching for a "perfect" fluorescent sensor, we synthesized 9-oxo-9,10-dihydro-acridine-4-carboxylic acid (4-ACA; see Fig. 1) that despite other interesting features possesses a dual fluorescence which is usually measured when ESIPT takes place [1]. In this work we demonstrate, by both experimental and computational arguments, that the dual fluorescence observed for 4-ACA may indeed be a consequence of ESIPT.

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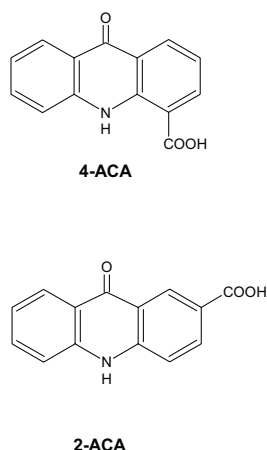
**4-ACA**



**2-ACA**

**Figure 1.** Chemical structure of 2-ACA and 4-ACA.

## MATERIALS AND METHODS

Absorption spectra were recorded using a Perkin Elmer Lambda 18 spectrometer, whereas steady state fluorescence spectra were measured at the excitation wavelength of 380 nm on a Perkin Elmer LS-50B spectrofluorimeter with 2.5 nm bandwidth for the emission and excitation. Fluorescence decay times were obtained using a time correlated single-photon counting apparatus at Center for Ultrafast Laser Spectroscopy, Adam Mickiewicz University (Poznań, Poland) [7]. The excitation source ($\lambda_{ex} = 277$ nm) was pico/femtosecond laser system (Ti:Saphire "Tsunami" laser pumped with an argon ion laser "BeamLok" 2060) with the half width of the instrument response of about 35 ps. Fluorescence decay data were fitted by the iterative convolution of the sum of exponents:

$$I(\lambda,t) = \sum_i \alpha_t(\lambda)\exp\left(\frac{t}{\tau_i}\right)$$

where $\tau_i$ is the decay time of the i-th component and $\alpha_i(\lambda)$ is the pre-exponential factor at the emission wavelength $\lambda$. The adequacy of the exponential decay was judged by examination of the plots of weighted residual and the statistical parameter $\chi^2$.

To gain an insight into molecular factors, that might be responsible for dual fluorescence observed, we performed PM3 [8] calculations concerning structure and energetics of the possible tautomers in the ground and first excited state. First, unconstrained geometry optimization have been carried out for the ground state of all species considered by EF procedure [9]. These structures were subsequently used as starting geometries in unconstrained optimization of their first singlet excited state ($S_1$). Due to the relatively large size of the relevant systems, we carried out CI [10] optimization in the minimal active space, restricted to the HOMO and LUMO orbitals. However, final estimation of the energy of fluorescence vertical transitions and energetics in the excited state have been obtained by single point CISDT (CI with Singles, Doubles and Triples) calculations in the active space that consisted of eight molecular orbitals (HOMO-3 to LUMO+3) [11]. For tautomer C we checked that the further extension of an active space to nine molecular orbitals changed the predicted fluorescence transitions and heat of formation of the excited state by less than 0.0001 eV and 0.01 kcal/mol, respectively. Therefore, in CISDT calculations we settled for eight orbitals. To correct for the well-know overestimation of transition energies, predicted by the semiempirical methods, 0.084 eV has been subtracted from all fluorescence transition energies obtained in the manner described above. This incremental factor was estimated as a difference between measured and calculated fluorescence transitions for the reference system (2-ACA). The influence of methanol on the predicted spectral and energetic characteristics was described within the self-consistent reaction field (SCRF) method [12]. All calculations were carried out with the MOPAC2000 code [13].

## RESULTS AND DISCUSSION

Spectral characteristics have been measured in methanol and acetonitrile solutions (see Fig. 2) of 4-ACA and 9-oxo-9,10-dihydro-acridine-2-carboxylic acid (2-ACA; for chemical structure see Fig. 1) employed as a reference system. In Table 1 we summarize emission characteristics of 4-ACA and 2-ACA in methanol. In the latter system, where the steric reasons (see Fig. 1) prevent formation of intramolecular hydrogen bond, only single fluorescence band was observed with emission maxima at 413 and 435 nm. Additionally, time-resolved measurements ($\lambda_{obs.}$ = 420 nm) reveal mono-exponential fluorescence intensity decay with life-time equals to 10.07 ns (see Table 1). In contrast, the emission characteristic of 4-ACA is more complicated. Namely, two well-separated emission bands appear, one with two maxima at 427 and 450 nm, and another one, stronger, with maximum at 509 nm (Table 1). Fluorescence intensity decay recorded at 520 or 500 nm observation wavelength is mono-exponential ($\tau$ = 6.44 ns), whereas this observed at 440 nm possesses two components with life-times 6.49 and 0.85 ns. One should note, that at 440 nm long and short wavelength emissions overlap (see Fig. 2) and, therefore, the bi-exponential decay confirms that both bands originate from different emitting species. Additionally, relatively long life-time (as for intramolecular proton transfer) of the short wavelength fluorescence suggests the existence of a kinetic barrier separating fluorescent species (work on the dependence of emission efficiency on the value of excitation wavelength in the red absorption region is in progress). Moreover, fluorescence excitation spectra measured for both 2-ACA and 4-ACA remain in a good agreement with their absorption features.
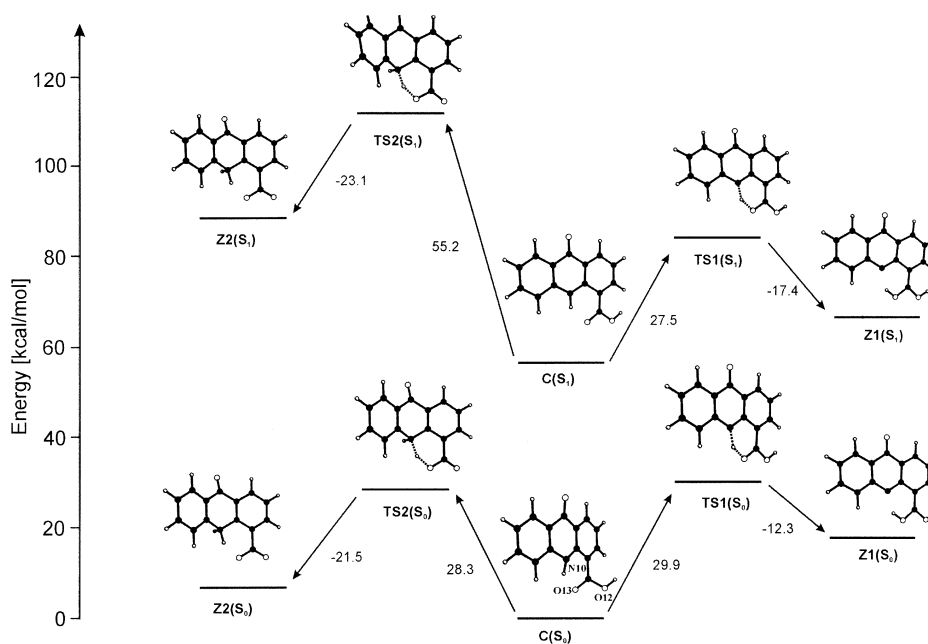
Experimental findings described above prove the existence of two emitting species, formed after excitation of 4-ACA. Taking into account, that internal hydrogen bond (N(10)–H··O(13)) is possible only for the 4-substituted isomer, ESIPT seems to be a very probable explanation of the dual fluorescence, observed for the title compound. To gain a deeper insight into this phenomenon, we carried out quantum-chemical calculations concerning structure and energetics of various 4-ACA tautomers in the ground ($S_0$) as well as first excited state ($S_1$). In Fig. 3 PM3 geometries together with relative energies of possible tautomeric forms of 4-ACA optimized in the ground and first excited state in methanol are depicted. Additionally, in Table 2 we gathered thermodynamic and kinetic barriers (in enthalpy scale) for tautomerization processes considered. In the most stable C tautomer, hydrogen bond is formed between N(10) and O(13) and, therefore, the proton transfer should be naturally connected to the formation of Z1 (see Fig. 3). Since in ACA derivatives -COOH is the most acidic group, we also considered the possibility of Z2 formation as a result of proton transfer between hydroxyl oxygen O(12) and nitrogen N(10) (see Fig. 3).
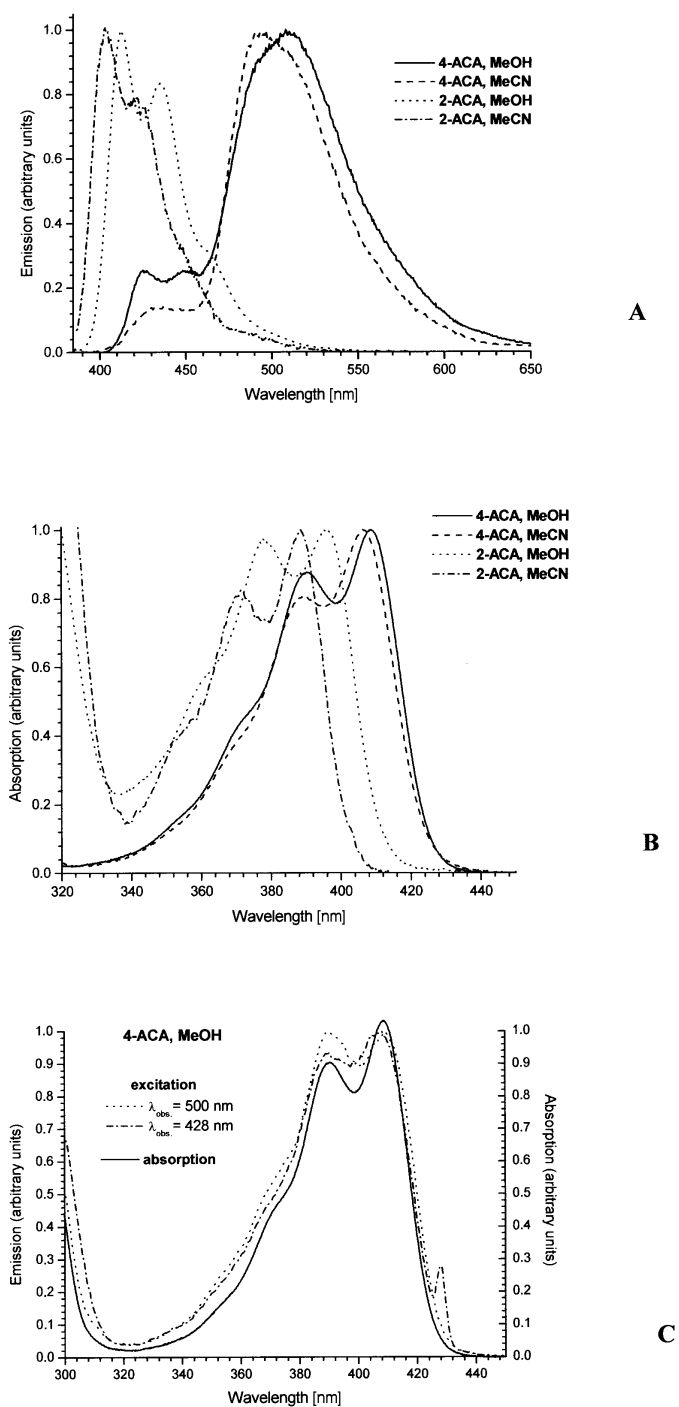
**Figure 2.** Normalized fluorescence (A; excitation wavelength equal to 380 nm) and absorption (B) spectra of 2-ACA and 4-ACA recorded in methanol and acetonitrile solutions together with excitation spectrum of 4-ACA (C) measured in methanol.

**Table 1.** Fluorescence characteristics of 2-ACA and 4-ACA measured in methanol solution.

| Compound | Emission decay characteristics | | | | Emission maxima [nm] |
|---|---|---|---|---|---|
| | $\lambda_{obs.}$ [nm] | $\tau$ [ns] | $\alpha$ | $\chi^2$ | |
| 2-ACA | 420 | 10.07 | 1.0 | 1.17 | 413 |
| | | | | | 435 |
| 4-ACA | 440 | 6.49 | 0.803 | 1.01 | 427 |
| | | 0.85 | 0.197 | | |
| | 520 | 6.44 | 1.0 | 0.94 | 450 |
| | 550 | 6.44 | 1.0 | 1.00 | 509 |



**Figure 3.** Relative energies and geometries of individual tautomers originating from 4-ACA together with respective transition states concerning tautomerization processes calculated for the ground and first excited state.

**Table 2.** Energy and kinetic barriers for tautomerization processes in the ground and first excited state calculated at the PM3 and PM3/CI levels, respectively. All quantities are given in kcal/mol.

| Process | Ground state barrier | | Excited state barrier | |
|---|---|---|---|---|
| | Energy | Kinetic | Energy | Kinetic |
| C → Z1 | 17.6 | 29.9 | 10.1 | 27.5 |
| C → Z2 | 6.8 | 28.3 | 32.1 | 55.2 |

As indicated by data shown in Table 2, energy barriers for proton transfer in the ground state amount to 17.6 and 6.8 kcal/mol for C → Z1 and C → Z2 transformations, respectively. If one disregards the change of entropy in the course of reaction (note that the tautomerization reactions studied are unimolecular processes), which implies equality $\Delta H_r = \Delta G_r$, these numbers show that in the methanol solutions of 4-ACA concentration of Z1 and Z2 tautomers should be close to zero, that remains in good agreement with the measured fluorescence excitation spectrum. On the other hand, in the first excited state energy barriers for proton transfer are 10.1 and 32.1 kcal/mol, respectively (see Table 2). First, it is worth noticing that the energy difference between first excited state of C and Z1 diminishes in comparison to the respective ground state value by 7.5 kcal/mol, that is probably connected to the increase of the acidity of N(10)–H bond. Moreover, the activation barrier for $C(S_1) \rightarrow Z1(S_1)$ transformation amounts to 27.5 kcal/mol and can be relatively easily overcome by excitation of C to the excited vibrational levels in the $S_1$ state. Note that the energy difference between transition state $TS1(S_1)$ and $C(S_0)$ (see Fig. 3) amounts to 83.8 kcal/mol, which is relatively close to 75.3 kcal/mol delivered by excitation photons of 380 nm used in the experiment (lacking 8.5 kcal/mol is probably connected to PM3/CI overestimation of excited states energies; recall scaling procedure applied to fluorescence transition energies). In contrast, formation of $Z2(S_1)$ seems to be improbable, because the energy barrier $C(S_0) \rightarrow Z2(S_1)$ amounts to 88.4 kcal/mol and additional portion of energy is necessary to overcome the activation barrier concerning $Z2(S_1) \rightarrow C(S_1)$ tautomerization (23.2 kcal/mol; see Table 2). Another computational argument, confirming ESIPT in 4-ACA comes from comparison of the calculated and measured fluorescence transition energies. Namely, for the C and Z1 tautomers, the PM3/CI method predicts fluorescence transitions at 428 and 505 nm that compares very well with the experimental emission maxima at 427 and 509 nm, respectively (see Table 1).

Thus, we demonstrated that the dual fluorescence measured for 4-ACA may be interpreted in the category of ESIPT process because: (i) only single fluorescence band is observed for 2-ACA, where internal hydrogen bond cannot be formed, (ii) there is a good agreement between fluorescence excitation and absorption spectra for 4-ACA, (iii) energetic factors calculated at the PM3/CI level admit formation of $Z1(S_1)$ through direct electronic excitation of the most stable C tautomer, (iv) measured and calculated fluorescence transition energies for C and Z1 tautomers compare very well.

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