

Fluorescence Study of 3-Acetoacetyl-7-methyl-2H,5H-pyranopyran-2,5-dione: Evidence for the Two *cis*-Enol Forms?

Gunvor Ellingsen,^a Karine Vercruysse,^a Vincent Spéziale,^a Maamar Hamdi^b and Suzanne Fery-Forgues^{a,*}

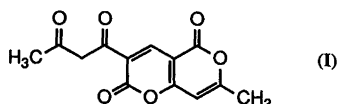
^aLaboratoire des Interactions Moléculaires Réactivité Chimique et Photochimique, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse, France and ^bLaboratoire des Composés Hétérocycliques, Institut de Chimie, Université des Sciences et de la Technologie, B.P. 32 El-Alia, Bab Ezzouar, Alger, Algeria

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The behaviour of the title compound has been investigated in different solvents by absorption and fluorescence spectroscopy. Absorption spectra showed that the enol form largely predominates in organic solvents, as confirmed by NMR data. Fluorescence was observable at room temperature (the quantum yield ranging from 10^{-2} to 10^{-3} according to the solvent) and was totally attributed to the enol tautomer. The emission spectrum was strongly shifted towards the blue, and its shape underwent significant changes when passing from toluene to water. This behaviour was mainly attributed to the involvement of two different species, assumed to be two *cis*-enol isomers, whose relative proportions vary with the nature of the solvent. Attributions were performed with the help of NMR data and MNDO calculations.

In the last decade, β -diketones have received special attention because of their increasing importance in photophysics and photochemistry. These compounds proved to be efficient in photostabilizing polymeric materials^{1,2} and for protecting the human skin,³ because of their capacity to absorb UV energy and to dissipate it through the keto–enol photoisomerization process. Moreover, their outstanding coordination ability allows β -diketones to be used as synergistic ligands for enhancing the fluorescence of rare-earth complexes,^{4,5} and stable β -diketonates have found potential applications as laser dyes and photocatalysts.⁶

Recently, a new β -diketone, 3-acetoacetyl-7-methyl-2H,5H-pyrano(4,3-*b*)pyran-2,5-dione (**I**, Scheme 1), has been synthesized in the laboratory.⁷



Scheme 1.

We anticipated that the conjugation of the acetylacetonone moiety with the heterocycle could lead to interesting photophysical properties, and that this compound could

* To whom correspondence should be addressed.

subsequently be used as a signalling agent for cation recognition. Before undertaking complexation studies, the behaviour of free ligand **I** was investigated by absorption and fluorescence spectroscopy, with the help of NMR data and MNDO calculations.

Results

It has been shown by crystallography that compound **I** is an enol in its solid state.⁸ However, like acetylacetonone and its congeners, in solution compound **I** should exist in a thermal equilibrium of the two tautomeric forms, the enol and the diketone. It must be noticed that all solutions were made and kept in the dark in order to avoid subsequent photoisomerization.^{9,10}

NMR spectra. The relative proportions of the keto and the enol forms were easily determined by ¹H NMR spectroscopy, which allowed a distinction to be made between the ketone methylenic protons (at 4.1 ppm in acetonitrile) and the enol ethylenic proton (between 8.55 and 8.75 ppm depending on the solvent), at least when deuteriated solvents were available. The data are collected in Table 1. The enol population was 100% in toluene and 98% in methanol. In acetonitrile, a polar solvent known to favour ketonization,¹¹ the proportion

Table 1. Spectroscopic characteristics of compound I in different solvents: percentage of enol as determined by NMR spectroscopy, maximum absorption of the short-wavelength band (λ) and of the long-wavelength band pertinent to the enol form (λ_E), corresponding apparent molar extinction coefficient (ϵ_{app}) and $\epsilon_{E(\text{app})}$, molar extinction coefficient of the pure enol form (ϵ_E), maximum emission wavelength (λ_F) and fluorescence quantum yield (Φ_F).

Solvent	Enol (%)	λ/nm	$\epsilon_{E(\text{app})}/\text{M}^{-1}\text{cm}^{-1}$	λ_E/nm	$\epsilon_{E(\text{app})}/\text{M}^{-1}\text{cm}^{-1}$	$\epsilon_E/\text{M}^{-1}\text{cm}^{-1}$	λ_F/nm	Φ_F
Toluene	100	—	—	404	23 700	23 700	477	1.2×10^{-2}
				389	23 600	23 600		
Ethyl acetate	—	276	7500	398	26 300	—	467	5.4×10^{-3}
				385	26 300	—		
Acetonitrile	90	278	8670	395	25 600	28 400	468	3×10^{-3}
				383	25 600	28 400		
Methanol	98	280	8900	395	18 900	19 300	469	2.4×10^{-3}
				383	18 600	19 000		
Water	—	288	—	376	—	—	437	1.4×10^{-3}

of the enol form was 90% immediately after dissolution and 84% after equilibration for 64 h.

It is also interesting to note that in acetonitrile and methanol the signal corresponding to the methyl group of the acetylacetonate moiety (2.22 ppm) is shielded with respect to the signal corresponding to the heterocyclic methyl group (2.35 ppm), and that the opposite is encountered in toluene, where the signals appear at 1.77 ppm and 1.40 ppm, respectively (Fig. 1).

Absorption spectra. The absorption spectrum of I recorded immediately after dissolution of the product in aqueous or organic solvent displays two distinct bands,

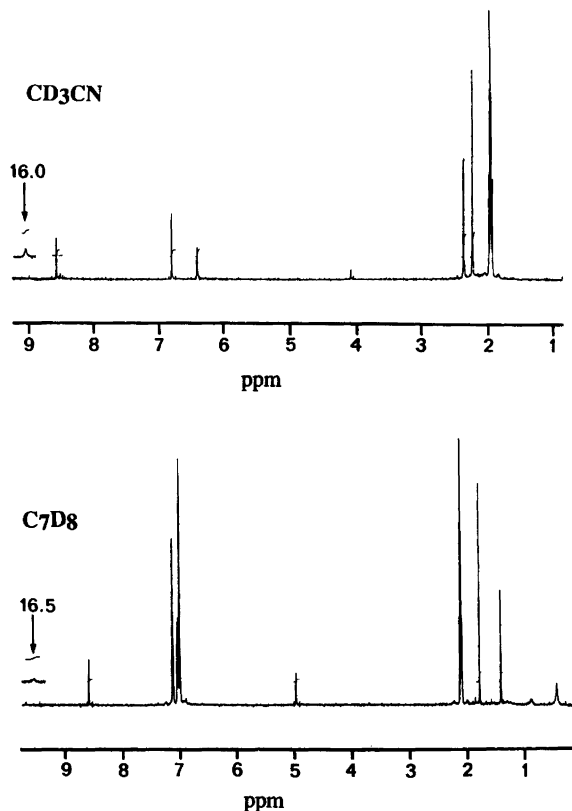


Fig. 1. ^1H NMR spectra of compound I (at saturation) in deuterated acetonitrile and toluene.

as shown for methanol in Fig. 2: a short-wavelength band situated around 280 nm, and an intense low-energy band lying around 380–400 nm, the latter being associated with the pure enol form.^{12–14} This enol band shows a plateau with two maxima in organic solvents but one only maximum was observed in water. The absorption maxima recorded in five different solvents are collected in Table 1. It was striking that the high-wavelength band underwent a significant blue shift when passing from toluene to water, the maximum moving from 389–404 to 376 nm.

Solutions were stable over 64 h in toluene, ethyl acetate and methanol. The enol band decreased very slightly in acetonitrile but it was checked that its variation was negligible over a few hours. In water, however, the proportion of the enol form quickly decreased, so spectroscopic measurements had to be performed immediately after dissolution.

The apparent molar absorption coefficients ϵ_{app} were calculated from the total concentration in compound I. They were of similar magnitude in each of the organic solvents considered (Table 1). The fast ketonization process encountered in water, together with the poor solubility of the product in this medium, did not allow an accurate determination of ϵ_{app} .

Knowing the proportion of enol from the NMR data, it was possible to calculate the molar absorption coefficient of the pure enol form in the corresponding solvents (Table 1). This coefficient ϵ_E increased when passing

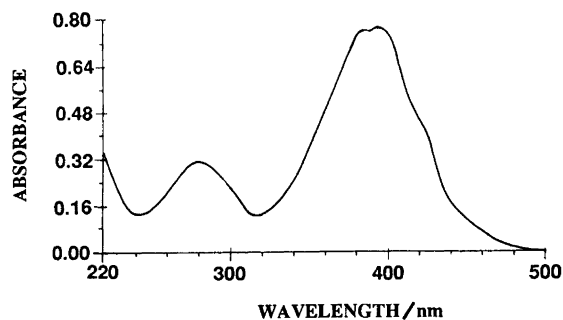


Fig. 2. Absorption spectrum of compound I (4×10^{-5} M) in methanol.

from toluene to acetonitrile, as expected for a charge-transfer band. In methanol, ϵ_E was found to be rather low, but this may be attributed to basic residues present in the solvent at a concentration close to that of the product,¹⁵ and which lead to a widening of the red edge of the spectrum, together with a decrease in intensity, characteristic of the formation of an enolate.

Fluorescence spectra. The fluorescence of compound I in the five solvents used above was observable at room temperature. Excitation spectra measured in each of the five solvents closely resembled the absorption spectra, displaying the strong enol band at 380–400 nm and the weaker band at around 280 nm (Fig. 3). No emission was observed in water when the enol-form band had disappeared.

The emission spectra underwent a strong blue shift when the polarity of the solvent increased, the peak moving from 477 nm in toluene to 437 nm in water (Fig. 4). It may be noticed that the peak was almost the same in the three 'intermediate' solvents (methanol, acetonitrile and ethyl acetate). However, large variations were found in the shape of the spectra according to the medium. In organic solvents the maximum lies between two shoulders whose relative proportions depend on the nature of the solvent. In toluene, a strong shoulder was observed at 500 nm, while the shoulder at 450 nm was large in methanol. In water, the spectra no longer showed any shoulder. This progression was evidenced using binary toluene–acetonitrile mixtures (Fig. 5). The shoulder above 500 nm vanished, while the short-wavelength shoulder increased as and when acetonitrile was added to the toluene. Then, when passing gradually from acetonitrile to water, it appeared that the shoulder at 450 nm first became the only band of the spectrum and secondly shifted towards the blue when the proportion of water increased (Fig. 6).

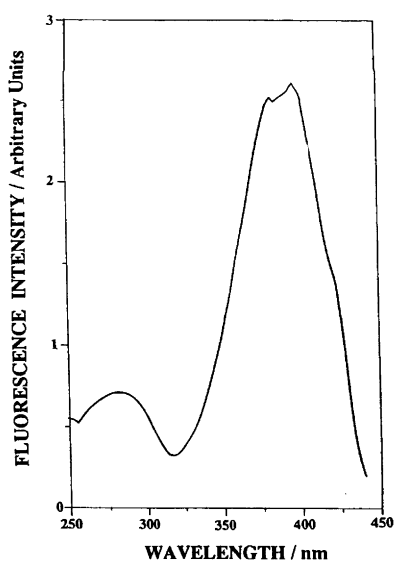


Fig. 3. Excitation spectrum of compound I (5×10^{-6} M) in acetonitrile. $\lambda_{em} = 468$ nm.

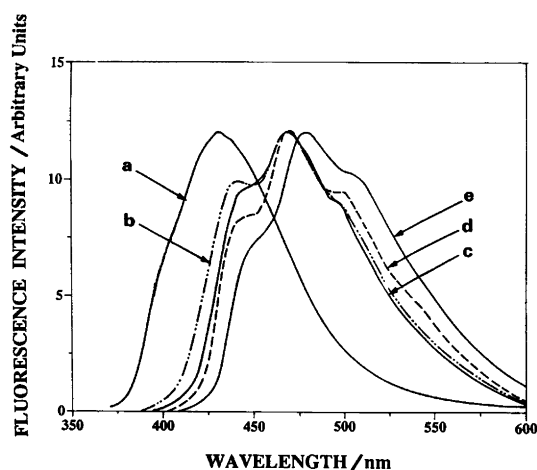


Fig. 4. Normalized emission spectrum of compound I (5×10^{-6} M) in (a) water, (b) methanol, (c) acetonitrile, (d) ethyl acetate and (e) toluene. $\lambda_{ex} = 350$ nm.

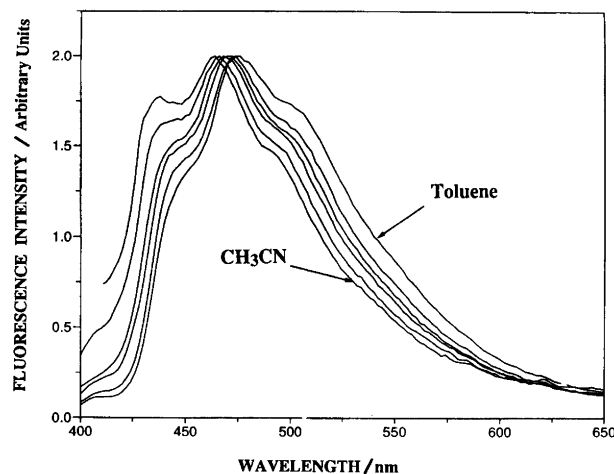


Fig. 5. Normalized emission spectra of compound I (5×10^{-6} M) in a toluene–acetonitrile binary mixture. From right to left: toluene (v/v): 100, 82, 50, 30, 11.5, 0%. $\lambda_{ex} = 350$ nm.

In toluene, the shape of the emission spectra was affected neither when changing the excitation wavelength nor when changing the temperature. In acetonitrile, excitation at 350 nm led to the appearance of a strong shoulder at 440–450 nm, compared with excitation at 420 nm (Fig. 7). Moreover, the relative intensity of the 440–450 nm shoulder seemed to be increased at 55 °C compared to 20 °C.

The fluorescence quantum yield was measured (Table 1). It ranged from 10^{-2} to 10^{-3} , being divided by ten when passing from toluene to water.

MNDO calculations. The enol form of β -dicarbonyl compounds usually exists as the conjugated *cis*-enol, stabilized by intramolecular hydrogen bonding.^{11,12,16} In the case of unsymmetrical structures, two isomers can be formed by transfer of an enol proton from one oxygen atom to the other.^{2,11} MNDO calculations were thus

that the ketone $n-\pi^*$ transition and another transition occurring in the heterocycle influenced by a CO group overlap in this region, as already noted for some substituted diketones.¹³ Knowing, from NMR spectroscopy, the keto/enol ratio in acetonitrile and in methanol, the respective contributions of the two forms to the absorption at 280 nm could be calculated for polar organic solvents. The molar absorption coefficient (ϵ_E) of the pure enol form at 280 nm was estimated to be $8560 \text{ M}^{-1} \text{ cm}^{-1}$, very close to that of the pure keto form (ϵ_K), i.e. $9600 \text{ M}^{-1} \text{ cm}^{-1}$, assuming that the intensity of the transitions is similar in the two solvents considered.

We now turn our attention to the fluorescence spectra. As indicated by the excitation spectra, which closely resemble the enol absorption spectra, and by the fact that no emission was observed from water as soon as ketonization had been completed, fluorescence could only arise from the enol form. In their fluorescence study of some substituted β -diketones, Gustav and Bartsh¹⁹ have reported the excitation spectra. By comparing these data with the corresponding enol absorption spectra given in the same paper, it appears that in this case too the enol form was the species responsible for fluorescence.

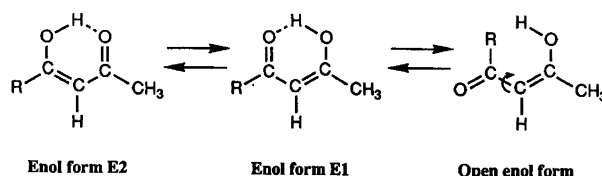
The low fluorescence efficiency can be explained by the enol intramolecular hydrogen bond, whose principal effect is an increase in the rate of internal conversion. Consequently, most of the absorbed energy is thermally dissipated.²⁰

At first sight, the considerable shift of the emission spectra may look like a negative solvatochromic effect. However, two observations prompted us to revisit this hypothesis. Firstly, such a solvatochromic effect would necessarily be accompanied by a decrease of the excited state dipole moment compared with the ground state dipole moment,²¹ which is not the case here. Secondly, varying the solvent induces drastic changes in the shape of the spectra. It seems that, far from being a pure solvatochromic effect, the observed phenomenon is mainly due to the involvement of different fluorescent species.

Only one species was detected in toluene, since varying the excitation wavelength or the temperature led to no change in the emission spectra. This species emitted at high wavelengths ($\lambda_{em} = 477 \text{ nm}$, with a shoulder at 500 nm) with a quantum yield close to 10^{-2} . When the polarity of the solvent was increased, a second species appeared, with an emission maximum at 437 nm and with a lower quantum yield.

The following hypothesis may be proposed to explain the occurrence of different species. Four different kinds of enol can be considered: the *trans*-enol form, two *cis*-enols and the open enol form. The presence of a *trans*-enol form is controversial¹¹ in the case of β -diketones, and would be more conceivable in the particular case of β -ketoaldehydes. In the same way, an open enol form, where no hydrogen bonding takes place, would be very unstable. On the contrary, the *cis*-enol form is generally stable due to the presence of the intramolecular hydrogen

bond, and it is fully possible that the two *cis*-enol forms **E1** and **E2** coexist in solution. According to the MNDO calculations, the enol form **E2** is planar. Its electronic system should then be more delocalized than that of twisted enol **E1**. This could make the absorption and emission spectra of enol **E2** shift towards the red. Moreover, this form should be stabilized by staking with a solvent such as toluene. On the other hand, the enol form **E1** (supposed to absorb and emit at shorter wavelength) is more polar and so should have more affinity for polar media. This hypothesis can be supported by NMR spectral data. When passing from toluene to acetonitrile, the order of the signals corresponding to the two methyl groups was flipped over. In toluene, the signal relative to the terminal methyl group was relatively unshielded, which may be attributed to the proximity of a ketone group (**E2** form). In polar solvents, the same signal was shifted to high field (with respect to the signal pertaining to the heterocyclic methyl group), which could indicate that the ketone was replaced by an enol group (**E1** form). In conclusion, the **E2** form should preferentially be encountered in toluene and the **E1** form in polar solvents. In protic media the equilibrium could be displaced towards formation of the open *cis*-enol form (Scheme 3), as it is well known that the water protons destroy the intramolecular hydrogen bond in the enol form of β -dicarbonyl compounds. The low fluorescence efficiency could then be attributed either to the dissipation of the absorbed energy through the rotation of the C-C single bond, which leads to the open form, or to the fact that the open form itself is non-fluorescent.



Scheme 3.

Determining the symmetry of the two *cis*-enol forms is not easy, because of the fast proton exchange (topomerization) process, with a rate estimated to range between 10^6 s^{-1} and $2 \times 10^5 \text{ s}^{-1}$.^{22,23} This is the reason why HPLC studies failed in distinguishing between these two forms.¹¹ ^1H and ^{13}C NMR studies at long timescales have given contradictory information, either supporting the existence of a single symmetric form or a fast interconversion equilibrium. Definitive experimental evidence has been given by X-ray photoelectron spectroscopy in favour of a fast topomerization process.²⁴ Only in some particular cases could ^1H , ^{19}F , ^{13}C and ^{17}O NMR spectral studies give evidence for the preferred enol form²⁵ or allow the measurement of the equilibrium constant between the two isomers.^{22,23} The present work has shown that such a challenge could also be taken up by steady-state fluorescence. By calculating the spectrum of the two pure *cis*-enol species (which, in our case,

must be close to the spectrum obtained, respectively, in toluene and water) and assuming that this spectrum is not very sensitive to solvent effects, any spectrum of the mixture may be analyzed in order to estimate the proportion of the two species in a given environment.

Experimental

3-Acetoacetyl-7-methyl-2*H*,5*H*-pyrano(4,3-*b*)pyran-2,5-dione (C₁₃H₁₀O₆) was prepared as previously described.^{7,26} Spectroscopic grade solvents (SDS and Merck) were used as received and contained up to 0.02% water in volume. Absorbance spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer recording the wavelength every 2 nm. Fluorescence work was performed on a PTI Quanta Master 1 spectrofluorometer. All fluorescence spectra were corrected. The fluorescence quantum yields were determined using 4-diethylamino-7-nitrobenzofurazan in dichloromethane ($\Phi_F=0.038$)²⁷ as standard. NMR spectra were recorded on a Bruker spectrometer operating at 200 MHz. Calculations were performed according to the MNDO method on a 4D30 Silicon Graphics computer by using Insight Discover Biosym software (Biosym Technologies, Inc.).

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