Journal of Photochemistry and Photobiology A: Chemistry 210 (2010) 31–35

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

The photostability and fluorescence of hydroxycoumarins in aprotic solvents

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ARTICLE INFO

Article history:
Received 5 August 2009
Received in revised form
23 November 2009
Accepted 12 December 2009
Available online 21 December 2009

Keywords:
Hydroxycoumarin
Fluorescence
Photostability
Spectra
Proton transfer

ABSTRACT

The absorption and fluorescence spectra of three hydroxycoumarins have been determined in anhydrous dimethylsulfoxide, tetrahyrofuran and acetonitrile. Minor absorption and fluorescence bands were observed at slightly longer wavelengths than the principal bands in dimethylsulfoxide. These bands were enhanced in dimethylsulfoxide which has a greater proton affinity than the other solvents studied. However, the minor bands were decreased by the presence of carbon dioxide in solution. These minor bands were ascribed to the deprotonated forms of the hydroxycoumarins with the deprotonation being facilitated by Lewis bases such as dimethylsulfoxide but suppressed by a Lewis acid such as carbon dioxide because, at the low concentrations of hydroxycoumarin used in this work, the carbon dioxide competes successfully for the electron pair/proton acceptor sites of the dimethylsulfoxide solvent.

The photostability of esculetin is less than that of scopoletin under UVA irradiation in all solvents studied. Because esculetin is more acidic than scopoletin, this order of photostabilities is consistent with the initial step in the photodegradation involving deprotonation of the hydroxycoumarins.

1. Introduction

Many hydroxycoumarins, including some naturally occurring members of the family that are present in plants, are both photoreactive and fluorescent. For example, coumarins such as 7-hydroxycoumarin (umbelliferone), 7-hydroxy-6-methoxycoumarin (scopoletin) and 6,7-dihydroxycoumarin (esculetin) emit blue fluorescence when excited in the UVA and UVB spectral regions and there has been interest in exploiting this fluorescence behaviour of suitable coumarins as fluorescent whitening and brightening agents [1]. However, coumarins are susceptible to light induced reactions including photooxidation and they can also photosensitise the degradation of the host or a substrate material [2,3]. Photooxidation can occur either by the ejection of an electron to give hydrated electrons in an aqueous environment or by excited state electron or proton-transfer reactions. Superoxide ions and hydrogen peroxide have been detected following photolysis of coumarins in aerated aqueous solution or on wet substrates and these products are suggested to be formed via an initial electron transfer from an excited state of the coumarin to molecular oxygen [4]. Such reactive oxygen species also degrade coumarins [5].

The formation of transient radical cations produced by the photoionization/oxidation of several methoxycoumarins has been observed in some aqueous environments [6,7]. In other work the rates of fluorescence quenching resulting from electron transfer between electron donor or acceptor species and the excited singlet states of several coumarins have been determined and the trends in the kinetics of fluorescence quenching correlate with different redox potentials of these coumarins as predicted by the Weller equation for excited state electron transfer reactions [8]. The quenching of aminocoumarin singlet and triplet states in solution by dissolved oxygen has also been observed [9].

The steady state fluorescence spectra of 7-hydroxycoumarin, 4-hydroxycoumarin and 7-hydroxy-4-methylcoumarin in protic solvents such as alcohol or water have been reported previously [10–12] in the presence of added acid or base. In these solvent systems, significant shifts and changes in their absorption and fluorescence spectra are observed. In a basic environment the absorption and fluorescence spectra have additional bands at longer wavelengths than those associated with the neutral molecule and these are attributed to absorption by the ground state and emission from the excited singlet states of deprotonated, anionic forms of the hydroxycoumarins. However, in a number of other aromatic molecules bearing acidic phenolic hydroxyls and basic keto groups or heterocyclic nitrogen atoms, intermolecular and/or intramolecular proton transfer can occur without added base [13–16]. This behaviour is very dependant on any hydrogen bonding species in the solvent, often in only trace amounts.

In some solvents, intramolecular excited state proton transfer can also occur via the singlet state with both tautomeric forms contributing to the total fluorescence spectrum. The emission from the 'enolic' form is at longer wavelengths than that of the corresponding 'ketonic' form [14]. The fluorescence emission bands

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doi:10.1016/j.jphotochem.2009.12.009

with maxima at 485 nm for 7-hydroxy-4-methylcoumarin [10] in slightly acidified water:methanol mixed solvent and 478 nm for 7-hydroxycoumarin [11] in acidified aqueous solution have been assigned to excited state, proton-transfer tautomers. It was proposed that a concerted process of proton exchange is responsible for tautomer formation under these conditions with the simultaneous deprotonation of the phenolic hydroxyl group and protonation of the ketonic oxygen atom from the acidic solvent [11]. A proton relay system involving alcohol solvent molecules may also contribute to the formation of tautomers of the hydroxycoumarins in alcohol solutions in a similar way to that proposed earlier by Kasha et al. for 3-hydroxyflavone in a hydrocarbon solvent containing traces of water [13].

To simplify the interpretation of the fluorescence and primary photochemical reactions associated with 7-hydroxycoumarins arising from such a varied ensemble of neutral, deprotonated and proton-transfer tautomer fluorophores, the emissions of 7-hydroxycoumarin (umbelliferone), 7-hydroxy-6-methoxycoumarin (scopoletin), and 6,7-dihydroxycoumarin (esculetin), have been studied in three dry, aprotic solvents in the absence of added acid or base. The relative rates of photodegradation of these coumarins were also established in the three solvents. In these systems, hydrogen bonding between the hydroxycoumarin and neighbouring solvent molecules that can facilitate or inhibit deprotonation and/or tautomerism, is expected to be significantly reduced or negligible.

2. Methods and materials

2.1. Materials

Anhydrous DMSO, THF and acetonitrile were obtained from Sigma Aldrich and used without further purification or drying. The levels of water, specified by the manufacturer are <0.005% for DMSO and THF and 0.002% for acetonitrile. The umbelliferone was 99% purity grade supplied by Chem Service (West Chester, PA) and the scopoletin and esculetin (98% purity) were supplied by Sigma Aldrich. Their structures are shown in Fig. 1.

2.2. Methods

Solutions of the coumarins were freshly prepared in oven-dried 10 mm spectrosil fluorescence cuvettes immediately before study. The anhydrous solvents were transferred under dry nitrogen gas and the coumarin solutions were made up to an absorbance at their absorption maxima of approximately 0.8 ODU, concentrations of $\sim 6 \times 10^{-5}$ mol dm^{-3}. Solutions saturated with carbon dioxide were prepared by gently bubbling with carbon dioxide gas for 2 min.

The absorption spectra were recorded with a Cary 100 spectrophotometer and the fluorescence spectra were recorded using a PerkinElmer MPF4 spectrofluorimeter.

The photostabilities of esculetin and scopoletin in air-saturated solutions of the three solvents were measured under irradiation from two NEC FL6BL-B 6W UV fluorescent lamps with a spectral distribution shown in Fig. 2. The absolute spectral irradiance of the lamps was determined using a spectroradiometer based on a Kratos double monochromator and calibrated by the Measurement Standards Laboratory of New Zealand. The cuvette was positioned in front of the lamps so the spectrally integrated intensity of radiation incident on the cuvette was 4 mW cm^{-2}.

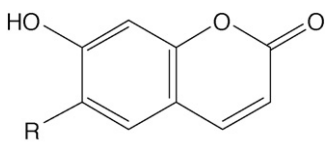

Fig. 1. Structures of the coumarins studied. R = H, 7-hydroxycoumarin (umbelliferone); R = OH, 6,7-dihydroxycoumarin (esculetin); R = OCH_3, 7-hydroxy-6-methoxycoumarin (scopoletin).

3. Results

3.1. Absorption spectra

The absorption spectra of umbelliferone, scopoletin and esculetin in anhydrous THF, acetonitrile and DMSO are shown in Fig. 3. In DMSO flushed with nitrogen gas, minor absorption bands or shoulders at wavelengths longer than the principal bands are

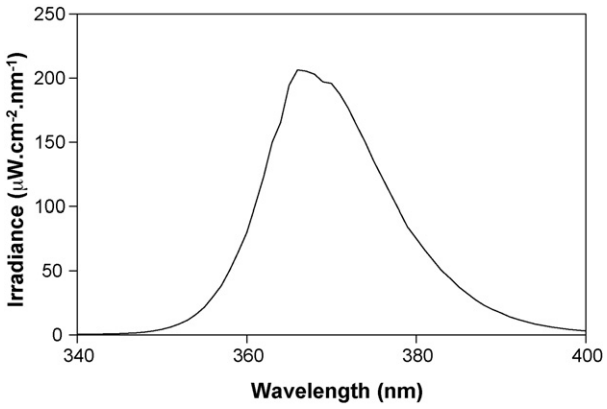

Fig. 2. Spectral irradiance distribution at the sample of the fluorescent UV lamps used for photostability studies.

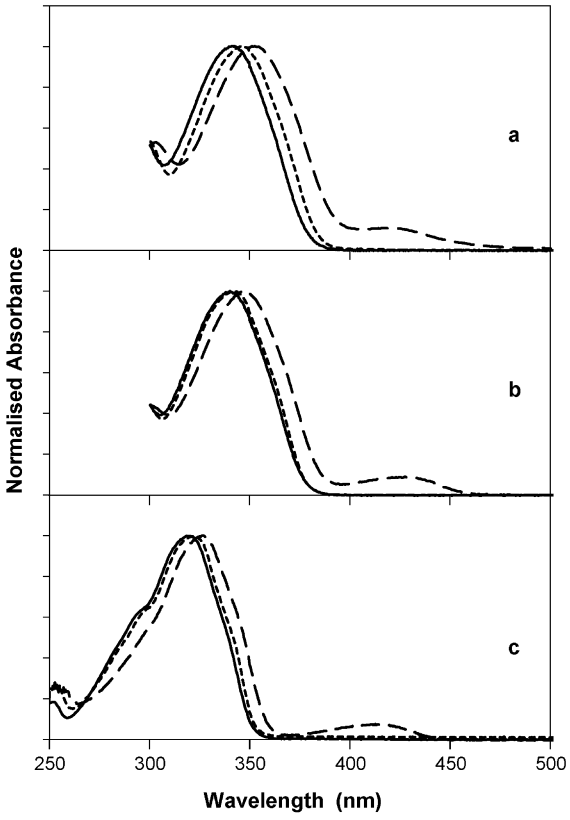

Fig. 3. Absorption spectra of (a) esculetin, (b) scopoletin and (c) umbelliferone. For comparison, spectra are normalised at the wavelength of maximum absorbance. Spectra were measured in three anhydrous solvents; acetonitrile (solid line), THF (dotted line) and DMSO (dashed line).

evident. These secondary bands have maximum absorbances at 415, 430 and 420 nm for umbelliferone, scopoletin and esculetin respectively. However, when the solutions were flushed with carbon dioxide, these subsidiary bands were absent for the two former compounds, and greatly reduced for esculetin. The effects of flushing a solution of scopoletin in DMSO with CO_2 and N_2 are shown in Fig. 4.

In acetonitrile a very weak absorption shoulder was also observed for umbelliferone. However, no absorption spectral shoulders were discernible for umbelliferone in THF or for esculetin or scopoletin dissolved in either acetonitrile or THF.

3.2. Fluorescence emission and excitation spectra

The fluorescence emission spectra of the hydroxycoumarins in anhydrous THF, acetonitrile and DMSO are shown in Fig. 5. In DMSO, excitation at the maxima of the main long wavelength absorption spectral band of all three coumarins gave fluorescence emission spectra that exhibited a 'mirror-image' relationship to the absorption spectra together with a shoulder at longer wavelengths than the main emission band (Fig. 5a, b and c). In the case of esculetin, this shoulder is largely obscured by the main band. The Stokes shift observed for esculetin fluorescence was greater than that for either scopoletin or umbelliferone.

A secondary weaker fluorescence emission band apparent at longer wavelengths was observed for all hydroxycoumarins in DMSO. When the coumarins were excited at wavelengths corresponding to the long wavelength shoulder in the absorption spectra, i.e. 415, 435 and 420 nm for umbelliferone, scopoletin and esculetin respectively, red-shifted fluorescence was observed that displayed a 'mirror-image' relationship with the subsidiary absorption shoulder band (Fig. 5d–f). Also, the fluorescence excitation

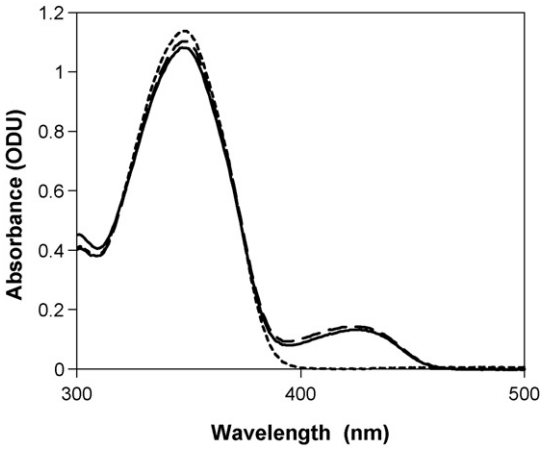

Fig. 4. Absorption spectra of scopoletin in anhydrous DMSO. The initial solution (solid line), flushed with CO_2 gas (dotted line) and then flushed with N_2 gas (dashed line).

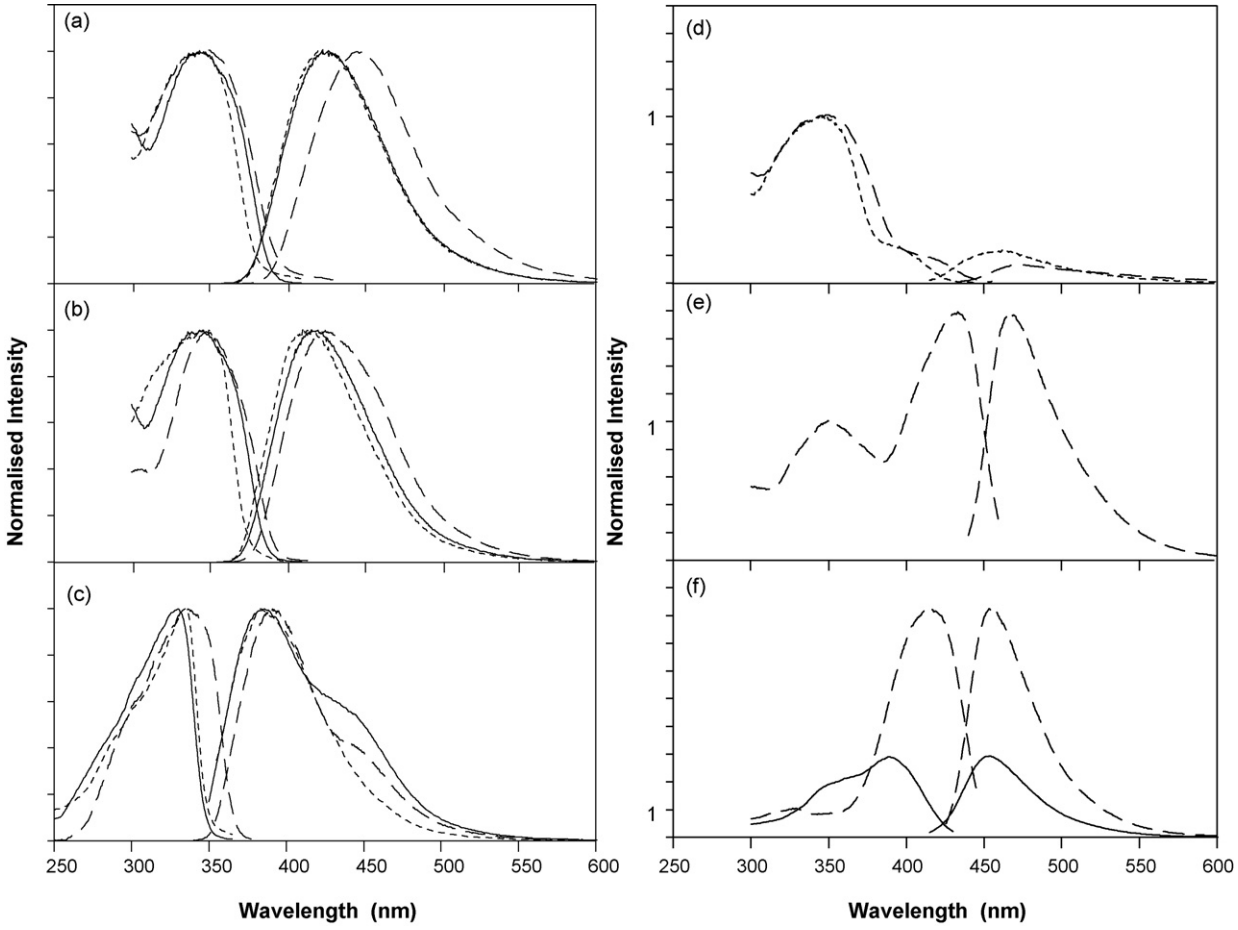

Fig. 5. Fluorescence and excitation emission spectra of esculetin (a and d), scopoletin (b and e) and umbelliferone (c and f) in three anhydrous solvents; acetonitrile (solid line), THF (dotted line) and DMSO (dashed line). Figures a, b and c show 'main' band spectra, normalised at the excitation band λ_{max}. Figures d, e and f show 'side' band spectra, again normalised at the main band excitation λ_{max}. Note the variation in *y*-axis scale of these three graphs. All excitation and detection wavelengths used for main band spectra corresponded to the maxima of the (shorter wavelength) 'main' bands, and excitation and detection wavelengths for 'side' band spectra corresponded to the maxima of the (longer wavelength) 'side' bands.

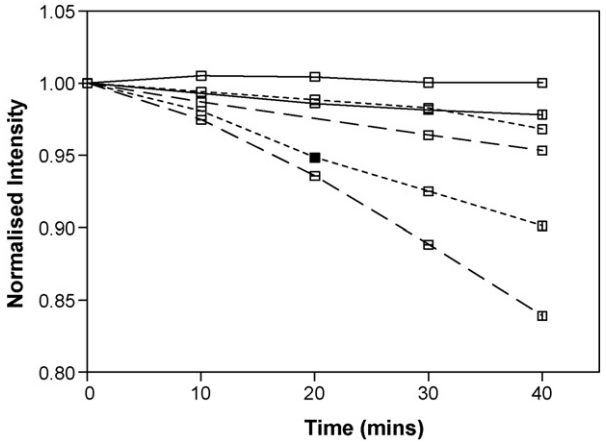

Fig. 6. Decreases in absorbance with increasing UVA radiation doses (irradiation time) for scopoletin (hollow symbols) and esculetin (solid symbols) in acetonitrile (solid line), THF (dotted line) and DMSO (dashed line).

spectra for the longer wavelength emissions are good matches to the absorption spectra of the species responsible for the shoulder bands.

In anhydrous acetonitrile, the fluorescence emission spectrum of umbelliferone excited at the maximum of the main absorption band at 330 nm exhibits a distinct shoulder with a maximum at ~450 nm. When this coumarin solution is excited at 390 nm, in the shoulder absorption band, the emission is dominated by the band with a maximum fluorescence at 455 nm as shown in Fig. 5f. However, no longer-wavelength fluorescence could be detected for esculetin and scopoletin in acetonitrile.

Although no longer-wavelength shoulders were apparent in the absorption spectra of esculetin, scopoletin or umbelliferone in THF, excitation at 400 nm, on the extreme long-wavelength edge of the absorption spectra of esculetin in THF did produce weak fluorescence with a maximum at 460 nm (Fig. 5d).

3.3. The photostabilities of scopoletin and esculetin

Scopoletin and esculetin were degraded in air-saturated solutions in THF, DMSO and acetonitrile by exposure to UVA radiation from 350 to 400 nm that covered their long wavelength absorption bands. The initial rates of degradation, expressed as the decays of absorbance normalised by the intensities of ultraviolet radiation absorbed by the solutions, are shown in Fig. 6. It is apparent that these initial rates of decay, i.e. relative quantum yields of degradation, depend on both the solvent and the structure of the hydroxycoumarin. In all solvents studied, esculetin degrades more rapidly than scopoletin. Both hydroxycoumarins follow the same trend in stability with solvent, with acetonitrile > THF > DMSO. Because umbelliferone does not absorb significantly in the same spectral range as the other two coumarins studied and the UVA radiation emitted by the source used, the photostability of umbelliferone was not determined.

4. Discussion

4.1. Absorption spectra

The minor absorption bands observed on the long wavelength side of the principal band in DMSO are attributed to the deprotonated forms of the hydroxycoumarins. This assignment is consistent with the previously reported absorption bands in the same position relative to the protonated forms of 7-hydroxycoumarin, 4-hydroxycoumarin and 7-hydroxy-4-methylcoumarin in alcohol or water that are enhanced by the addition of base [10–12]. It also explains the appearance of the absorptions in nitrogen flushed solutions and their absence in DMSO flushed with carbon dioxide. It is proposed that DMSO, being a relatively strong Lewis base [17], accepts protons from the hydroxycoumarins to produce the deprotonated forms. NMR, IR and Raman spectroscopic studies and carbon dioxide gas solubilities in various solvents [18–21] indicate carbon dioxide can undergo Lewis acid (electron pair acceptor) – Lewis base interactions with some electron pair donor functional groups such as carbonyl and sulfoxide. Thus, at the concentrations of hydroxycoumarins used in this work, the carbon dioxide (Lewis acid) – DMSO (Lewis base) interaction competes successfully with the phenolic coumarin – DMSO interaction in solutions saturated with carbon dioxide. As a consequence, no solvent-facilitated hydroxycoumarin deprotonation occurs in such solutions.

No minor absorption bands were observed in nitrogen flushed, i.e. in the absence of carbon dioxide, THF or acetonitrile. This behaviour is consistent with the formation of the deprotonated hydroxycoumarin only in DMSO because THF and acetonitrile are weaker Lewis bases than DMSO [17] and therefore do not accept a phenolic proton from the ground states of the hydroxycoumarins.

4.2. Fluorescence spectra

Like the absorption spectra, the fluorescent emission spectra observed from the hydroxycoumarins in the anhydrous solvents can be explained in terms of protonated and deprotonated forms, in this case, their emission from excited singlet states. For all the hydroxycoumarins studied, the strongest fluorescence originates from the protonated forms when they are excited at wavelengths corresponding to the maximum of the main absorption bands. The larger Stokes shift observed for esculetin in DMSO compared with the other two solvents, Fig. 5a, is consistent with the greater polarizability of DMSO which therefore elicits a greater non-specific coulombic interaction and stabilization of the lowest excited $\pi\pi^*$ singlet state than is the case for the other solvents. (Solvent polarizabilites, expressed in terms of dielectric constants, are DMSO $\varepsilon = 46.6$, acetonitrile $\varepsilon = 37.5$, THF $\varepsilon = 7.5$ [22].) Further, the Stokes shift for esculetin is greater than for umbelliferone and for scopoletin in DMSO indicating that the polarity/polarizability of the lowest excited singlet state of esculetin is greater than those of the other two hydroxycoumarins (Fig. 5b and c).

When excited at their absorption maxima, weaker emission bands were observed at longer wavelengths for all the hydroxycoumarins studied in DMSO. However, in the case of esculetin, the band is very weak and it is largely obscured by the tail of the main fluorescence band. This longer wavelength emission band is attributable to the deprotonated forms of the hydroxycoumarins. In THF, only esculetin displays the longer wavelength fluorescence which is only visible by excitation at the long wavelength edge of the absorption spectrum where the deprotonated forms of ground states are expected to absorb. In acetonitrile, no secondary, longer wavelength fluorescence could be observed from scopoletin or esculetin. These results are in accord with the relative proton accepting powers (basicities) of DMSO (6.6 kcal mol^{-1}) > THF (5.6 kcal mol^{-1}) > acetonitrile (4.2 kcal mol^{-1}) expressed as the enthalpies of formation of a complex with p-fluorophenol [17]. Hence, a greater concentration of the deprotonated forms of the hydroxycoumarins, and hence higher intensity fluorescence is expected, and observed, in DMSO.

4.3. Photostabilities of scopoletin and esculetin

There have been a number of reported examples of photoionizations and excited state proton/electron transfer reactions of

coumarins and phenolics in solvating molecular environments such as water and micelles with polar head groups [6,7,23–27]. In these systems that favour the stabilization of ionic species, including hydrated electrons, transient radical ions of several methoxycoumarins as well as some easily oxidized phenolics have been observed by flash photolysis [6,7,23–27]. However, in the presence of water, basic entities or electron acceptors, the radical ions undergo facile proton transfer or electron transfer to form phenoxyl radicals [23,24]. In the case of the phenolics, the rate of deprotonation correlates with the acidity of the phenol [24]. Similar behaviour has been reported in the presence of proton acceptors where the phenoxyl radical is produced directly [25]. Self-sensitized photooxidation by reactions of the excited singlet or triplet states of some coumarins with oxygen in air-saturated solution have been reported [8,9]. Evidence has been presented indicating these reactions are mediated by singlet oxygen and/or the superoxide free radical [4,8].

The relative photostabilities of scopoletin and esculetin in DMSO, THF and acetonitrile presented in Fig. 6 are consistent with the primary event in the decomposition of the hydroxycoumarins being the ionization and deprotonation of the 7-hydroxyl substituent group. The ground-state pK_a values calculated for esculetin and scopoletin associated with their 7-hydroxyl groups are 6.9 and 7.6 respectively [28]. Because the excited singlet state energies of these compounds are similar, the ground-state pK_a values are expected to reflect the relative proton donating capacities of their respective excited states. Further, both compounds degraded more rapidly in DMSO than in THF or acetonitrile which is consistent with DMSO being the best Lewis base or proton accepting solvent [17] if the oxidative degradation is caused by intramolecular excited state proton transfer to the solvent. In contrast, both compounds displayed very little degradation resulting from 4 mW cm^{-2} doses of UVA radiation in acetonitrile which is the solvent with the lowest proton accepting power [17].

5. Conclusions

7-Hydroxycoumarin (umbelliferone), 6,7-dihydroxycoumarin (esculetin) and 7-hydroxy-6-methoxycoumarin (scopoletin) in dry DMSO are subject to excited state deprotonation as a result of intramolecular proton transfer to the solvent molecules which are an effective Lewis base. These coumarins are also photodegraded in all solvents studied in this work and the quantum yields of degradation are correlated with the basicities of the solvent and the acidities of the hydroxycoumarin indicating that photodegradation proceeds through the deprotonated hydroxycoumarins.

Acknowledgements

This work was carried out under contract VICX0803 from the New Zealand Foundation for Research, Science and Technology.

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