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The dissipation of excitation energy in methoxyflavones by internal conversion

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Abstract

Flavonoids are found in all higher plants, and many display strong absorption in the UVB spectral region. It has been suggested that they play a role in the protection of plants by screening vital cellular components from damaging UV radiation. Some flavonoids can effectively dissipate the absorbed energy by intramolecular proton transfer. For example, the 3-hydroxyflavones undergo excited state intramolecular proton transfer from the 3-hydroxyl group to the neighbouring keto group. However, evidence of another mechanism of energy dissipation, involving enhanced non-radiative internal conversion, is presented in this paper. The fluorescence of monomethoxyflavones, which do not have hydroxyl groups adjacent to the keto group, and therefore are not subject to intramolecular excited state proton transfer, was examined. The fluorescence spectra and yields of 5-, 7- and 8-monomethoxyflavones in 3-methylpentane solvent exhibited considerable changes over a range of temperature from 200 to 77 K. The yield decreased with increasing temperature for all three flavones, and the fluorescence spectral maximum of 5-methoxyflavone shifted from 440 to 418 nm. This is interpreted as resulting from thermally induced vibronic coupling and concomitant splitting of the two lowest excited singlet states of the flavones. An increase in internal conversion relative to intersystem crossing will result, thereby reducing both the triplet state population and flavone participation in damaging photochemical reactions.

Keywords: Flavonoids; Fluorescence; Plants; Proximity effect; UV protection

1. Introduction

The flavonoids are a group of natural pigments found in all land-based green plants. They are biosynthesized under enzymatic control from the amino acid phenylalanine, via the cinnamic acids, and malonic acid [1,2]. It is notable that this synthesis is activated by UV radiation, with higher levels of flavonoids being found in many plants which have been exposed to elevated levels of UV radiation [3].

Flavonoids exhibit strong absorption in the UV spectral region. The two principal absorption bands are designated as band I (380–300 nm) and band II (285–240 nm) [4]. Many flavonoids are effective quenchers of free radicals [5]. These characteristics represent the basis for the putative role of flavonoids in the screening and protection of UV-sensitive molecules in plant tissue. To be an effective screen of UV radiation, a molecule must exhibit high UV absorption and must have the facility to dissipate this energy in a harmless fashion without participating in damaging photochemical reactions. For example, the cinnamates (used in cosmetic sunscreens) accomplish energy dissipation by trans–cis photoisomerization [6]. Many flavones have a hydroxyl group at the C-3 position. Kasha and coworkers [7,8] have shown that these flavonols can undergo proton transfer (tautomerism) in their excited singlet states from the 3-hydroxyl group to the adjacent ketone group. The principal experimental evidence for this behaviour is the shift in the fluorescence spectra and the changes in the singlet state decay exhibited by a number of flavonoids (or model flavonoid compounds) in various solvents. Energy dissipation results from the lower excited state energy associated with the tautomer.

Another possible mechanism by which the dissipation of the excess energy of excited states can occur is through a phenomenon known as the pseudo-Jahn–Teller or proximity effect [9,10]. When two excited electronic states are nearly degenerate and have the correct symmetry, they can undergo thermally induced vibronic coupling with a resultant splitting of the states. In such molecules, the proximity effect is evident as a red shift in the fluorescence as the temperature is increased, and this is accompanied by a substantial decrease in the fluorescence yield resulting from increased internal conversion. Examples of this behaviour have been reported in molecules with energetically similar $\pi\pi^*$ and $n\pi^*$ states,

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such as the psoralens (molecules which are related to the flavonoids) and the indoles [10,11].

The absorption spectra of some flavonoids show signs of two lower excited state levels in close energetic proximity [12]. However, to obtain unambiguous evidence for the proximity effect in flavonoids, molecules must be selected in which no excited state intramolecular proton transfer is possible. In addition, they must be photostable and soluble in non-polar solvents in order to avoid possible confounding effects of polar solvent–solute coulombic interactions. In this paper, 5-, 7- and 8-monomethoxyflavones were used to investigate this issue.

2. Experimental details

The three flavonoids selected for study were 5-methoxyflavone, 7-methoxyflavone and 8-methoxyflavone. Pure, synthetic samples of these compounds were generously supplied by Professor H. Geiger, formerly of the Department of Chemistry, University of Stuttgart. Purum grade 3-methylpentane, ex Fluka, was purified by passage down an activated silica gel column to remove unsaturated hydrocarbons and water. Solutions of the methoxyflavones (1×10^{-5} mol dm^{-3}) were prepared under low intensity light and degassed by a series of freeze–pump–thaw cycles.

The absorption spectra of solutions of the compounds in 3-methylpentane at room temperature were determined using a Hewlett Packard 8451 A diode array spectrophotometer.

The luminescence and fluorescence excitation spectra were recorded using a Hitachi 3010 spectrofluorometer over a range of temperature from 77 to 300 K. The excitation wavelength used for the emission spectra was 295 nm, and emission was monitored at 410 nm for the excitation spectra. Temperature control was achieved using an Oxford Instruments DN1704 cryostat fitted with Suprasil fused silica windows. The wavelength calibration of the spectrophotometer and spectrofluorometer was performed as recommended by the manufacturers. The relative fluorescence yields were determined by measurement of the area under the fluorescence emission spectra.

3. Results

In the non-polar, hydrocarbon solvent used in this work, no fluorescence from any of the methoxyflavones studied can be observed at room temperature, which is in agreement with a previous report [12]. However, in all cases, the fluorescence yield increases rapidly with decreasing temperature at temperatures below about 200 K. The fluorescence excitation spectra at temperatures below 200 K are red shifted relative to the room temperature absorption spectra, with band I shifted by as much as 30 nm, as shown in Fig. 1 for 7-methoxyflavone. Band II is shifted to a smaller extent in all three compounds studied. The emission spectra display a lesser red shift with respect to the excitation spectra in all cases (see Fig. 1). The emission maxima for 7-methoxyflavone and 8-methoxyflavone are at 420 nm and 410 nm respectively. Although no spectral shift is apparent over the temperature range from 200 to 77 K for these flavones, the position of the fluorescence maximum for 5-methoxyflavone moves from 440 to 418 nm as the temperature is reduced from 200 to 77 K. The temperature dependence of the fluorescence yield and emission spectral maximum of 5-methoxyflavone is shown in Fig. 2 and Fig. 3. Phosphorescence at wavelengths longer than about 450 nm is also evident in the emission spectra of the three flavones studied at low temperature.

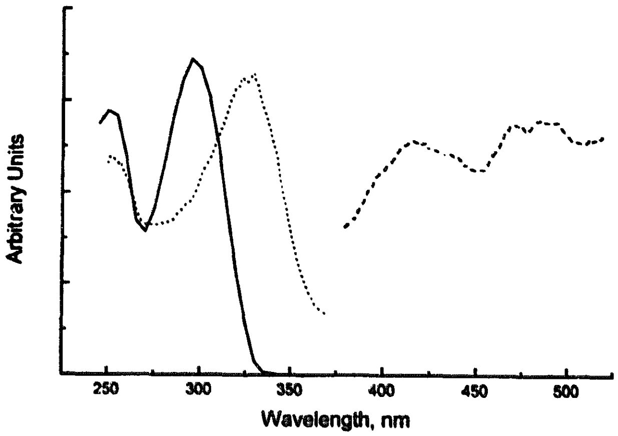

Fig. 1. Absorption spectrum of 7-methoxyflavone at room temperature (———). Fluorescence excitation (···) and emission (– – –) spectra of a 1×10^{-5} mol dm^{-3} solution of 7-methoxyflavone in 3-methylpentane at 170 K.

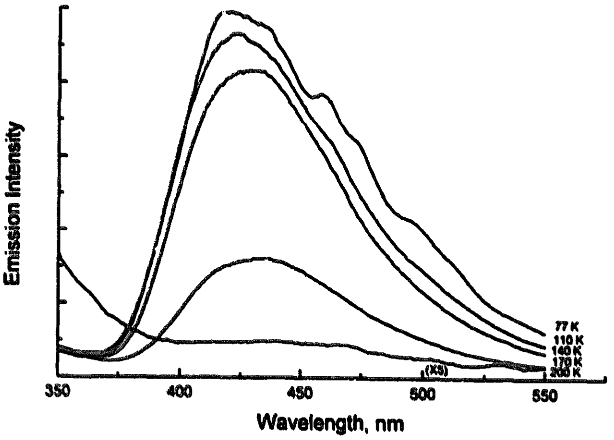

Fig. 2. Emission spectra of 5-methoxyflavone at 77, 110, 140, 170 and 200 K.

4. Discussion

4.1. Previous work on the relaxation of flavone singlet states

Mechanisms for the relaxation of the excited singlet states of a number of hydroxyflavones have been reported previ-

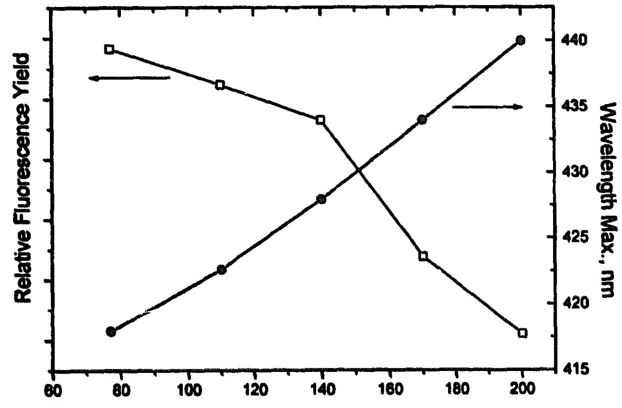

Fig. 3. Temperature dependence of the relative fluorescence yield (□) and fluorescence spectral maximum (•) of 5-methoxyflavone.

ously, in which the hydroxyl group plays a significant role and impacts on the fluorescence spectra and yield. In 3-hydroxyflavone, the spectral shifts were attributed to contributions to the observed emission from two tautomers of the flavone, which were formed as a result of temperature-dependent excited state intramolecular proton transfer between the hydroxyl group and the neighbouring carbonyl group [7,8]. A temperature-dependent fluorescence spectral shift has also been observed for 7-hydroxyflavone in a polar solvent, and was explained in terms of the ionization of the 7-hydroxyl group and solvation of the dipolar excited singlet state, which occurs when the solvent molecules are able to undergo rotational relaxation as the temperature is increased [13,14]. However, in the present study, none of the selected flavones possesses hydroxyl substituent groups, and therefore another mechanism must be responsible for the observed temperature-dependent fluorescence of these compounds. Anomalous fluorescence behaviour has also been reported for 4′-*N*,*N*-dimethylamino derivatives of 3-methoxyflavone and 3-hydroxyflavone in polar solvents, which was attributed, in part, to the formation of a twisted charge transfer state [15].

Molecular aggregation can result in changes in the fluorescence yield and in the position of the fluorescence spectra [16] as the temperature (and solubility) is reduced. However, aggregation results in a red shift in the fluorescence emission spectral position [16], whereas a blue shift in the fluorescence spectrum of 5-methoxyflavone is observed with decreasing temperature. Furthermore, the fluorescence spectra of concentrated solutions of methoxyflavones (i.e. above 1×10^{-4} mol dm^{-3}) at low temperature are characteristic of molecular aggregates with a sharp, well-resolved structure and are very different from those reported in this work for dilute (1×10^{-5} mol dm^{-3}) solutions. It is therefore concluded that molecular aggregation does not contribute to the fluorescence from dilute solutions of methoxyflavones.

Another possible mechanism for excited state relaxation/energy dissipation which involves vibronic coupling of nearly degenerate states is the proximity effect.

4.2. *The proximity effect in model flavones*

The lowest energy absorption band (band I) in a number of flavones is believed to be associated with a state resulting from the mixing of zero-order nπ^* and $\pi\pi^*$ states [12]. In molecules possessing lowest excited singlet states which are nearly degenerate, or become degenerate as a result of perturbation by a polar solvent–solute interaction, thermally activated vibronic mixing via out-of-plane bending modes, i.e. the proximity effect, can remove this degeneracy, e.g. 8-methoxypsoralen and a number of indoles [10,11]. As a result of this mixing, the lower perturbed states lie at energies below that of the lowest zero-order excited state. Provided that the perturbed states are fluorescent, the observed consequence of this phenomenon is a red shift in the fluorescence emission spectrum, accompanied by a decrease in the fluorescence yield, with increasing temperature, as the thermally activated vibronic coupling of the zero-order excited states takes place [9,10,17]. The decrease in the fluorescence yield is a result of the increased non-radiative internal conversion as the S_1^*–S_0 energy gap is reduced through vibronic coupling of the lowest zero-order excited singlet states [10,16]. The red shift in the fluorescence emission spectrum and the concomitant reduction in the fluorescence yield with increasing temperature, observed with 5-methoxyflavone, are therefore consistent with increased thermally induced vibronic coupling of degenerate excited singlet states. No shifts in the fluorescence emission spectra were observed for 7- and 8-methoxyflavones over the temperature range from 77 to 200 K, although there were substantial falls in the fluorescence yields. This suggests that, from a temperature below 77 K, the thermally induced coupling forms non-fluorescent vibronic excited states in a number of molecules, which have energies lower than those of the ensemble of fluorescent molecules. As the temperature is increased, the partitioning between fluorescent and non-fluorescent molecules changes, with an increase in the population of molecules present in the non-fluorescent excited states and a consequent fall in the fluorescence yield.

4.2.1. *Temperature dependence of absorption/excitation spectra*

Band I in the room temperature absorption spectra occurs at a wavelength about 20–30 nm shorter than in the low temperature fluorescence excitation spectra. These observations can be explained in terms of the vibronic coupling model by reference to an energy level diagram (Fig. 4). Absorption band I is dominated by a transition to a state of primarily $\pi\pi^*$ character, which is blue shifted as a result of vibronic coupling with a lower nπ^* state. However, the nπ^* state makes no observable contribution to the absorption (or excitation) spectrum because of its very low oscillator strength. Because the $\pi\pi^*$ state is subject to less thermally activated coupling to the nπ^* state at lower temperatures, it experiences a smaller shift to higher energies than at higher temperatures. It therefore appears at longer wavelengths in the low temperature

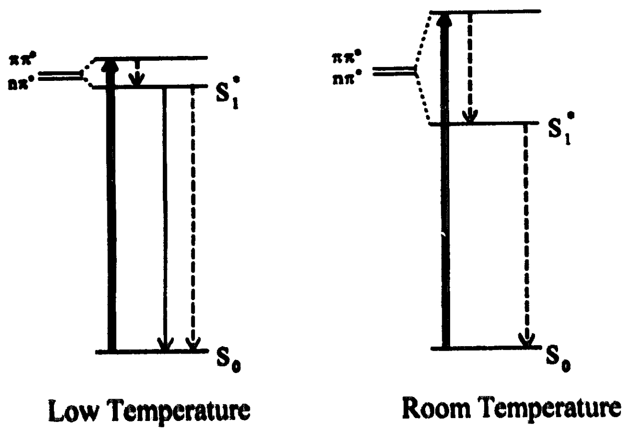

Fig. 4. Energy diagram depicting the thermally induced splitting of nearly degenerate lowest singlet states which occurs at low temperatures (less than 200 K) and at room temperature. The vertical lines represent transitions between the ground state S_0 and the perturbed excited singlet states. The full lines represent radiative transitions, i.e. absorption and fluorescence. The broken lines represent non-radiative transitions, i.e. internal conversion.

excitation spectra than in the room temperature absorption spectra.

In supersonic jet experiments with molecules having two nearly isoenergetic excited states, there are lines missing in the fluorescence excitation spectrum associated with transitions to higher vibrational levels in the excited state [18]. This is interpreted as indicating that these higher vibrational levels are effective in coupling the zero-order excited states, and are therefore subject to efficient non-radiative internal conversion and low fluorescence yield. This phenomenon could also account for at least a small part of the red shift apparent in the fluorescence excitation spectra with respect to the room temperature absorption spectra observed with the monomethoxyflavones, i.e. a reduction in the fluorescence yield originating from the excitation of higher vibrational levels.

4.2.2. Effect of solvent polarity

The previously reported [12] effect of solvent polarity on the room temperature fluorescence yield of the monomethoxyflavones studied in this work can also be rationalized in terms of the proximity effect. Because the dipole moments of ππ* states are greater than those of nπ* states, polar solvents have a greater stabilizing effect on ππ* states than nπ* states. The magnitude of the proximity effect is inversely proportional to the energy gap between the zero-order states. If the ππ* and nπ* states are almost degenerate in the isolated, unperturbed molecule, when it is dissolved in a polar solvent the ππ*–nπ* energy gap will be increased, thereby reducing the proximity effect and its associated impact on non-radiative internal conversion. Therefore it is expected that the fluorescence yield will be greater in polar solvents.

4.2.3. Triplet state yield and photoreactivity

Triplet states are relatively reactive species, and the thermally induced vibronic coupling of the excited singlet states of the methoxyflavones (shown in this work) is expected to reduce intersystem crossing to the triplet state. This is because the energy separation between the lowest excited singlet state S_1^* and the ground state is much greater than that between the lowest singlet and triplet states; the energy gap rule predicts that a lowering of the energy of S_1^* will have a greater effect on non-radiative relaxation (internal conversion) to the ground state than intersystem crossing [17]. As a result of a low triplet state population, the photochemical stability of these molecules will be correspondingly enhanced and participation in potentially damaging reactions will be reduced.

4.3. Relevance of the proximity effect in plant flavones

The mechanism of energy dissipation proposed here for a range of synthetic methoxyflavones, which do not occur naturally, may also operate with plant-based flavonoids. Related flavonoids of low polarity occur naturally, being commonly encountered on leaf surfaces, in resins, waxes and farinose exudates and occasionally on the surfaces of other plant parts such as flowers and fruits [19]. The key structural feature required to favour the involvement of this mechanism is the lack of unsubstituted phenolic hydroxyl groups, especially at the 3-, 5- and 7-positions on the flavonoid nucleus. Surface flavonoids of this type are not uncommon [19] and, because of their location in the plant, would be exposed to high levels of UV radiation. It is thus probable that the proximity effect will be a significant mechanism involved in the dissipation of absorbed energy in such naturally occurring flavonoids.

Acknowledgements

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References

[1] J. Braun and M. Tevini, Regulation of UV-protective pigment synthesis in epidermal layer of rye seedlings, *Photochem. Photobiol.*, *57* (1993) 318–323.

[2] W. Heller and G. Forkmann, Biosynthesis of flavonoids, in J.B. Harborne (ed.), *The Flavonoids—Advances in Research Since 1986*, Chapman and Hall, London, 1994, pp. 499–535.

[3] M. Tevini and A.H. Teramura, UVB effects on terrestrial plants, *Photochem. Photobiol.*, *50* (1989) 479–487.

[4] K.R. Markham, *Techniques of Flavonoid Identification*, Academic Press, London, 1982, pp. 36–51.

[5] W. Bors, C. Michel and M. Saran, Flavonoid antioxidants: rate constants for reaction with oxygen radicals, *Methods Enzymol.*, *234* (1994) 420–429.

[6] J. Saltiel, J. D'Agostina, E.D. Megarity, L. Metts, K. Neuberger, M. Wrighton and O.C. Zafiriou, The cis–trans photoisomerization of olefins, *Org. Photochem.*, *3* (1973) 1–113.

[7] P.K. Sengupta and M. Kasha, Excited-state proton transfer spectroscopy of 3-hydroxyflavone and quercetin, *Chem. Phys. Lett.*, *68* (1979) 382–385.

[8] D. McMorrow and M. Kasha, Intramolecular excited-state proton transfer in 3-hydroxyflavone. Hydrogen-bonding solvent perturbation, *J. Phys. Chem.*, *88* (1984) 2235–2243.

[9] E.C. Lim, Proximity effect in molecular photophysics: dynamical consequences of pseudo Jahn–Teller interaction, *J. Phys. Chem.*, *90* (1986) 6770–6777.

[10] T. Lai, B.T. Lim and E.C. Lim, Photophysical properties of biologically important molecules related to proximity effects: psoralens, *J. Am. Chem. Soc.*, *104* (1982) 7631–7635.

[11] G.J. Smith and W.H. Melhuish, Effect of temperature and viscosity on the excited singlet state of indoles in polar solvents, *J. Phys. Chem.*, *95* (1991) 4288–4291.

[12] O.S. Wolfbeis, M. Leiner, P. Hochmuth and H. Geiger, Absorption and fluorescence spectra, p*K* values, and fluorescence lifetimes of monohydroxyflavones and monomethoxyflavones, *Ber. Bunsenges. Phys. Chem.*, *88* (1984) 759–767.

[13] M. Sarkar and P.K. Sengupta, Luminescence behaviour of 7-hydroxyflavone: temperature-dependent effects, *J. Photochem. Photobiol. A: Chem.*, *48* (1989) 175–183.

[14] R. Schipfer, O.S. Wolfbeis and A. Knierzinger, pH-dependent fluorescence spectroscopy. Flavone, 7-hydroxyflavone and 7-methoxyflavone, *J. Chem. Soc., Perkin Trans. II* (1981) 1443–1448.

[15] S.M. Ormson, R.G. Brown, F. Vollmer and W. Rettig, Switching between charge- and proton-transfer emission in the excited state of a substituted 3-hydroxyflavone, *J. Photochem. Photobiol. A: Chem.*, *81* (1994) 65–72.

[16] M. Kasha, H.R. Rawls and M.A. El-Bayoumi, The exciton model in molecular spectroscopy, *Pure Appl. Chem.*, *11* (1965) 371–392.

[17] T. Lai and E.C. Lim, Temperature effects on $S_1 \rightarrow S_0/S_1 \rightarrow T_1$ branching ratio in radiationless transitions of N-heterocyclic compounds, *Chem. Phys. Lett.*, *62* (1979) 507–510.

[18] Y. Nibu, H. Abe, N. Mikami and M. Ito, Electronic spectra of hydrogen-bonded indoles in a supersonic free jet, *J. Phys. Chem.*, *87* (1983) 3898–3901.

[19] E. Wollenweber, Flavones and flavonols, in J.B. Harborne (ed.), *The Flavonoids—Advances in Research Since 1986*, Chapman and Hall, London, 1994, pp. 259–335.