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Journal of Photochemistry and Photobiology A: Chemistry 136 (2000) 87–91

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.nl/locate/jphotochem

The photostabilities of naturally occurring 5-hydroxyflavones, flavonols, their glycosides and their aluminium complexes

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Received 31 May 2000; accepted 7 June 2000

Abstract

The photostabilities of luteolin, in solution, in the presence of aluminium ions, and deposited on a cellulosic substrate have been determined and compared with those of quercetin and other 5-hydroxyflavonols and their 3-O-glycosides.

In aqueous methanol solution, luteolin and flavonol 3-glycosides exhibited no degradation over periods of up to 15 h of UV irradiation. However, the flavonols studied were all found to degrade and their relative photostabilities correlate with their redox potentials. Quercetin was the least stable. In the presence of aluminium ions, all the flavonoids, including luteolin, were degraded by UV irradiation.

In contrast to the absorption spectra in dilute solution, the reflectance spectra of both quercetin and luteolin deposited on a cellulosic substrate exhibited strong absorptions beyond 400 nm. On this substrate these flavonoids displayed the characteristic yellow colour associated with flavonoids in some environments. Although the quercetin yellow faded rapidly on exposure to UV radiation, the colour of luteolin darkened. This was due to the formation of a photoproduct absorbing maximally at 450 nm.

The relevance of these observations to cellulosic dyeing and flower colouration are discussed.

Keywords: 5-Hydroxyflavones; Flavonols; Luteolin; Quercetin

1. Introduction

The flavonoids are members of a class of polyphenolics, with structures shown in Fig. 1, that are found in virtually all terrestrial plants. In dilute aqueous or alcoholic solutions, the most common flavonoids, the flavones and flavonols, have a long wavelength absorption band, designated ‘Band I’, with a maximum between 325 nm and 370 nm [1]. However, in a number of other molecular environments such as those encountered in plants, e.g. in flowers [2] and cell walls [3], this absorption band can be shifted to longer wavelengths and thus, in these situations, the flavonoids may exhibit a yellow colour. The reasons for this absorption spectral shift are not clear at present but it may be related to molecular aggregation at high concentrations, and/or complex formation with metal ions.

The yellow colour of some of these plant-derived flavonoids has been exploited by mankind, who have used such pigments as dyestuffs since antiquity. The most widely used plant in early days was weld (*Reseda luteola*) containing luteolin and its 7-glucoside. Saw-wort (*Serratula tinctoria*) was also used, which in addition to luteolin, contains 3-methylquercetin [4]. Plants containing the more common plant flavonoids, quercetin and kaempferol, were not favoured as dyes as they are subject to comparatively rapid photobleaching, i.e. fading on exposure to light. To obtain a wash-fast finish in dyeing keratin fibres, the flavonoids were usually mordanted with aluminium ions [5] which chelate either with the hydroxy-keto functionality formed between 3-hydroxyl or 5-hydroxyl and 4-carbonyl groups or where they exist, the catecholic di-hydroxyl grouping [6]. The resultant complexes have absorption bands shifted to longer wavelengths than those of the uncomplexed flavonoid. These spectral shifts are characteristic of the nature of the complex and indeed tests for the hydroxyl substitution patterns in flavonoids, have been developed, based on this behaviour [7].

It has been proposed that plant flavonoids serve a protective, UV-screening function in plants [8]. To be effective in this role, it is necessary for a flavonoid to have both a

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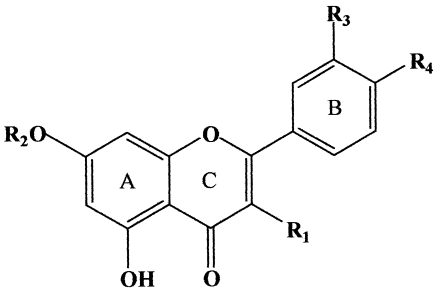
PII: S1010-6030(00)00320-8



Flavonoid	R_1	R_2	R_3	R_4
Luteolin	H	H	OH	OH
Luteolin-7-O-glucoside	H	glc	OH	OH
Kaempferol-3-O-sophoroside	O-soph	H	H	OH
Quercetin-3-O-galactoside	O-gal	H	OH	OH
Galangin	OH	H	H	H
Quercetin-4′-O-glucoside	OH	H	OH	O-glc
Kaempferol	OH	H	H	OH
Quercetin	OH	H	OH	OH

Fig. 1. The structure of the flavonoids studied in this work.

high extinction coefficient in the UVB spectral region and a degree of photostability. We have previously reported that in aqueous solution, quercetin and kaempferol 7-glucosides are more photostable at high concentrations than at low concentrations. This higher photostability was attributed to greater non-degradative dissipation of absorbed excitation energy by intermolecular excited state proton transfer [9].

The purpose of the work described in this report is to study the relative photostabilities of luteolin, quercetin, kaempferol and some other flavonols, both in solution and on a cellulosic substrate. Such flavonoid systems may serve as useful models for rationalising the photostability of the yellow colouration associated with some flavonoids, both in flowers and when used as dyestuffs.

2. Experimental details

The flavonoids used were isolated from natural sources by one of the authors (KRM) and were purified by column chromatography and recrystallisation. Solutions of the flavonoids were prepared in 1:1 mixtures of methanol-distilled water at concentrations of $\sim 1\times10^{-4}$ mol dm^{-3} to give absorbances of 1.0 at the maxima of their 'Band I' absorptions. Equal quantities of quercetin and luteolin in solution were deposited on Whatman filter paper and dried. Their reflectance spectra, referenced to a compressed Halon powder scatterer, were recorded using a McPherson 2051, 1 m monochromator with an integrating sphere input optics and the changes in these spectra after periods of 17 and 85 h exposure to UV radiation at an intensity of 90 μW cm^{-2} over the 300–400 nm spectral range from a 500 W xenon arc lamp passed through a Schott WG305 glass filter were determined.

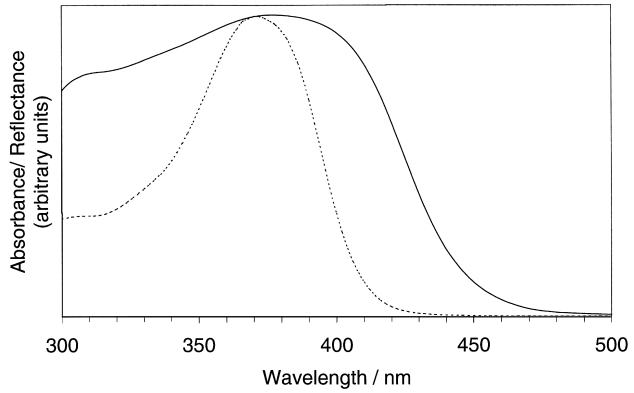


Fig. 2. The reflectance spectrum of dried quercetin on cellulose (—); the absorption spectrum of quercetin in aqueous methanol solution (- - -).

Air saturated solutions of uncomplexed flavonoids in the presence of 2×10^{-4} mol dm^{-3}, aluminium nitrate (aluminium, flavonoid ratio of ~2:1) were irradiated in 10 mm quartz absorption cells, using the same radiation source as that for the irradiation of the flavonoids on cellulose, and their absorption spectra determined at intervals using a Hitachi 8452A diode array spectrophotometer for a period of 12–15 h.

3. Results

3.1. Flavonoids on dry cellulose

On cellulose, both quercetin and luteolin exhibit broader reflectance (absorption) spectra than in solution, with their absorptions extending well beyond 400 nm as shown in Figs. 2 and 3, respectively. The effect of this absorption in the blue spectral region is lower relative reflectance at longer visible wavelengths, thus, producing the familiar yellow colour of flavonoids on a solid substrate.

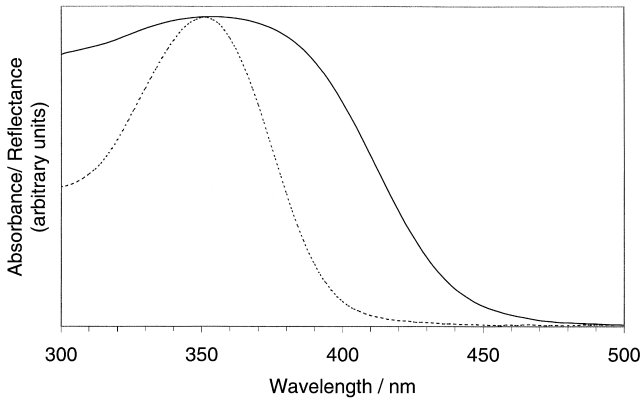


Fig. 3. The reflectance spectrum of dried luteolin on cellulose (—); the absorption spectrum of luteolin in aqueous methanol solution (- - -).

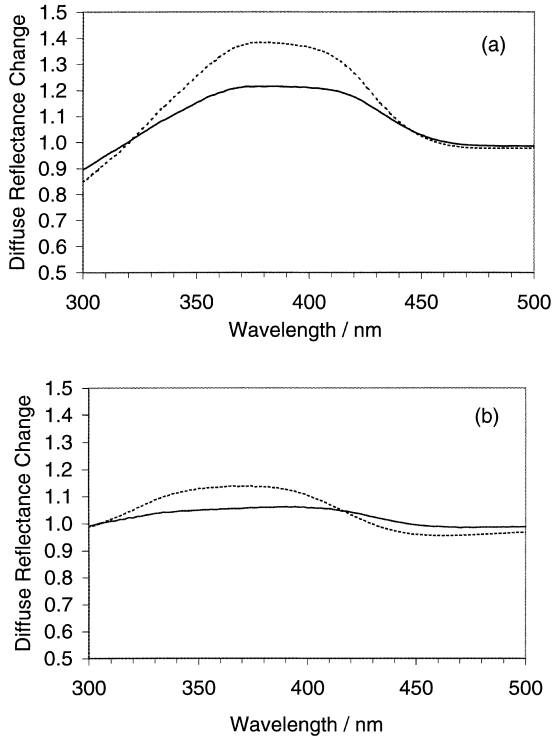


Fig. 4. (a) The change in the reflectance spectrum of quercetin on cellulose after 17 h UV irradiation (—), and after 85 h UV irradiation (- - -). (b) The change in the reflectance spectrum of luteolin on cellulose after 17 h UV irradiation (—), and after 85 h UV irradiation (- - -).

Quercetin on cellulose was substantially degraded and its colour faded after periods of exposure to radiation from the xenon arc lamp. In contrast, after the same doses of radiation, luteolin on cellulose was slightly darker than before irradiation. The changes in the reflectance spectra of both compounds resulting from irradiation are shown in Fig. 4(a) and (b). The photobleaching of quercetin appears as a decrease in reflectance across the whole spectrum from about 320–450 nm, with a greater quantum efficiency for degradation of the longer wavelength chromophore with an absorption centred around 400 nm. The reflectance spectrum of luteolin has small losses of reflectance centred about 350–390 nm which is accompanied by greater reflectance at wavelengths longer than 430 nm with a maximum at 450 nm.

3.2. Flavonoids in solution

The reductions in absorbances of 'Band I' for each flavonoid studied in this work after 15 hours irradiation are given in Table 1. The most susceptible to photobleaching is quercetin, while luteolin is the most resistant. The extent of photobleaching of solutions of the other flavonoids was found to depend on the positions of the the hydroxyl group substitution on the aromatic nucleus (B-ring), and the order of stability found was, luteolin≈kaempferol-3-sophoroside≈quercetin 3-galactoside>galangin>quercetin-4′-glucoside>kaempferol>quercetin.

Table 1
Radiation induced absorbance reduction for a number of flavenoids

Flavonoid	Absorbance reduction at λ_{max} after 15 h irradiation
Luteolin	0.01
Luteolin-7-glucoside	0.03
Kaempferol-3-sophoroside	0.02
Quercetin-3-galactoside	0.02
Galangin	0.08
Quercetin-4′-glucoside	0.10
Kaempferol	0.13
Quercetin	0.17

3.3. Aluminium–flavonoid complexes

In the presence of aluminium ions in the ratio of 2:1 aluminium:flavonoid, the absorption maxima of the complexes, comprising two flavonoid molecules linked by an aluminium ion [7,10] are red shifted 50 nm with respect to uncomplexed flavonoids. Irradiation resulted in substantial photobleaching of luteolin, kaempferol and quercetin complexes. The kinetics of the decreases in absorbances at the absorption spectral maxima with irradiation time are shown in Fig. 5.

4. Discussion

4.1. Flavonoids in solution

It is apparent from the trend in photostabilities of the flavonoids studied, that the main determinant of photoreactivity is the 3-OH group. Without such a group, e.g. luteolin, or when the group is glycosylated, e.g. quercetin-3-galactoside the flavonoid has a high photostability. The relative photoreactivities of the flavonols, possessing free 3-OH groups, can be explained in terms of an excited state electron transfer rate determining step. The energy change,

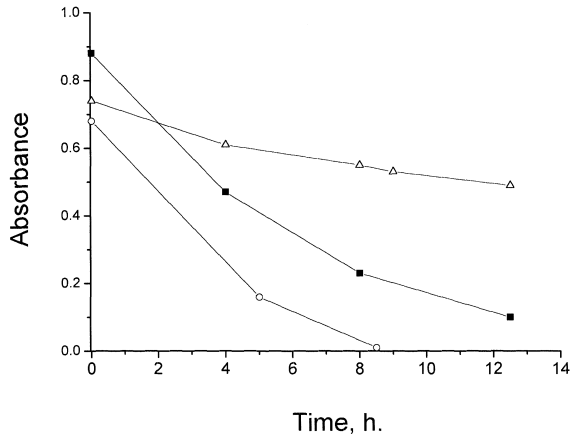


Fig. 5. The dependance of absorbance at the spectral maxima, of aqueous methanol solutions of flavonoids in the prescence of aluminium ions on UV irradiation time. (○) quercetin, (■) kaempferol, (△) luteolin.

ΔE, associated with such a process is given by

$$\Delta E = E_{ox} - E_{red} - E^* - C \quad (1)$$

where E_{ox} is the oxidation potential of the electron donor, E_{red} the reduction potential of the electron acceptor, in this case oxygen, E^* the energy of the excited state and C is the term associated with the coulombic interaction between the ion pair intermediate [11]. Provided the reaction is not diffusion controlled, the rate constant for the photoinduced electron transfer is exponentially related to ΔE [10]. Because the lifetimes of singlet states are extremely short, the probability of bimolecular encounters with oxygen in homogeneous solutions are low and redox reactions involving excited singlet states are not important. Thus, most photooxidation involves the longer-lived triplet states. The relative photostabilities of the flavonols are in the order of their redox potentials given in paratheses [12] in accordance with Eq. (1); i.e. galangin (0.34 eV)>kaempferol (0.17)>quercetin (0.06 eV). Luteolin, without a 3-OH group, has a redox potential of 0.16 eV [12] and does not fit this photostability trend suggesting that it either has a lower triplet state yield than the flavonols or electron transfer is not the rate determining step in its photooxidation/degradation.

The lowest energy zeroth-order, n,π^* and π,π^* excited states in 5-hydroxyflavonoids have similar energies [13,14]. Therefore, small perturbations to the energy of the n,π^* states resulting from hydrogen bonding interactions with the carbonyl oxygen atom involving the 5-OH group, the 3-OH group and/or protic solvents can result in near degeneracy of the n,π^* and π,π^* states which results in pseudo Jahn Teller splitting of the states. This has profound effects on the rate of internal conversion relative to intersystem crossing and hence the triplet state population i.e. the 'proximity effect' [15,16].

Another phenomenon which could contribute to the lower photostability of flavonols compared with that of flavonoids without a free 3-OH group is the higher reactivity of flavonols with singlet oxygen. Singlet oxygen is frequently produced as a result of reactions of ground state oxygen with triplet states and singlet oxygen has been detected following photolysis of oxygenated solutions of a range of flavonoids. The rates of reaction of singlet oxygen with flavonols are 1–2 orders of magnitude greater than those with luteolin or 3-glycosylated flavonol because of the higher electron density of the C_2–C_3 double bond in flavonols [17]. Thus, if singlet oxygen is involved as an intermediate in the photooxidation of flavonoids, this high reactivity with singlet oxygen would contribute to the relatively low photostability of flavonols compared to luteolin.

4.2. Aluminium–flavonoid complexes

The 2:1 aluminium ion complexes of quercetin and kaempferol have been demonstrated to comprise two flavonoid molecules linked by an aluminium ion and involve the 5- or 3-hydroxy groups and the 4-carbonyl group of the flavonoids [7,10]. When aromatic chromophores associate in such a way that their molecular orbitals interact, coupling of their ground-excited singlet state transition dipoles can occur (exciton coupling), resulting in splitting of the excited singlet states and consequent shifts in the absorption spectra. Kasha et al. [18] have found that exciton coupling can have a substantial effect on intersystem crossing to the triplet state which is the principal excited state involved in photooxidation reactions.

An increase in the rate of photooxidation/bleaching resulting from association of flavonoids mediated by metal ion complexing contrasts with previous research findings on quercetin and kaempferol in concentrated solution, where we found that these flavonoids effected efficient and non-degradative dissipation of excitation energy by an intermolecular, dual proton transfer involving the 3- or 5-OH and the 4-carbonyl groups of molecular pairs [9]. However, in the aluminium ion–flavonoid complexes, which are the subject of the present work, the energy-dissipating, excited state proton transfer is prevented by the intervening metal ion [19].

4.3. Flavonoids on dry cellulose

Inspection of the difference reflectance spectra of luteolin and quercetin on dry cellulose before and after irradiation shows greater degradation of the chromophores responsible for the absorptions with maxima about 400 nm, than for the monomer chromophores. The longer wavelength chromophores are assigned to exciton coupled, molecular aggregates which are present in dried flavonoids deposited on solid substrates such as delignified paper and even in the flower petals of Lisianthus (*Eustoma grandiflorum*). These normally white petals contain high levels of kaempferol glycosides but acquire a yellow colouration on dessication which is not a result of oxidation/degradation [20]. As discussed above, the exciton coupling, associated with molecular aggregates can result in enhanced intersystem crossing, higher triplet state yields and therefore, greater susceptibility to oxidation [18].

As is the case in solution, the monomer luteolin with an absorption maximum at 350 nm is significantly more stable than the quercetin monomer. The formation of photoproduct (derived largely from luteolin aggregates) which absorbs in the blue region of the spectrum with a maximum at 450 nm, gives the luteolin-dyed cellulose a darker yellow appearance after irradiation. In contrast, the only photoproducts evident in the difference reflectance spectrum of irradiated quercetin on cellulose absorb at wavelengths shorter than 300 nm and they therefore, have no effect on the visible reflectance spectrum (colour).

5. Conclusions

The lower photostability of the flavonols possessing a free 3-OH group compared with the flavonols and flavones lack-

ing this group, is attributed to a greater triplet state population and/or a higher reactivity with singlet oxygen.

It is proposed that an interaction between the 3-OH group and the carbonyl group perturbs the n,π* zeroth-order state in such a way as to either introduce more n,π* character into the singlet state and hence greater intersystem crossing to the triplet state or reduce psuedo Jahn Teller splitting and concommitant internal conversion [13]. Further, the 3-OH group raises the electron density of the C_2–C_3 bond and hence its reactivity with electron-affinic singlet oxygen [14].

The reduced photostability of aluminium-complexed flavonoids suggests that interactions between the 5-hydroxyl and carbonyl groups have a photostabilising effect through energy dissipation by excited state proton transfer.

The practical consequences of the photochemistry described herein are:-

1. Luteolin, or flavonol-3-glycoside, used to dye cellulosic materials without a metal ion mordant will be substantially more resistant to light-induced fading than flavonols such as quercetin or kaempferol. These flavonols normally occur in plants as 3-O-glycosides which are labile in weak acid and are likely to be converted to the aglycones during their extraction and application to textiles.
2. Yellow colouration in flower petals, and its stability, are dependent upon the oxygenation/hydroxylation patterns of the constituent flavonoids, and on the presence/absence of metal chelation. For example, the kaempferol glycoside based yellow in *Lathyrus chrysanthus* is deep and stable and does not involve metal complexing.

Acknowledgements

This work was partly funded by the New Zealand Foundation for Research Science and Technology contracts, NO88133, CO8636 and CO8804 and a Marsden Fund grant (IRL 701). We are grateful to the French Ministry of Foreign Affairs for a grant for scientific cooperation to facilitate collaborative research between the New Zealand Institute for Industrial Research, the University of Montpellier and CNRS, France.

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