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Light Sensitivity of Cochineal. Quantum Yields for Photodegradation of Carminic Acid and Conjugate Bases in Aqueous Solution

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ABSTRACT

The quantum yield for photobleaching of carminic acid, the major constituent of cochineal, has been determined at 25·0°C for air-saturated aqueous 1·0M NaCl solution at different pH, using monochromatic light at each of the three wavelengths 254 nm, 366 nm, and 436 nm, respectively, in continuous photolysis experiments, in order to provide an objective measure of the sensitivity of this important food colourant to ultraviolet and visible light. For each wavelength of irradiation, the photobleaching increases with increasing pH, the effect being most significant for 254 nm irradiation. Reaction quantum yields for the fully protonated form of carminic acid and each of three deprotonated forms were calculated from the pH-dependence of the photobleaching in combination with potentiometrically determined acidity constants (LH_3: $\mathrm{p}K_{a,1} = 2{\cdot}81 \pm 0{\cdot}09$; LH_2^-: $\mathrm{p}K_{a,2} = 5{\cdot}43 \pm 0{\cdot}04$; LH^{2-}: $\mathrm{p}K_{a,3} = 8{\cdot}10 \pm 0{\cdot}03$*, the constants being based on concentrations in 1·0M NaCl), and have the value* $\Phi_{LH_3} = 2 \times 10^{-5}$ *mol einstein*$^{-1}$, $\Phi_{LH_2^-} = 2 \times 10^{-5}$, $\Phi_{LH^{2-}} = 6 \times 10^{-5}$, *and* $\Phi_{L^{3-}} = 4 \times 10^{-4}$ *for 436 nm irradiation. For 254 nm irradiation, the values range from* $\Phi_{LH_3} = 5 \times 10^{-5}$ *mol einstein*$^{-1}$ *to* $\Phi_{L^{3-}} = 0{\cdot}01$ *mol einstein*$^{-1}$*, with the values for 366 nm irradiation being intermediates. The increased photolability of the deprotonated forms and an apparent linear free energy relationship between lowest energy transition and photoreactivity resulting from 436 nm excitation are discussed in relation to the nature of the lowest electronically excited state.*

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INTRODUCTION

As a result of increasing restrictions on the application of synthetic food colours, the 'ancient' colourant cochineal has gained renewed interest as a colour additive to foods and beverages (Lloyd, 1980; Kearsley & Katsaboxakis, 1980). Ironically, owing to toxicological concerns, products derived from cochineal are now, in several countries, replacing the synthetic food colours which, for economic reasons, replaced cochineal a century ago (Marmion, 1979).

The major constituent of cochineal is carminic acid, a β-C-glycosyl derivative of anthraquinone, for which the complete stereochemistry has recently been assigned (Fig. 1) (Fiecchi *et al.*, 1981), although a total synthesis of carminic acid has not yet been accomplished. Cochineal and carminic acid have, in general, been found to be stable and safe colourants in foods (Marmion, 1979; Coulson, 1980), although concern about a possible role as prooxidant for carminic acid has been expressed (Gutteridge & Quinlan, 1986). Kearsley & Katsaboxakis (1980) and Tanaka *et al.* (1986) have, however, found that the stability decreased with increasing pH and that the colour is sensitive to exposure to light. In order to provide an objective measure of the light sensitivity under different sets of conditions, we have determined the photobleaching quantum yield for carminic acid in aqueous solution at varying pH for monochromatic light of three different wavelengths. Besides being of immediate interest to food technology, the variation of photoreactivity with solution pH, also allows, when combined with acid dissociation constants determined under similar conditions, assignment of the relative photoreactivity of the fully protonated form of carminic acid and of the three conjugate base forms which are of importance in aqueous solution (Schwing-Weill, 1986). A photochemical exploration of the different acid/base forms is also of interest in the use of carminic acid as a fluorescent probe in enzyme kinetics.

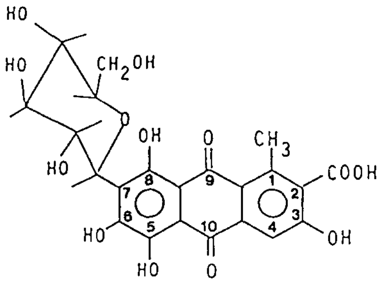

Fig. 1. Carminic acid with numbering of the anthraquinone moiety.

MATERIALS AND METHODS

Materials

Carminic acid (Merck) was dried at 100°C to constant weight (weight loss approximately 10%) prior to use. Astaxanthin was from Roche Inc., Copenhagen. Other chemicals were of analytical grade and were used without further purification.

Determination of pK_a-values

Twenty-five millilitre portions of 1·0 mM solutions of carminic acid in aqueous 0·00100M HCl, 1·00M NaCl were titrated at $25{\cdot}0 \pm 0{\cdot}1$°C with aqueous 0·0100M NaOH, 0·99M NaCl, and pH was measured with a combination glass electrode (Radiometer GK 2322C in conjugation with a Radiometer PHM84). The electrode was standardized against titrated solutions of hydrochloric acid in 1·00M NaCl, employing the definition $\mathrm{pH} = -\log[\mathrm{H}^+]$ throughout. The calculations were made within the framework of non-linear regression analysis and a model including a tribasic acid could fully account (χ^2-goodness-of-fit test) for the observed titration curve ($2 < \mathrm{pH} < 10$).

Photolysis experiments

Weighed amounts of carminic acid were dissolved in 0·010M HCl, in 0·010M NaOH, or in buffered solutions (0·020M acetate or tris(hydroxymethyl)-aminomethane buffer), each medium containing 1·00M NaCl, to yield a total carminic-acid concentration of 6×10^{-5}M. The solutions were air-saturated or saturated with oxygen (1 atm) or with nitrogen (1 atm). pH was measured in each solution, and 5·0 ml of solution was transferred to a quartz cell with a 2 cm light path and exposed to monochromatic light (wavelength 366 nm or 436 nm) selected from an Osram HBO 100/2 high pressure Hg lamp (line spectrum), mounted as part of an optical train, which also included a light-condenser, a heat filter, an interference filter, a shutter connected to an electronic timer, and lenses focusing the light into a thermostatted ($25{\cdot}0 \pm 0{\cdot}5$°C) cell-holder. An Oriel 6035 low-pressure Hg–Ar penlight equipped with an Oriel 6041 short-wave filter mounted in a thermostatted cell-holder ($25{\cdot}0 \pm 0{\cdot}5$°C) was used as a diffuse light-source for 254nm irradiation. Light intensities were determined by ferrioxalate actinometry (Hatchard & Parker, 1956). The extent of photodegradation was monitored at regular intervals by spectrophotometric measurements (Hewlett-Packard 8452 A Diode Array Spectrophotometer). The photolysis solution was air-saturated and in contact with air during photolysis, or saturated with oxygen or nitrogen for 30 min prior to photolysis.

The apparent photodegradation quantum yield

$$\Phi_{\text{app}} = \frac{\text{number of carminic acid degraded}}{\text{number of photons absorbed by carminic acid}} \tag{1}$$

was calculated from the degree of bleaching of the colour of the solution monitored at the absorption maximum in the visible range (480 nm < λ_{max} < 580 nm, dependent on pH) for up to 20% photodegradation in a typical experiment, in combination with I_0, the light intensity as determined by actinometry and expressed in quanta s^{-1}. The number of photons absorbed by carminic acid, Q_{CA}, was calculated by adding the light absorbed in small, but finite, time intervals, $t_i - t_{i-1}$ for a solution with a total carminic acid concentration, c_0:

$$Q_{CA} = \frac{1}{V \times c_0} \sum_i \frac{I_0}{N_A}(1 - 10^{-\bar{A}_{\text{irr}}})(t_i - t_{i-1}) \tag{2}$$

where N_A is Avogadro's number, V is the volume (in litres), and $\bar{A}_{\text{irr}}$ is the average absorbance at the wavelength of irradiation at the time $\frac{1}{2}(t_i - t_{i-1})$. The quantum yield was calculated from:

$$\Phi_{\text{app}} = \frac{(A(t_0) - A(t_i))/A(t_0)}{Q_{CA}(t_i)} \tag{3}$$

and the means of at least three determinations are reported.

Dark reactions

Thermal reactions during the time span of photolysis were monitored spectrophotometrically for solutions prepared as the photolysis solutions and kept at 25·0°C, excluded from light.

Photosensitization experiments

The potential of carminic acid to act as a sensitizer for the photodegradation of other food components was tested with the carotenoid astaxanthin as substrate in the solubilized system previously developed (Jørgensen & Skibsted, 1990).

RESULTS AND DISCUSSION

Carminic acid dissolved in water was found to degrade as a result of light-absorption. The photochemical degradation could be observed as a slow bleaching of the reddish colour of the carminic acid solution during exposure to light and was followed spectrophotometrically. The photochemical stability was found to be strongly dependent on pH and on the oxygen partial pressure of the carminic acid solution, and less dependent on the wavelength of irradiation. For a given set of conditions, the photolability of carminic acid solution was expressed as the apparent quantum yield as defined in eqn (1), providing an objective measure of the effectiveness with which monochromatic light promotes the degradation of carminic acid. The quantitative information presented in Fig. 2 is expected to be of immediate interest in the selection of the optimal packaging conditions for beverages and foods to which carminic acid has been added as a colourant. The transparency of the packaging material to different wavelength regions of light is often selected as a compromise between materials which convey to the consumers a direct impression of the product during retail display and materials which yield efficient protection against light-induced oxidation

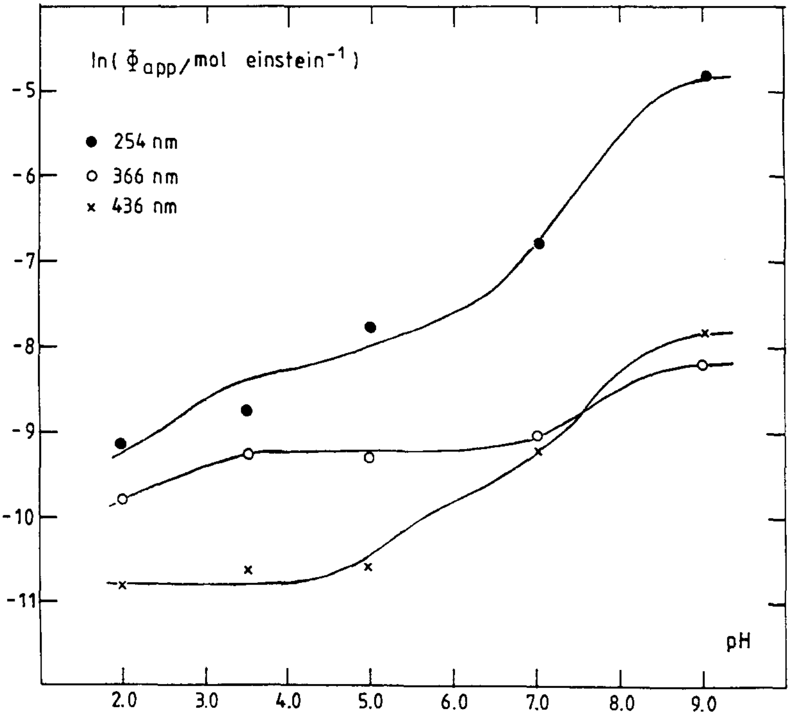

Fig. 2. Apparent reaction quantum yields for photodegradation of carminic acid in air-saturated aqueous 1·0M NaCl at varying pH for monochromatic light of three different wavelengths at 25·0°C. The full line is calculated by non-linear regression according to eqns (7)–(9), including independently determined value for $pK_{a,n}$ ($n = 3,2,1$), in effect resolving the photoreactivity into quantum yield for the fully protonated form of carminic acid and each of the three conjugate base forms, see Table 1.

processes in the product. In conclusion, carminic acid was found to be sufficiently light-fast for most food applications. It is, however, notable that little protection is to be expected from the application of UV-light absorbers in packaging materials. The largest difference noted is a factor of 20 between the reaction quantum yield for irradiation with 254 nm and for irradiation with 436 nm monochromatic light (pH = 9·06, see Fig. 1). This is in marked contrast to the dramatic wavelength-dependence found for the photooxidation of other food pigments such as β-carotene and lutein (Jørgensen & Skibsted, 1990) and oxymyoglobin (Bertelsen & Skibsted, 1987), which warrants the use of UV-absorbers to prevent photodegradation.

The thermal degradation of carminic acid was found to obey first-order kinetics with $k_{obs} < 1 \times 10^{-8}\,s^{-1}$ at $pH \leq 5{\cdot}0$, $k_{obs} = 2{\cdot}5 \times 10^{-7}\,s^{-1}$ at pH = 7·03, and $k_{obs} = 7{\cdot}6 \times 10^{-6}\,s^{-1}$ at pH = 9·06, for air-saturated solutions at 25·0°C. This 'dark reaction' is very slow and is significant only in alkaline solution, and it is of no relevance to conditions prevailing in foods and beverages. For a fixed value of solution pH, the rate of bleaching of carminic acid solutions protected against light was proportional to the oxygen partial pressure (Fig. 3A). This proportionality, however, identifies the bleaching process as an oxidation. For the light-induced bleaching, a similar linear relationship between oxygen partial pressure and reaction quantum yield likewise identifies the degradation process as an oxidation. The intercept noted in Fig. 3B could indicate a competitive photodegradation process which does not require oxygen in the primary light-activated step. Such complex kinetics of the photooxidation could also involve the generation of activated

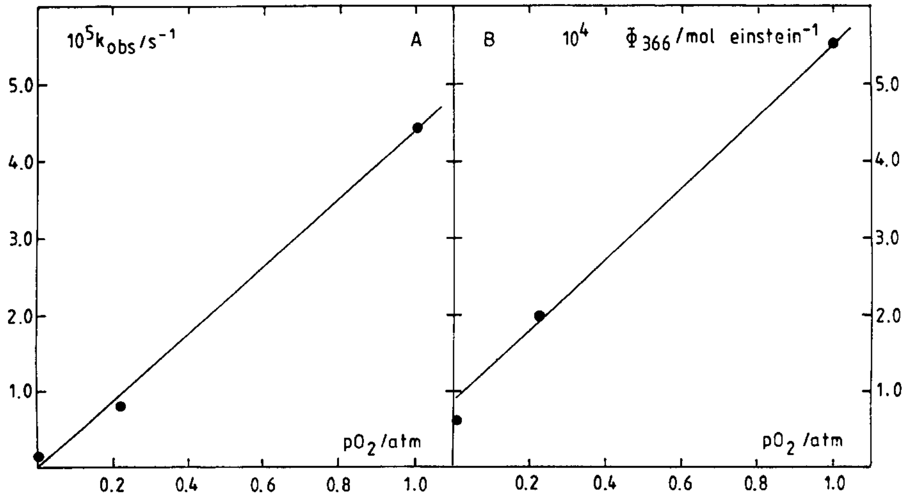

Fig. 3. A: First-order rate constant for bleaching of carminic acid at pH = 9·06 and 25·0°C as a function of oxygen pressure. B: Quantum yield for photobleaching of carminic acid at pH = 9·06 and 25·0°C as a function of oxygen pressure.

oxygen species, as has been suggested by Gutteridge and Quinlan (1986), and could provide the basis for a coupling between photooxidation of carminic acid and degradation of other food constituents. However, carminic acid was not found to act as a sensitizer for the degradation of the carotenoid astaxanthin in the solubilized aqueous system previously developed ($\lambda_{\mathrm{irr}} = 436$ nm, Tween-20 in aqueous citrate buffer with pH = 5·5).

Besides being of interest in the use of cochineal and carminic acid as a food colourant, the results obtained also afford the information required for the assignment of the relative photoreactivity of different acid/base forms of the pigment. In aqueous solution, carminic acid is a tribasic acid (*cf.* Fig. 1):

$$\mathrm{H_3L} \rightleftharpoons \mathrm{H_2L^-} + \mathrm{H^+} \quad K_{a,1} \tag{4}$$

$$\mathrm{H_2L^-} \rightleftharpoons \mathrm{HL^{2-}} + \mathrm{H^+} \quad K_{a,2} \tag{5}$$

$$\mathrm{HL^{2-}} \rightleftharpoons \mathrm{L^{3-}} + \mathrm{H^+} \quad K_{a,3} \tag{6}$$

for which the acidity constants ($n = 3,2,1$):

$$K_{a,(4-n)} = \frac{[\mathrm{H_nL}^{(3-n)-}][\mathrm{H^+}]}{[H_{(n-1)}\mathrm{L}^{(4-n)-}]} \tag{7}$$

were determined by titration in the 1·0M NaCl reaction medium also used for the photochemical investigations. The values determined, and given in Table 1, are 0·1–0·2 units lower than the corresponding values for aqueous solution with an ionic strength of 0·01 determined by Schwing-Weill (1986). The increase in acidity at the higher ionic strength is, however, in accordance with the Debye-Hückel theory for non-ionic and negatively charged acids.

TABLE 1

Photobleaching of Carminic Acid and Conjugate Bases[a] in Aqueous Solution of 1·0M NaCl at 25°C. pK_a-values[b] and Quantum Yields for Photodegradation Resulting from Exposure to Monochromatic Light of Each of Three Different Wavelengths

Reactant[a]	*p*K$_a$[b]	λ_{max}[c] (*nm*)	Φ_{254} (*mol einstein*$^{-1}$)	Φ_{366} (*mol einstein*$^{-1}$)	Φ_{436} (*mol einstein*$^{-1}$)
$\mathrm{H_3L}$	$2{\cdot}81 \pm 0{\cdot}09$	492	$< 2 \times 10^{-4}$[d]	$4{\cdot}6 \pm 2{\cdot}9 \times 10^{-5}$	$2{\cdot}1 \pm 0{\cdot}7 \times 10^{-5}$
$\mathrm{H_2L^-}$	$5{\cdot}43 \pm 0{\cdot}04$	488	$2{\cdot}6 \pm 1{\cdot}2 \times 10^{-4}$	$1{\cdot}0 \pm 0{\cdot}3 \times 10^{-4}$	$1{\cdot}9 \pm 0{\cdot}5 \times 10^{-5}$
$\mathrm{HL^{2-}}$	$8{\cdot}10 \pm 0{\cdot}03$	530	$4{\cdot}9 \pm 1{\cdot}2 \times 10^{-4}$	[e]	$6{\cdot}5 \pm 0{\cdot}7 \times 10^{-5}$
$\mathrm{L^{3-}}$		560	$8{\cdot}8 \pm 0{\cdot}1 \times 10^{-4}$	[e]	$4{\cdot}3 \pm 0{\cdot}1 \times 10^{-4}$

[a] Carminic acid is considered to be a tribasic acid in aqueous solution: $\mathrm{H_3L}$.

[b] $\mathrm{p}K_a = -\log(K_a/\mathrm{mol\ litre^{-1}})$ for $H_n\mathrm{L}^{(3-n)-} \rightleftharpoons \mathrm{H}_{(n-1)}\mathrm{L}^{(4-n)-} + \mathrm{H^+}$; for $n = 3, 2, 1$.

[c] From Schwing-Weill (1986).

[d] Upper limit.

[e] Could not be resolved with reasonable accuracy because of spectral similarities of the different forms.

The apparent quantum yield as defined in eqn (1) is, for each wavelength of irradiation, a function of the distribution between the four acid/base forms of carminic acid,

$$\Phi_{\mathrm{app}} = \alpha_{\mathrm{HL_3}} \Phi_{\mathrm{irr}}^{\mathrm{H_3L}} + \alpha_{\mathrm{HL_2^-}} \Phi_{\mathrm{irr}}^{\mathrm{H_2L^-}} + \alpha_{\mathrm{HL^{2-}}} \Phi_{\mathrm{irr}}^{\mathrm{HL^{2-}}} + \alpha_{\mathrm{L^{3-}}} \Phi_{\mathrm{irr}}^{\mathrm{L^{3-}}} \qquad (8)$$

in which the fraction of light absorbed by each form ($\alpha_{\mathrm{H}_n\mathrm{L}^{(3-n)-}}$) depends on the molar absorptivities ($\varepsilon_{\mathrm{H}_n\mathrm{L}^{(3-n)-}}$) and the acidity constants according to ($n = 3,2,1,0$):

$$\alpha_{\mathrm{H}_n\mathrm{L}^{(3-n)-}} = \frac{(\varepsilon_{\mathrm{H}_n\mathrm{L}^{(3-n)-}})[\mathrm{H}_n\mathrm{L}^{(3-n)-}]}{\sum_n (\varepsilon_{\mathrm{H}_n\mathrm{L}^{(3-n)-}})[\mathrm{H}_n\mathrm{L}^{(3-n)-}]} \qquad (9)$$

The resolution of the apparent quantum yields into quantum yields for each of the four acid/base forms for each of the three wavelengths of irradiation was made by a general non-linear regression procedure combining the independently determined pK_a values and molar absorptivities in accordance with eqns (7)–(9). The resolved quantum yields are presented in Table 1, and the experimental data are fully accounted for by this simple model, not including any contribution from secondary reactions, as may be seen from Fig. 2. At 366 nm, however, the absorptivities have almost identical values, especially for the di- and tri-deprotonated forms of carminic acid, thereby rendering the resolved quantum yields undetermined.

Visible light is absorbed by the anthraquinone moiety of the carminic acid producing π^* excited states of the conjugated system, among which the reactive states should be found. Accordingly, reaction yields for 436 nm irradiation, in contrast to reaction quantum yields resulting from the higher energy and more non-specific 254 nm irradiation, should provide the basis for a correlation between the nature and the lifetime of the reactive excited state of the different acid/base forms of carminic acid and their photochemical reactivity. The linearity observed between the low energy transition in the anthraquinone moiety and the logarithm of the reaction quantum yield (Fig. 4) could indicate a proportionality between the available excitation energy in the lowest singlet state and the reactivity. Assuming a constant Stokes shift, this correlation could be understandable on the basis of the energy gap law (Englman & Jortner, 1970). However, the luminescence intensity, and in particular, the presence of phosphorescence for dyes belonging to several series of amino-substituted and hydroxy-substituted anthraquinones has been found to correlate with photolability of the dyes (Allen & McKellar, 1976; Allen *et al.*, 1978). A high luminescence intensity is indicative of a long-lived excited state and a less effective non-radiative deactivation, which also allows the reactive state a longer reaction time for reaction with oxygen. A luminescence study of the acid/base forms of

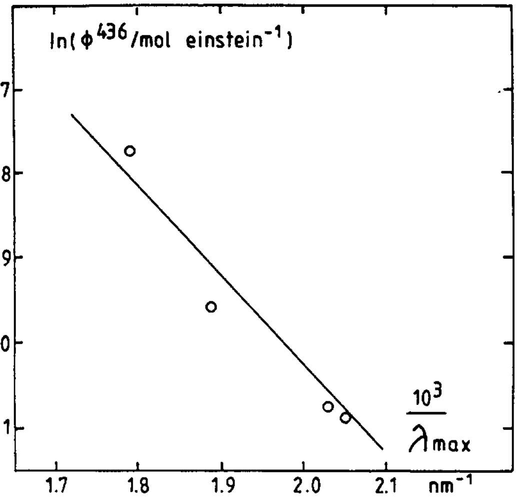

Fig. 4. Dependence of photooxidation quantum yields on the absorption maximum of the different acid/base forms of carminic acid for irradiation with visible light (436 nm) according to the semi-logarithmic regression line: $\ln \Phi_{app} = \ln A - \alpha/\lambda_{max}$, *cf.* Table 1.

carminic acid, using phase-resolution, is in progress in order to provide the photophysical information required to correlate energy and lifetime of the reactive state with the observed reactivity.

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