

4-Carboxyflavylium Salts: Stable Red Dyes?

Duřanka Davidović-Amić, Dragan Amić

Faculty of Agriculture, University of Osijek, P. O. B. 117, HR-54001 Osijek, Croatia

and

Nenad Trinajstić

The Rugjer Bošković Institute, P. O. B. 1016, HR-41001 Zagreb, Croatia

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The effects of ring substitution on the stability and spectral characteristics of synthetic 4-substituted flavylium salts have been studied. Several 4-carboxyflavylium salt model compounds are proposed, which are expected to exhibit improved stability and red coloration in slightly acidic and neutral solution.

INTRODUCTION

The safety of synthetic food dyes has been questioned for a number of years, leading to interest in the use of natural pigments in food applications.¹ The anthocyanins are one group of natural pigments which have been used with some commercial success.² They provide a wide range of colors, from orange to reddish blue, but they usually incur the disadvantages of instability and sensitivity to changes in pH. The common anthocyanins³ (colored flavylium cation AH⁺ and anhydrobase A) are subject to changes in colour with pH because of the formation of a colorless pseudo-base B and/or chalcone C at pH near 4. These transformations in aqueous media are illustrated in Figure 1.

There is a continuing effort to produce anthocyanin-type colorant which would show more stability. A new group of flavylium salts, structurally very close to anthocyanins have been reported to show both stability and increased coloration at high pH values.⁴ The presence of the substituent at C-4 is highly desirable for a food colorant because it would be highly colored at pH above 4. The presence of extra substituents attached to the flavylium core contributes to different colour hues. Our objective was to develop a substitution model compound that would exhibit stability and stable red coloration. To achieve this goal, we combined our experimental results and known experimental facts in flavylium salt literature⁵ with theoretical calculations using simple MO methods.

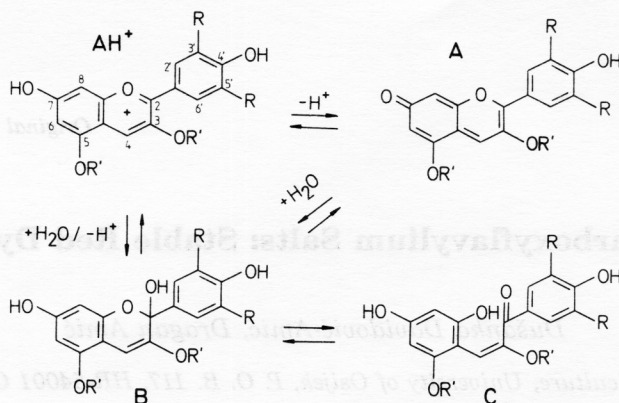


Figure 1. Structural transformations of flavylium salts. R = H, OH, OCH₃; R' = H, Sugar

This paper is organized as follows. In the second, third and fourth section, we give brief outlines of the experimental work, the absolute hardness concept and the absorption spectra calculations. The fifth section contains evaluation of favourable model compounds. The paper ends with some concluding remark.

EXPERIMENTAL

The solutions were prepared in Britton-Robinson buffers of constant ionic strength (0.2 mol dm⁻³) and were not exposed to light. The electronic absorption spectra were recorded immediately after the preparation of solutions on a Pye Unicam SP8-100 UV-vis spectrophotometer. All measurements were carried out at a temperature of 18 °C.

The Absolute Hardness Concept

The absolute hardness η is a theoretical quantity, useful for predicting the aromatic stability of conjugated systems.^{6,7} The absolute hardness η of a molecule is defined as

$$\eta = \frac{1}{2} \left(\frac{\partial^2 E}{\partial N^2} \right)_V$$

where E is the electronic energy of the molecule, N is the number of electrons in the molecule and V is the external potential due to the nuclei. The corresponding finite difference equation is given by

$$\eta = (I - A)/2$$

where I is the ionization potential and A is the electron affinity. Alternatively,

$$\eta = (E_{\text{LUMO}} - E_{\text{HOMO}})/2$$

where E_{LUMO} and E_{HOMO} represent, respectively, the energy of the lowest unoccupied molecular orbital (LUMO) and the energy of the highest occupied molecular orbital (HOMO). The E_{LUMO} and E_{HOMO} may be computed by means of any MO model. In the present study, we have used the Hückel model. Absolute hardness η is also a reliable criterion for predicting reactivity (a larger HOMO – LUMO gap means a lower reactivity of the molecule). It may also be considered as a good measure of aromatic stability.⁸ Therefore, η is a theoretical quantity that may be regarded as a unifying criterion for both the aromatic stability (thermodynamic stability) and reactivity (kinetic stability). In this sense, the harder the conjugated molecule, the more aromatic and less reactive it is.

Theoretical Calculations of Electronic Absorption Spectra

All calculations were carried out on an Atari 1040ST computer using the standard version of the PPP (Pariser-Parr-Pople) method.^{9,10} Idealized, planar geometries have been assumed, with the rings taken as regular hexagons. All C-C bonds within the rings were set equal to 1.39 Å. All valence angles have been taken to be equal 120°.

We have selected a consistent set of valence state energy parameters (Table I). These parameters have been developed and tested in calculations of various 4-carboxyflavylium structures. In Table I, I_{μ} and A_{μ} are the ionization potential and electron affinity of atom μ in the atomic valence state, respectively. The monocentric electronic repulsion integrals and the core resonance integrals between nearest neighbors are designated as $\gamma_{\mu\mu}$ and $\beta_{c-\mu}$, respectively. n_{π} is the number of electrons contributed to the π -system and $l_{c\mu}$ (Å) is the distance between atoms C and μ . The bicentric electronic repulsion integrals were calculated using the Mataga-Nishimoto formula.¹⁶

RESULTS AND DISCUSSION

Depending on their particular substitution pattern and pH, flavylium salts can exist in the form of the yellow or red flavylium cation AH^+ , red or blue anhydrobase A and colorless pseudobase B and chalcone C. Typical UV-vis spectra of 7-hydroxy-4'-methoxy-4-carboxyflavylium chloride are presented in Figure 2. The intensity of the absorption at 458 nm (the λ_{max} of flavylium cation AH^+) decreased as the pH was

TABLE I
Parameters used in the PPP calculations

Atom, μ	I_{μ} / eV	A_{μ} / eV	$\gamma_{\mu\mu}$ / eV	$\beta_{c-\mu}$ / eV	n_{π}	$l_{c\mu}$ / Å	Ref.
C	11.16	0.03	11.13	-2.349	1	1.390	11
O ⁺	34.95	15.30	19.65	-2.800	1	1.360	12
=O	17.28	2.70	14.58	-3.070	1	1.240	13
-O-	35.76	17.70	18.06	-2.270	2	1.360	13
OH	26.14	7.32	18.82	-1.950	2	1.360	12
OCH ₃	33.00	11.47	21.53	-1.808	2	1.370	11
CH ₃	28.43	12.99	15.44	-1.673	2	1.500	14
OH from							
COOH	25.43	10.85	14.58	-2.550	2	1.360	15

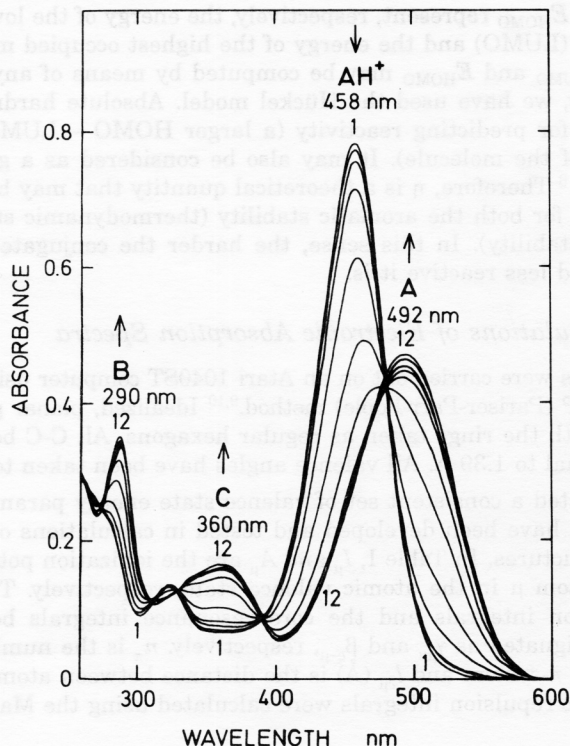


Figure 2. Absorption spectra of 7-hydroxy-4'-methoxy-4-carboxyflavylium chloride in solutions of pH 2.00 – 7.50, concentration $1.68 \times 10^{-5} \text{ mol dm}^{-3}$. Each spectrum was taken at successive 0.50 pH intervals: curve 1 – pH 2.00; curve 12 – pH 7.50.

raised. Simultaneously, the absorption at 492 nm increased as a result of the formation of the anhydrobase form A. This change in absorbance with increasing pH reflects the conversion of the colored AH^+ form to the colored A form. Lack of well-defined isobestic points between the spectral curves indicates that there were more than two chemical species in the solutions. Small amounts of the colorless chalcone C ($\lambda_{\text{max}} \approx 360 \text{ nm}$) and colorless pseudobase B ($\lambda_{\text{max}} \approx 290 \text{ nm}$) were also present. Thus, the most favoured species for this flavylium salt are colored AH^+ and A forms. Permanent coloration is due to the presence of carboxy group in position C-4. Substituent attached to position C-4 stabilize the colored forms by suppressing the hydration reactions. By varying the substitution pattern of the flavylium ring, salts that can exist primarily in the red colored flavylium cation AH^+ and anhydrobase A can be proposed.

In Table II, published absorption maxima in UV-vis spectra, λ_{max} , for some 4-unsubstituted and 4-substituted flavylium compounds are given. As it can be seen, the lowest values were derived for 4- CH_3 salts: substitution of the H atom at the C-4 position by a CH_3 group causes a hypsochromic shift by 14–24 nm. It is inter-

TABLE II

Experimental absorption maxima λ_{\max} (in nm) for flavylum salts

Substitution model	4-H	4-CH ₃	4-Ph
7-OH	429 ^a	414 ^b	428 ^b
5,7-diOH	450 ^c	431 ^d	450 ^d
7,4'-diOH	458 ^a	444 ^b	460 ^b
7-OH-4'-OCH ₃	458 ^a	444 ^e	459 ^b
5,7-diOH-4'-OCH ₃	466 ^c	446 ^b	468 ^b
5,7,4'-triOH	466 ^c	447 ^b	470 ^b
7,4'-diOH-3'-OCH ₃	469 ^a	454 ^b	474 ^b
5,7,4'-triOH-3'-OCH ₃	482 ^f	458 ^b	484 ^b

a) Ref. 17; b) Ref. 18; c) Ref. 19; d) Ref. 20; e) Ref. 21; f) Ref. 22

esting to note that λ_{\max} for 4-H and 4-phenylflavylium salts are practically the same. In this respect, the methyl group seems to be of disadvantage in producing long wavelength absorbing flavylium salts. However, the methyl group in position C-4 produces higher stability of the flavylium chromophoric system than the phenyl one and particularly than the hydrogen one.²³

It has been shown that the presence of a carboxy group at C-4 results in unusually stable colorants.⁴ Stability of flavylium compounds can be expressed quantitatively by the equilibrium constant, *i.e.*, the pK value of the overall transformation $AH^+ \rightleftharpoons (A + B + C) + H^+$. Table III presents the pK values of some 4-substituted flavylium salts as well as the observed and calculated spectral properties. From these data it is apparent that the 4-carboxyflavylium salt is the compound of choice. This can be additionally supported by the fact that carboxy group is ionizable: at least two ionizable groups attached to flavylium nuclei produce nontoxic colorants.²⁶

The position of substitution, and the nature of the substituent are both critical in determining the effect of substitution on the stability and reactivity of the flavylium chromophore.²⁷ Positive charge of the flavylium core, delocalized over the en-

TABLE III

Calculated and experimental indices for flavylium salts: pK values of the overall transformation $AH^+ \rightleftharpoons (A + B + C) + H^+$; absorption maxima λ_{\max} (in nm); experimental intensities ($\log \epsilon$) and oscillator strengths (f)

7-OH-4'-OCH ₃ flavylium core with	Observed			Calculated	
	pK	λ_{\max}	$\log \epsilon$	λ_{\max}	f
4-H	3.14 ^a	458	4.57	448	1.028
4-CH ₃	4.80 ^b	444	4.62	437	0.994
4-Ph	4.44 ^c	459	4.63	462	0.943
4-COOH	4.78 ^d	458	4.67	462	1.008
4- <i>p</i> -anisyl	3.10	460	4.48	457	0.927

a) Ref. 17; b) Ref. 24; c) Ref. 23; d) Ref. 25;

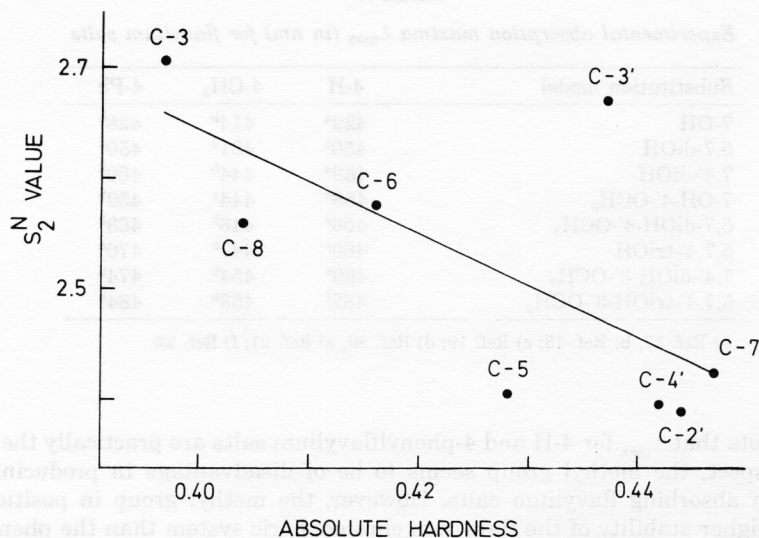


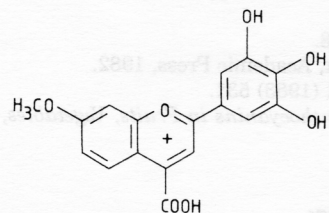
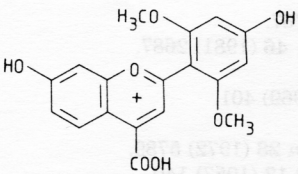
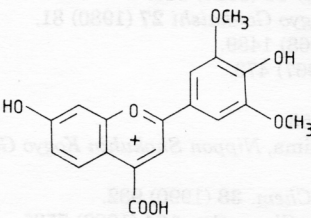
Figure 3. Correlation of superdelocalizability (nucleophilic) (in β^{-1}) with absolute hardness (in $-\beta$) for a cationic form of 4-carboxyflavylium chromophore with a single OH group in a specified position.

tire heteroaromatic system, has the highest partial value at positions C-2 and C-4.¹² This causes the high reactivity of these positions to nucleophilic reagents (such as H_2O), which results in the formation of colorless forms, *i.e.*, fading of the solution colors.

In the simple MO theory, the quantity η is half the energy gap between the HOMO and the LUMO. Stable molecules have a large HOMO-LUMO gap and reactive molecules have a small HOMO-LUMO gap. The superdelocalizability index, S_2^N , for nucleophilic attack on position C-2 of the flavylium chromophore is the appropriate reactivity index.²⁸ A higher value of the S_2^N index indicates that the nucleophilic attack will proceed more easily.

If a single H atom in the 4-carboxyflavylium chromophore is replaced by an OH group, then some useful conclusions can be drawn from the relationship between the absolute hardness and S_2^N index of the compounds obtained (see Figure 3). For example, one can see that high reactivity and low stability are related to the presence of an OH group in position C-3.

However, if an OH group is introduced in position C-7, it is expected that such flavylium salt will be much more stable. The influence of various substituents (OH, OCH_3 , CH_3), their positions and combinations on the stability of 4-carboxyflavylium chromophore were studied. We have calculated electronic absorption spectra for a number of postulated structures, both cationic and anhydrobase forms. Figure 4 presents the model compounds which are predicted to exhibit high stability, low reactivity and stable red coloration in slightly acidic and neutral solutions.

	AH ⁺ form		A-7 form		A-4' form	
	λ_{\max} nm	f	λ_{\max} nm	f	λ_{\max} nm	f
	528.6	0.631	—	—	586.6	0.195
	525.7	0.049	—	—	559.3	1.533
	377.6	0.513	—	—	371.8	0.020
	473.6	1.209	531.4	1.363	505.1	1.786
	370.2	0.057	374.8	0.087	361.6	0.100
	326.2	0.150	339.6	0.102	354.0	0.019
	479.7	1.234	542.7	1.366	518.3	1.889
	375.7	0.041	386.9	0.077	429.3	0.009
	342.3	0.005	347.5	0.003	370.3	0.055

The A-7 (A-4') form is the anhydrobase formed by loss of a proton from the hydroxy groups at C-7 (C-4')

Figure 4. Proposed stable red 4-carboxyflavylium model compounds. Calculated absorption maxima in UV-vis spectra λ_{\max} (in nm) and the corresponding oscillator strengths (f).

CONCLUSION

In this paper, the stabilities and spectral properties of many postulated 4-carboxyflavylium structures were calculated. On the basis of this calculations, model compounds are proposed, predicted to exhibit improved stability and red coloration. Thus, unlike natural anthocyanins, these 4-carboxyflavylium salts could be of advantage to coloring foods in slightly acidic and neutral media.

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SAŽETAK

4-Karboksiflavilijeve soli: postojana crvena bojila?

D. Davidović-Amić, D. Amić i N. Trinajstić

Proučavan je utjecaj supstitucije u prstenu na stabilnost i spektralne značajke sintetskih 4-supstituiranih flavilijevih soli. Predloženo je nekoliko modelnih 4-karboksiflavilijevih soli za koje se očekuju povećana stabilnost i pojačano crveno obojenje u blago kiselim i neutralnim otopinama.