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Substituent effects on the pH-dependent multiequilibria of flavylum salt analogs of anthocyanins

Adilson A. Freitas^{a*}, Luis G. Dias^b, António A.L. Maçanita^a
and Frank H. Quina^c



Substituent effects on the hydration, tautomerization, and isomerization equilibria of flavylum salts can be described by a series of linear free energy relationships (LSER) based on Hammett correlations. The positions on the flavylum rings were classified as either activated (*para*-like) or nonactivated (*meta*-like) to decide which σ value to employ (σ_R or σ_{m^+} , respectively), while the steric effects of substituents at C-3 were included via the E_s parameter. Based on these relationships, we then show that it is possible to predict values of the “apparent pK_a ” (pK_{ap}) of flavylum ions that were not included in the original data set, as well as those of several naturally occurring anthocyanins. The value of pK_{ap} provides a measure of the thermodynamic stability of the flavylum cation as a function of pH and is directly related to the pH range in which the color of the flavylum cation form of anthocyanins persists in aqueous solution. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: anthocyanins; flavylum salt; hydration reaction; linear free energy relationships; substituent effects

INTRODUCTION

Flavylum cations (2-phenyl-benzopyrylium ions) are the synthetic analogues of anthocyanins, the major group of water-soluble pigments in the plant kingdom.^[1] In the last two decades, these compounds have been studied in applications such as laser dyes,^[2] thermochromic sensors,^[3] chemical actinometers,^[4] optical media for information storage,^[5] dye-sensitized solar cells^[6,7] and solvatochromic gel sensors for humidity, ammonia, and volatile organic compounds.^[8,9] Paradoxically, the richness of the chemistry of anthocyanins and flavylum salts hampers such potential applications. In aqueous media at pH below 2, the flavylum cation, AH^+ , is the sole species present in solution (Scheme 1). In weakly acidic and neutral media, however, AH^+ converts to the quinonoidal base A (if a deprotonable OH group is present) and to the hemiketal species B (formed by hydration of AH^+), which subsequently ring opens to the *Z*-chalcone C_Z (tautomerization of B) that can then isomerizes to the *E*-chalcone C_E . The *E*-chalcone is the thermodynamically most stable species in most cases. If additional OH groups are present, ionized forms of the quinonoidal base, the hemiketal and the chalcone isomers may also be present at higher pHs.^[10] All of these equilibria are dependent on the substitution pattern of the compound. For instance, flavylum cations with methyl, phenyl, or carboxyl groups at the C-4 position are particularly stable with respect to the hydration reaction; OH groups at C-5 also stabilize the cation.^[11–14] Consequently, understanding the role of substituents on flavylum salt reactivity is fundamental for the development of new compounds with tailored stabilities and properties.

Amic and Trinajstić^[12] and subsequently Lietz *et al.*^[15] treated flavylum cation hydration in terms of the concept of chemical

hardness. Amic *et al.*^[16] also employed QSAR/QSPR models for prediction of, among other properties, the pK_{ap} of flavylum salts. The pK_{ap} or “apparent pK_a ” values are functions of the individual equilibrium constants,^[17] and can be expressed as:

$$pK_{ap1} = -\log(K_a + K_h + K_h K_t)$$

when chalcone isomerization is very slow or the *E*-isomer nonexistent or as

$$pK_{ap2} = -\log(K_a + K_h + K_h K_t + K_h K_t K_i)$$

when the equilibrium amount of *E*-chalcone is significant. The value of pK_{ap} provides a measure of the thermodynamic stability of the flavylum cation as a function of pH^[11] and is directly related to the pH range in which the color of the flavylum ion form of anthocyanins persists in aqueous solution. However, the pK_{ap} provides no information about the mechanism or the substituent effects on each of the individual chemical equilibria.

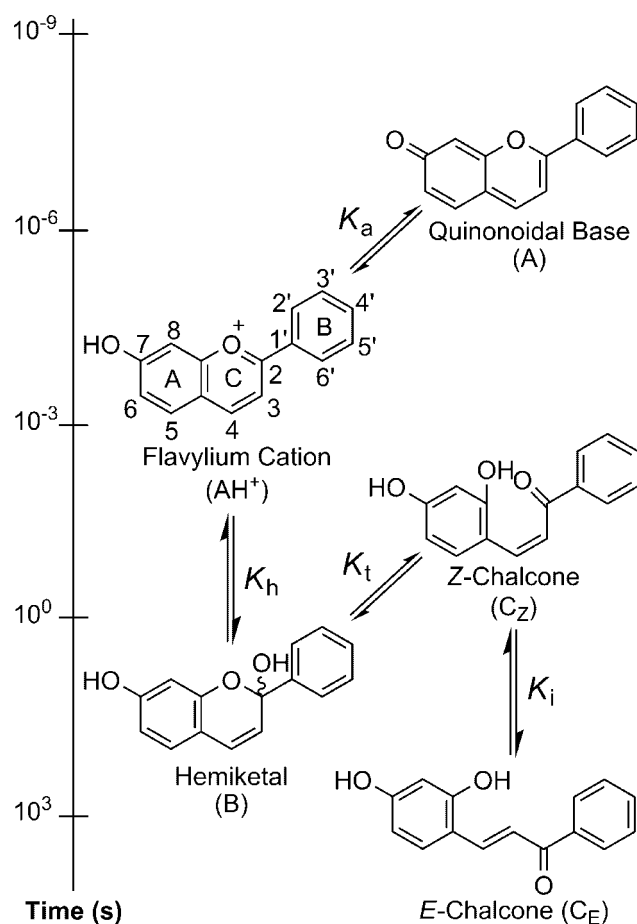
With respect to natural anthocyanins, Redus *et al.*^[18] employed Hammett correlations to explain the hydration of cyanidin

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Scheme 1. Chemical transformations of the flavylium salts and the approximate timescale of the reactions.

3-*O*- β -galactoside derivatives (equilibrium constant K_h and rate constants k_h and k_{-h}) in which the substituents were in the β -galactose residue. Recently,^[19] we described a procedure for evaluating the acidity constants, pK_a , and the one-electron reduction potentials of synthetic flavylium salts by quantum chemical calculations. In the present work, we develop a series of linear free energy relationships (LSER), based on Hammett σ values plus a steric parameter, for the three individual equilibria of flavylium ions, i.e., hydration, tautomerization, and chalcone isomerization. Using these relationships, we then show that it is possible to predict values of pK_{ap} for flavylium ions that were not included in the original data set and for several naturally occurring anthocyanins.

METHODOLOGY

Selection of the data set

The equilibrium constant values were culled from the literature and refer to data obtained in water at 20–25 °C. Particular attention was paid to exclude pseudo or apparent equilibrium constants from the data set, as well as data evaluated in water–organic solvent mixtures, in micellar media, at high buffer concentrations or in the presence of copigments.

Hammett correlations

The correlations were carried out by employing Hammett σ parameters combined with the Taft steric hindrance parameter E_s and equations of the type $\log(K_X/K_X^0) = \rho\Sigma\sigma + \delta E_s$.

Multiple regression analysis was performed with Excel and the goodness of fit was evaluated via statistical parameters such as the square of the correlation coefficient R^2 ($0 \leq R^2 \leq 1$, where $R^2 = 1$ is a perfect correlation), the standard errors of the coefficients, and the *F*-test.

Minimization of the flavylium salt structures

Geometry optimizations of the substituted flavylium cations were performed without any geometry constraint with the hybrid functional mPW1PW91^[20] in vacuum using the 6-31+G(d,p) basis set. This functional was selected based on our previous study,^[19] which found that the electronic transitions of flavylium cations and quinonoidal bases calculated with this functional were in better agreement with experimental results than those calculated with B3LYP. Harmonic frequency calculations indicated that all stationary points were minima (no imaginary frequencies) on the electronic potential energy surface. The electronic contribution to the gas phase free energy was obtained by single-point calculations with the 6-311+G(2d,2p) basis set. All computational calculations were carried out with the Gaussian03 package.^[21]

RESULTS AND DISCUSSION

Chemical transformations of the flavylium salts

Table 1 lists the hydration (pK_h), tautomerization (K_t), and isomerization (K_i) equilibrium constants of the compounds employed in this study, as well as the positions of the substituents on the flavylium cation structure (Scheme 1). Particularly noteworthy is the high degree of correlation ($R^2 = 0.904$) between pK_h and $\log(K_t)$ shown in Fig. 1, which points to opposite substituent effects on these two equilibria.

Inspection of the pK_h values in Table 1 shows that, in all cases, the presence of electron-donating groups in rings A and/or B stabilizes the flavylium cation relative to the unsubstituted compound, **1**, presumably reflecting the increased electron density of the molecule. The pK_h values for **2**, **3**, **10**, and **11** clearly show that the inclusion of an OH group at positions C-6 or C-8 increases pK_h less than a hydroxyl group at C-7 or C-4'. Comparing the hydration constants of compounds **1** and **7** with those of **12** and **14** indicates that a methyl group at C-3 reduces the pK_h of flavylium salts by ca. 2 units. The inclusion of a methyl group at C-3 enhances the torsion angle between the B and C rings, reducing the charge delocalization into the B ring and facilitating nucleophilic attack at C-2.

The K_t values for the hemiketal/Z-chalcone tautomerization indicate that electron-donor substituents shift the equilibrium towards the Z-chalcone form. The electron delocalization in C_z and the presence of the electron-accepting C=O group permit a favorable conjugation with electron-donating substituents, which has no equivalent in the hemiketal. The inclusion of a methyl group at C-3 decreases K_t by a half, as shown by compounds **1**, **14**, **7**, and **12**.

Substituents at C-3 and C-4 hinder the formation of the E-chalcone.^[11,29] Compounds **4** and **5** have large pK_h values, but K_i is negligible, pointing to the effect of the substituent at position

Table 1. Flavylum salts studied (Scheme 1) and equilibrium constants measured in water at 20–25 °C

Compound	R3	R4	R6	R7	R8	R4'	pK _h	K _t	K _i
1	H	H	H	H	H	H	3.01 ^a	0.06 ^a	400 ^b
2	H	H	H	OH	H	H	5.10 ^b	0.48 ^e	500 ^b
3	H	H	H	H	H	OH	5.44 ^b	1.00 ^b	3500 ^b
4	H	Me	H	OH	H	H	5.97 ^c	2.00 ^f	—
5	H	COO [−]	H	OH	H	OMe	6.37 ^c	7.08 ^c	—
6	H	H	H	H	H	Me	3.65 ^a	0.16 ^a	800 ^g
7	H	H	H	H	H	OMe	4.47 ^a	0.50 ^a	—
8	H	H	H	H	H	NH ₂	7.55 ^d	21.6 ^d	130 ^d
9	H	H	H	H	H	NHCOMe	3.68 ^d	0.19 ^d	14 ^d
10	H	H	OH	H	H	H	4.00 ^h	0.57 ^h	25 ^h
11	H	H	H	OH	OH	H	4.64 ^j	—	600 ^j
12	Me	H	H	H	H	OMe	2.36 ^k	0.20 ^k	—
13	Me	H	H	OH	H	OH	4.02 ^j	0.53 ^j	1.2 ^j
14	Me	H	H	H	H	H	1.22 ^k	0.02 ^k	—

^a Ref. [10].
^b Ref. [22].
^c Ref. [23].
^d Ref. [17].
^e Evaluated from equilibrium constants in Ref. [22].
^f Ref. [24].
^g Ref. [25].
^h Ref. [26].
^j Ref. [27].
^k Ref. [28].

C-4. Thus, K_i data for compounds with substituents at the C-4 position are absent in Table 1.

Linear free energy relationships (LFER) for the equilibrium constants K_h and K_t

In applying Hammett LFERs to flavylum salts, we examined the resonance structures of AH⁺ to separate the carbon atoms of the rings into two classes: (1) activated positions, to which the positive charge on C-2 can be delocalized via resonance

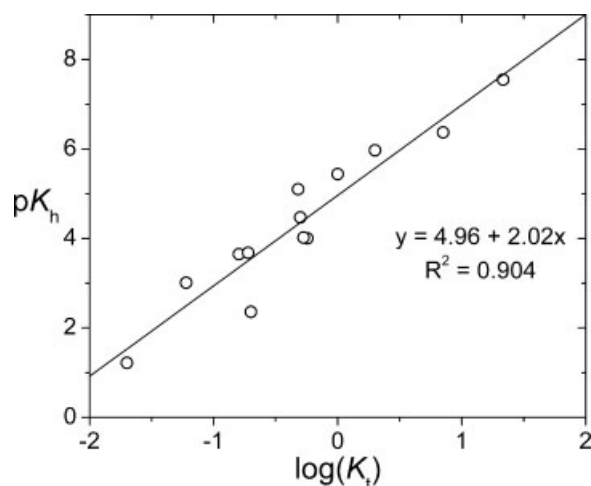


Figure 1. Correlation between hydration equilibrium pK_h and the logarithm of the tautomerization equilibrium constant K_t. The straight line corresponds to the linear regression.

(positions C-4, C-5, C-7, and C-4'); and (2) nonactivated positions, to which the positive charge is not delocalizable by resonance (positions C-3, C-6, C-8, and C-3'). Because the effect of the former type of substituent should be analogous to that of a *para* substituent in a benzene ring system, we employed the values of σ_p for substituents at these positions. For substituents at the nonactivated positions, we employed the values of σ_m . The electronic effect of *para*-like substituents can be further factored into inductive σ_I and resonance σ_R components^[32] ($\sigma_p = \sigma_R + \sigma_I$) in order to decide whether resonance or the inductive effect makes the greater contribution to the reactions of flavylum cations.

After testing different sets of σ parameters for K_h and K_t with the condition of maintaining a common set for both reactions, the best group found was the following: σ_I for substituents at C-3, σ_m for those at C-6 or C-8, and σ_R for those at C-7, C-4, or C-4' (σ_R^+ for the amino group). The steric parameter value of $E_s = -1.24$ was employed for compounds with methyl groups located at the C-3 position, which exerts an important steric effect. When the flavylum cation had more than one substituent, the appropriate σ values of each substituent were summed to give a combined value, i.e., the effect of multiple substituents on the reactivity was considered to be additive. Table 2 lists the Hammett σ parameters for each substituent employed in this study.

For the hydration of AH⁺, we found the correlation:

$$\log\left(\frac{K_h}{K_h^0}\right) = 5.49\Sigma\sigma - 1.83E_s \quad (R^2 = 0.845)$$

with σ_p and σ_m as descriptors for the activated and nonactivated sites, respectively (Supplementary Information). This correlation points to a common reaction mechanism for the hydration of the

Table 2. Hammett σ parameters for the substituents

Substituent	σ_p	σ_m	σ_l	σ_R
Me	-0.17	-0.07	0.01	-0.18
OMe	-0.27	0.12	0.29	-0.56
OH	-0.37	0.12	0.33	-0.7
O ⁻	-0.81	-0.47	-0.26	-0.55
COO ⁻	0	-0.1	-0.1	0.1
NH ₂	-0.66	-0.16	0.08	-1.38 ^a
NHCOMe	0	0.21	0.31	-0.31

The σ -values were compiled from reference [32].
^aThrough-conjugation parameter σ_R^+ ($\sigma_R^+ = \sigma^+ - \sigma_l$); $\sigma_R = -0.74$ for NH₂.

flavylium cation, sensitive to the pattern of substitution. The large positive value of $\rho = 5.49$ clearly reflects the decrease in electron delocalization when going from the flavylium ion (highly sensitive to substituent effects) to the hemiketal (much less

sensitive). Consequently, electron-donor substituents stabilize the flavylium cation in relation to the hemiketal form. The correlation with σ_R instead of σ_p , $\log(K_h/K_h^0) = 2.93\Sigma\sigma - 1.90E_5$ (presented in Fig. 2a), follows the same trend observed for σ_p , but with a better correlation coefficient ($R^2 = 0.901$ and reduced standard errors) and a smaller ρ value. The deviation observed for compound **8** when σ_R was employed was greatly diminished by utilizing the Hammett parameter for the NH₂ group modified to take into account the 'through-resonance' ($\sigma_R^+ = -1.38$)^[32,33]. Furthermore, the inductive effect is only relevant for substituents at nonactivated positions and the Hammett correlation can be substantially improved by replacing the σ_m parameter for the 6-OH group (0.12) of compound **10** by that for a more electron-rich oxygen, i.e., by using the value of σ_m for O⁻ (-0.47) for this group. For compounds **2** and **13**, replacement of σ_R (-0.70) for the 7-OH group by the value of σ_R for O⁻ (-0.55) also improved the final correlation $\log(K_h/K_h^0) = 3.02\Sigma\sigma - 1.83E_5$, $R^2 = 0.960$ (presented in Fig. 2b). The use of σ_m and σ_p values for a more electron-rich oxygen than OH for **10**, **2**, and **13** suggests

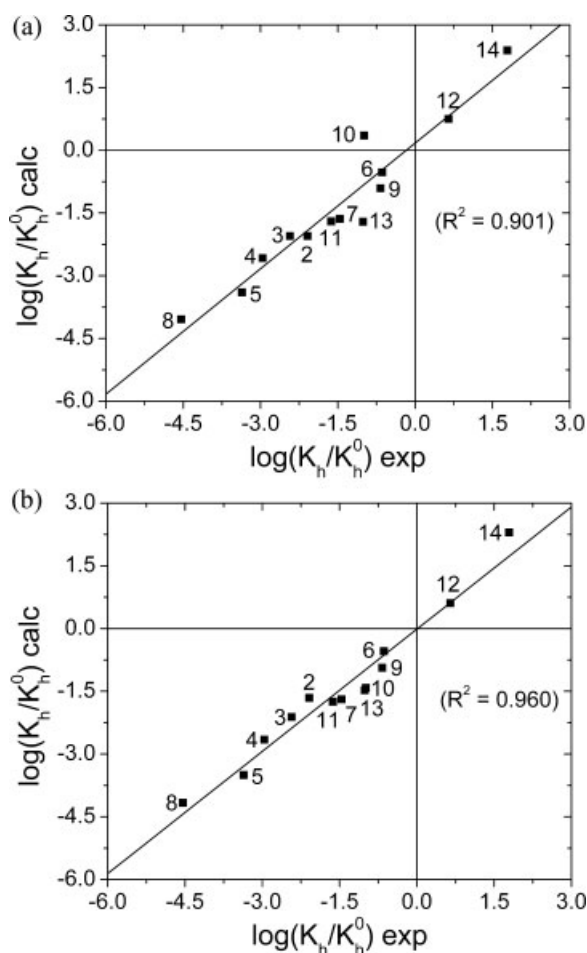


Figure 2. Correlations between the experimental hydration equilibrium constants K_h for the flavylium cations and the corresponding values calculated from the Hammett equation employing: (a) σ values for 6- and 7-OH groups for compounds **2**, **10**, and **13** or (b) σ values for 6- and 7-O⁻ groups for these three compounds. The straight lines correspond to the best-fit multiparametric regression line. A summary of the statistical data is given in Supplementary Material.

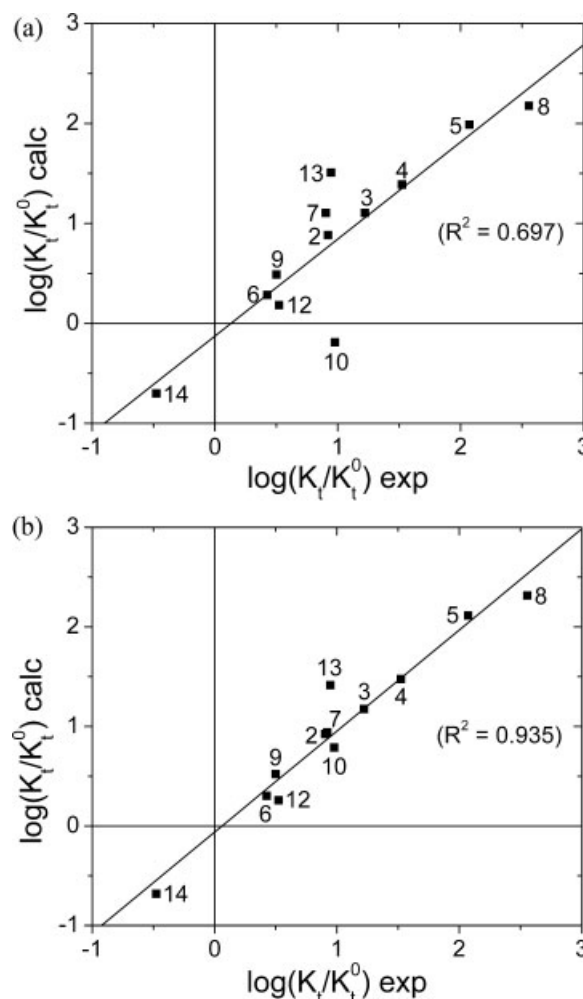


Figure 3. Correlations between the experimental tautomerization equilibrium constants K_t and the corresponding values calculated from the Hammett equation employing: (a) σ values for unionized OH groups for compounds **2**, **10**, and **13** or (b) σ values for ionized OH groups for these three compounds. The straight lines correspond to the best-fit multiparametric regression line. A summary of the statistical data is given in Supplementary Material.

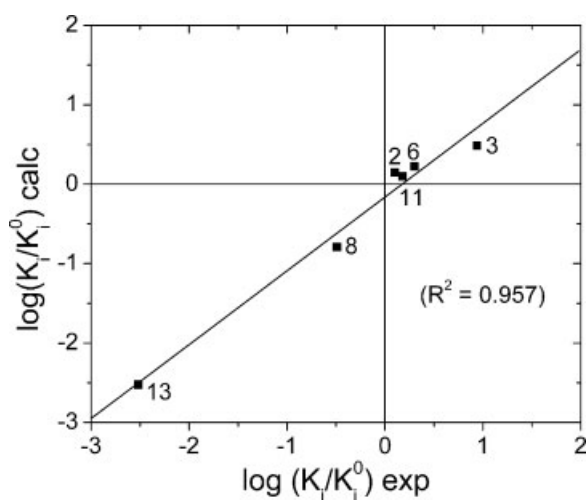


Figure 4. Relationship between the experimental C_Z-C_E isomerization equilibrium constants K_t and the corresponding values calculated from the Hammett equation.

that the corresponding pK_h values of these compounds have a contribution from quinonoidal and hemiketal-like resonance structures, respectively. This interesting result indicates that the same reaction mechanism prevails for both quinonoidal bases and flavylum cations.^[31] The Supplementary Material summarizes the coefficient values, the standard errors and the regression statistics. The partial F -test values show that both the steric and electronic descriptors are statistically important in the correlations.

When σ_p and σ_m are employed as descriptors, the value of ρ for the hydration of flavylum salts (5.49) is slightly smaller than those for hydration of 6- and 7-substituted benzopyrylium ions ($\rho = 5.64$ and 5.90, respectively).^[34] The fact that flavylum cations are 2-phenyl-benzopyrylium ions points to the importance of charge delocalization into the phenyl ring for AH^+ stability. Indeed, compounds with a methyl group at C-3, which reduces conjugation with the phenyl ring, have considerably lower pK_h values than other substituted flavylum ions, reflected in the correlation as a large negative coefficient δ of the steric hindrance term.

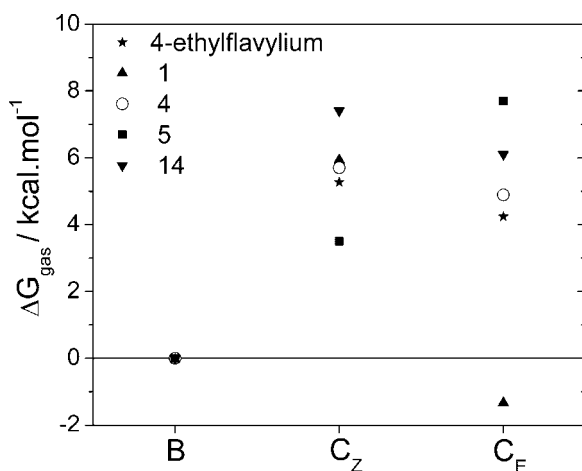


Figure 5. Relative free energies calculated in vacuum (kcal/mol) for the hemiketal (B), Z-chalcone (C_Z) and E-chalcone (C_E) forms of several flavylum salts.

Resonance structures show that the activated positions for tautomerization remain the same as those for the hydration reaction. These expectations are mirrored in the LSER equation for K_t obtained by using σ_p and σ_m as descriptors for groups at activated and nonactivated positions plus the E_s parameter for those at C-3:

$$\log\left(\frac{K_t}{K_t^0}\right) = -2.95\Sigma\sigma + 0.15E_s$$

Nonetheless, the overall quality of the correlation ($R^2 = 0.562$) was substantially lower than that found for K_h (Supplementary Information). Figure 3 shows plots of the correlation between experimental and calculated tautomerization equilibrium constants K_t using the same σ values employed for the correlations of K_h . Despite the difficulties in obtaining

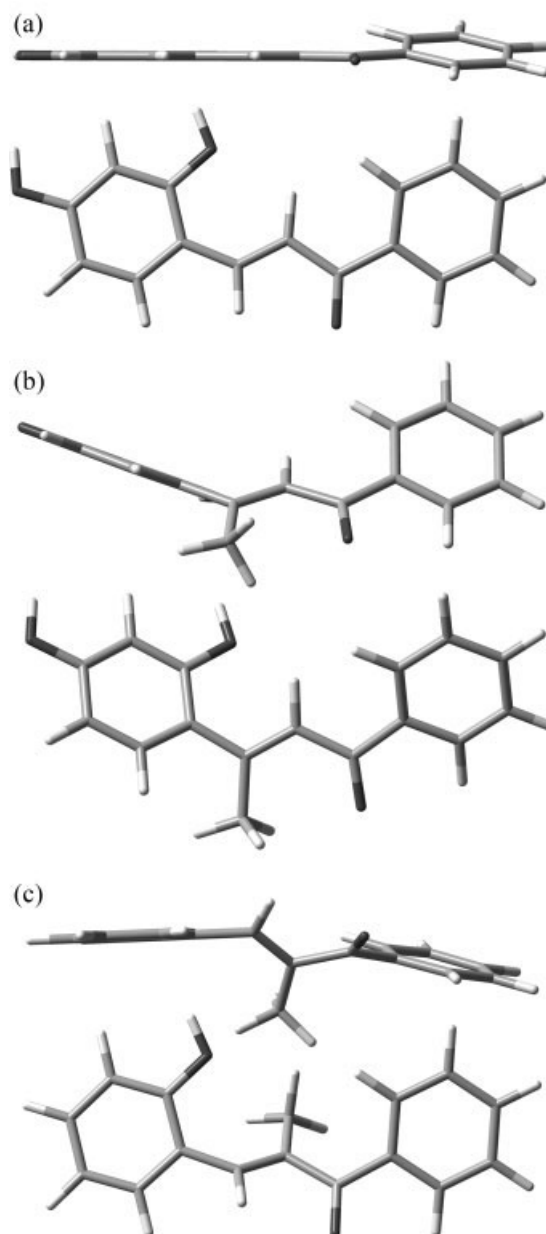


Figure 6. Side and top views of the optimized geometries in vacuum of the E-chalcone isomer of compounds (a) 2, (b) 4, and (c) 14.

accurate equilibrium constants for tautomerization, Figure 3a shows a reasonable correlation $\log(K_t/K_t^0) = -1.58\sum\sigma + 0.55E_S$ ($R^2 = 0.697$), indicating that resonance plays an important role in tautomerization. Again, the quality of the correlation $\log(K_t/K_t^0) = -1.68\sum\sigma + 0.55E_S$ increased substantially ($R^2 = 0.935$, Fig. 3b) when σ values for ionized OH groups were used for compounds **2**, **10**, and **13**. This phenomenon suggests a contribution of deprotonated *cis*-chalcones to K_t (due to extended electron delocalization in the phenolate anions, *cis*-7-hydroxychalcones must indeed be more acidic than the corresponding hemiketals). The statistical data, summarized in the Supplementary Material, confirm the robustness of the multiparametric regressions.

The negative ρ coefficient reflects the recovery of at least partial conjugation between the A and B rings upon going from the hemiketal to the Z-chalcone and substituents in either ring contribute to the stabilization of the latter. The steric coefficient δ is much less important for the keto-enol tautomerization of flavylum salts, presumably reflecting the lack of coplanarity of the hemiketal form, even without substituents at C-3.

Linear free energy relationship (LFER) for the equilibrium constants K_i

The experimental K_i constants in Table 1 indicate that substituents in both rings affect the value of K_i . Assuming that a simple Hammett equation is valid for each ring,^[30] the following

modified equation $\log\left(\frac{K_i}{K_i^0}\right) = \rho_A\sigma_A + \rho_B\sigma_B + \delta E_S$ was employed,

where the index A refers to substituents in the phenyl ring of the chalcone and the index B to substituents in the benzoyl ring. The values of σ_p and σ_m were employed for substituents at C-7 and C-8, respectively, σ_p for substituents located at C-4' and $E_S = -1.24$ for a CH_3 group at C-3. Compounds **9** and **10** were outliers in all attempts to correlate the substituent effect on the isomerization equilibrium; since natural anthocyanins do not have substituents at C-6 nor do they have acetamide substituents at C-4', these two compounds were excluded. Figure 4 shows the resultant correlation $\log(K_i/K_i^0) = -0.40\sum\sigma_A - 1.32\sum\sigma_B + 2.54E_S$

($R^2 = 0.957$) for K_i and the Supplementary Material gives the regression statistics. The partial *F*-test indicates that substituents at C-7 in ring A have little influence on the isomerization equilibrium. Necessarily, however, any equation with three parameters and only six observables must be regarded with caution and the results as qualitative at best.

Figure 5 presents the calculated free energy profiles in vacuum for the hemiketal and chalcone forms of some of the flavylum salts studied here, as well as for 4-ethylflavylum. Although these results should be regarded as qualitative because solvent effects were not taken into account, the profiles show that the relative energy of the C_E isomer is strongly influenced by the presence of substituents at C-3 and C-4. Indeed, as pointed out earlier, a substituent at C-4 strongly inhibits hydration and tautomerization of the flavylum cation form in water. These calculations^[19] also reveal that the main difference between the substituted and unsubstituted chalcones lies in the geometry of the C_E isomer. The molecular structure of C_E is almost planar for unsubstituted compounds, but is nonplanar when a substituent is located at C-3 or C-4 (Fig. 6). These computational results are consistent with the LFER for isomerization, which points to a large steric stabilization of the *E*-chalcone and a greater effect of substituents in the benzoyl ring (derived from the B ring of the flavylum ion).

Estimation of substituent effects on the pK_{ap} of flavylum salts and anthocyanins

Taken individually, the correlations of the substitution effects on the multiequilibria of the flavylum salts provided by the LFERs developed here might facilitate studies of the pH-dependent speciation of flavylum ions in aqueous solution. However, more importantly, by combining the correlations for all three equilibria, one can in principle begin to predict the thermodynamic stability of flavylum cations and, hence, of anthocyanins in aqueous solution. It is known that when significant amounts of the *E*-chalcone are present at equilibrium, the "apparent pK_{ap} " is given by $\text{pK}_{\text{ap}2} = -\log(K_a + K_h + K_h K_t + K_h K_i K_t)$. On the other hand, if the *E*-chalcone is not formed or under pseudo-equilibrium conditions when isomerization is

Table 3. Experimental pK_{ap} and pK_a for some synthetic flavylum salts and natural anthocyanins^a

Compound	pK_{ap}	pK_a	Ref.	Compound	pK_{ap}	pK_a	Ref.
7,4'-OH	3.05	4.30	[24]	4'-OMe	3.95 ^c	—	[15]
5,7,4'-OH	4.00	4.23	[24]	4'-N(Me) ₂	5.97 ^c	—	[15]
5,7-OH,4-Me,4'-OMe	4.10 ^c	4.3	[24]	7-OMe	2.54	—	[15]
7-OH,4-Me,4'-OMe	4.80 ^c	4.85	[24]	7-OMe,4'-Me	2.69	—	[15]
7,3',4'-OH	2.91	4.35	[35]	7,4'-OMe	3.08	—	[15]
Oenin (5,7,4'-OH,3',5'-OMe,3-OGl)	2.50 ^b	3.7	[36,13]	7-OMe,4'-N(Me) ₂	4.83 ^c	—	[15]
Malvin (7,4'-OH,3',5'-OMe,3,5-OGl)	1.80 ^b	3.88	[36,37]	7-N(Et) ₂ ,4'-Me	5.25 ^c	—	[15]
Cyanin (7,4'-OH,3'-OMe,3,5-OGl)	2.10 ^b	4.09	[36,37]	7-N(Me) ₂ ,4'-OMe	5.51 ^c	—	[15]
7-OH,4'-N(Me) ₂	3.00	4.10	[38]	7-N(Et) ₂ ,4'-OMe	5.28 ^c	—	[15]
7,4'-OH, 5-OMe	3.80	4.00	[39]	7,4'-N(Me) ₂	6.10 ^c	—	[15]
8,4'-OMe	3.65 ^c	—	[16]	7,4'-OMe,4-Ph	5.44 ^c	—	[15]
4'-Me	3.43 ^c	—	[15]				

^a The σ and E_S parameters were taken from Refs. [32,33], respectively.

^b Using the parameters of *t*-butyl as surrogates for the *O*-glucoside residue.

^c Calculated as $\text{pK}_{\text{ap}1} = -\log(K_a + K_h + K_h K_t)$.

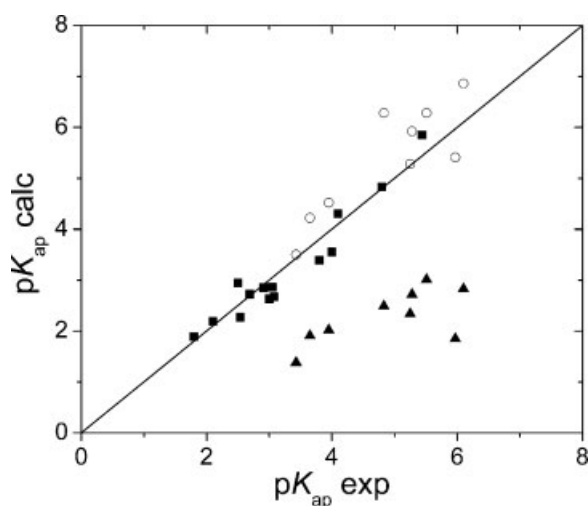


Figure 7. Relationship between experimental and calculated apparent pK_{ap} for some flavylum salts and anthocyanins reported in the literature. The full squares and triangles depict $pK_{ap2} \text{ calc} = -\log(K_{ap} = K_a + K_h + K_h K_t + K_h K_t K_i)$; the open circles are values of $pK_{ap1} \text{ calc} = -\log(K_{ap} = K_a + K_h + K_h K_t)$ calculated for the triangle dataset. The straight line represents $x = y$ and was included just to guide the eye.

very slow and the *E*-chalcone has not yet been formed, $pK_{ap1} = -\log(K_a + K_h + K_h K_t)$. Since K_h , K_t , and K_i can be estimated from the Hammett correlations given above, the only additional piece of data needed is the pK_a of the flavylum cation (when it is deprotonable). Experimentally, the value of pK_a can be determined by rapidly measuring the absorption spectra of the flavylum ion as a function of solution pH before significant hydration occurs or from the pH dependence of the apparent hydration rate constant, especially with naturally occurring anthocyanins. Alternatively, one can calculate the pK_a rather well by quantum chemical methods.^[19] Table 3 lists the experimental pK_{ap} and pK_a for some synthetic flavylum salts and for several natural anthocyanins.

Figure 7 shows the relationship between experimental and calculated values of pK_{ap} . When the *E*-chalcone was assumed to be formed for all of the flavylum salts listed, some of the compounds clearly deviated by ~ 2 pK units from the experimental value. This was expected for 4-substituted compounds, where the amount of *E*-chalcone in solution is typically very small and the simplified equation for pK_{ap1} should be used. The application of the pK_{ap1} equation to all the outliers corrected the offset, indicating that the values reported for these compounds are those for the pseudo-equilibrium condition.

It should be noted that the pK_{ap} correlation shown in Fig. 7 includes compounds with substituents at positions that were not represented in the data set used to establish the Hammett relationships. This emphasizes the robustness and the consistency of the Hammett relationships and the conclusions regarding the electronic and steric effects of substituents at activated and nonactivated positions on K_h , K_t , and K_i . Finally, quite good results were obtained for several natural anthocyanins (oenin, malvin, and cyanin) with an *O*-glucoside residue attached to the C-3 carbon. In this case, the electronic and steric parameters of the *t*-butyl group proved to be adequate surrogates for representation of the effects of an *O*-glucoside group. In fact, *t*-butyl must be bulkier but, unlike *O*-glucoside, is weakly electron donating rather than weakly electron withdrawing by the

inductive effect. Apparently, these differences in steric and electronic effects cancel out.

CONCLUDING REMARKS

Substituent effects on the position of the hydration, tautomerization, and chalcone isomerization equilibria of flavylum salts can be correlated by LFERs employing Hammett σ parameters. The positions on the flavylum rings are classified as either activated (*para*-like) and nonactivated (*meta*-like) to decide which σ value to employ (σ_R or σ_m , respectively) and a simple additivity of the σ values is assumed when multiple substituents are present. Steric effects of substituents at C-3 are included via the E_s parameter. Finally, the resultant Hammett relationships are shown to be a potentially important tool for predicting the pH range of color stability (directly related to the value of pK_{ap}) of new flavylum salts and of naturally occurring anthocyanins. This last application can be improved by expanding the study of the steric effects to position C-2' and by determination of the isomerization equilibrium constants for a much wider variety of flavylum ion-derived chalcones. Unfortunately, kinetic and thermodynamic data for the latter are limited, which reflects the difficulties of obtaining reliable results in such complex multiequilibrium systems.

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