A Theoretical Study of Flavylium Salts

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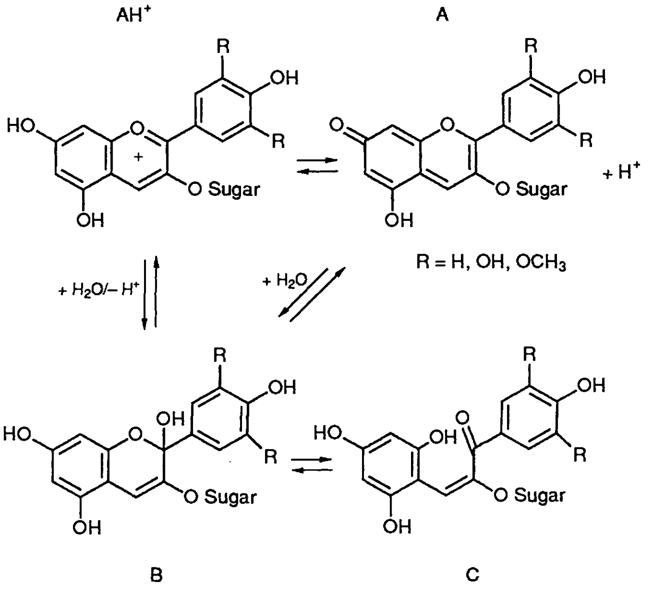
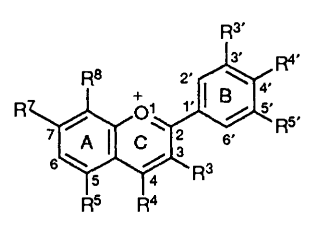
The aromatic stabilities of cationic and anhydrobase forms of 30 synthetic flavylium salts are predicted using the concepts of absolute hardness and relative hardness. These predictions are supported by other theoretical measures of aromaticity and reactivity, and are in good agreement with experimental findings. A flavylium salt model compound is also proposed, which is expected to exhibit high stability and low reactivity.

During the last decade a large number of papers have been published dealing with the study of the flavylium chromophoric system.[1] The reason for this is the wide distribution of flavylium salts in the plant kingdom and the immeasurable number of orange, red and blue hues they give to flowers, fruits and vegetables. When separated from cell sap the flavylium salts (anthocyanins and anthocyanidins) are stable only in strongly acidic solutions.[2] In a weakly acidic medium, equilibrium between two coloured forms (flavylium cation AH^+ and anhydrobase A) and two colourless forms (pseudobase B and chalcone C) is rapidly achieved, resulting in discoloration[3] (see Scheme 1). The weak colour of flavylium salt solutions in the pH interval 4–6 is the main limitation for the application of these plant pigments in the food industry, as substitutes for the potentially carcinogenic food colourants currently used. On the other hand, synthesized flavylium salts, which are not found in nature, demonstrate higher colour stability.[4,5] Recently, natural flavylium salts which maintain their coloration over a wider pH interval have been isolated from vegetable material, and mechanisms explaining the stability of these natural flavylium salts have been suggested.[6,7] In spite of numerous efforts, however, flavylium salts which could be widely used as food colourants have not yet been developed.



Scheme 1

This study is a continuation of our previous work,[8–10] and reports an attempt to design a substituted model flavylium chromophore which would have high stability and low reactivity. In order to achieve this, the aromatic stabilities and reactivities of 30 flavylium salts were studied. In Table 1 the structures of the investigated flavylium salts **1**–**29** are given.



1–29

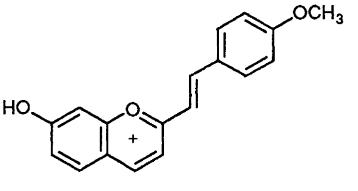


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Table 1 Structures of studied flavylium salts **1**–**29**

Compound	Substituent							
	R-3	R-4	R-5	R-7	R-8	R-3′	R-4′	R-5′
1	H	H	H	OH	H	H	H	H
2	H	H	H	H	H	H	OH	H
3	H	H	H	OH	H	H	OH	H
4	H	H	H	OH	H	H	OCH_3	H
5	H	H	H	H	OCH_3	H	OCH_3	H
6	H	H	H	H	H	OCH_3	OH	H
7	H	H	OH	OH	H	H	OH	H
8	H	H	H	OH	H	OH	OH	H
9	H	H	H	OH	H	OCH_3	OH	H
10	H	H	H	OH	H	OCH_3	OCH_3	H
11	H	H	OCH_3	OH	H	H	OH	H
12	OH	H	OH	OH	H	H	OH	H
13	H	H	H	OH	H	OH	OH	OH
14	OH	H	H	OH	H	OCH_3	OH	H
15	H	H	OH	OH	H	OCH_3	OH	H
16	H	H	OH	OH	H	OH	OCH_3	H
17	H	H	H	OH	H	OCH_3	OCH_3	OCH_3
18	OH	H	OH	OH	H	OCH_3	OH	H
19	H	CH_3	H	OH	H	H	H	H
20	H	CH_3	H	OH	H	H	OH	H
21	H	CH_3	OH	OH	H	H	H	H
22	H	CH_3	H	OH	OH	H	H	H
23	H	CH_3	H	OH	H	H	OCH_3	H
24	H	CH_3	OH	OH	H	H	OCH_3	H
25	H	Ph	H	OH	H	H	H	H
26	H	Ph	H	OH	H	H	OH	H
27	H	Ph	CH_3	OH	H	H	H	H
28	H	Ph	H	OH	H	H	OCH_3	H
29	H	CO_2H	H	OH	H	H	OCH_3	H

Table 2 Calculated and experimental indices for flavylium salts[a]

Compound	Absolute hardness	Relative hardness	E_{HOMO}	TREPE	S_2^N	λ_{max} AH^+/ nm	pK
1	0.450	0.186	0.653	0.0277	2.441	429	2.70
2	0.445	0.183	0.642	0.0278	2.381	437	4.33
3	0.430	0.168	0.584	0.0235	2.197	458	3.30
4	0.440	0.176	0.606	0.0237	2.210	458	3.14
5	0.449	0.205	0.638	0.0250	2.317	434	3.65
6	0.396	0.165	0.542	0.0247	2.373	458	4.09
7	0.426	0.162	0.547	0.0201	2.041	468	4.23
8	0.384	0.152	0.492	0.0211	2.191	469	3.18
9	0.397	0.160	0.519	0.0212	2.191	469	3.20
10	0.413	0.172	0.552	0.0213	2.204	468	2.98
11	0.433	0.167	0.561	0.0202	2.050	462	3.92
12	0.363	0.136	0.420	0.0188	2.019	505	2.63
13	0.364	0.158	0.453	0.0190	2.183	475	3.07
14	0.355	0.145	0.434	0.0197	2.199	499	1.39
15	0.405	0.160	0.504	0.0182	2.036	482	4.17
16	0.407	0.162	0.508	0.0183	2.046	480	3.96
17	0.398	0.180	0.521	0.0193	2.197	458	2.56
18	0.354	0.139	0.403	0.0172	2.014	515	1.82
19	0.507	0.206	0.652	0.0286	1.810	414	4.44
20	0.487	0.185	0.579	0.0244	1.707	442	4.84
21	0.483	0.187	0.578	0.0243	1.724	433	4.38
22	0.413	0.163	0.457	0.0256	1.791	438	4.25
23	0.498	0.193	0.603	0.0246	1.712	444	4.80
24	0.489	0.184	0.556	0.0211	1.639	448	4.10
25	0.448	0.186	0.653	0.0320	2.413	429	4.27
26	0.428	0.168	0.584	0.0285	2.199	459	4.49
27	0.453	0.185	0.629	0.0309	2.218	432	3.32
28	0.438	0.176	0.606	0.0286	2.212	458	4.44
29	0.437	0.175	0.606	0.0197	2.222	458	4.30
30	0.367	0.133	0.517	0.0211	2.222	508	3.64

[a] For experimental data and preparative work see refs. 9, 19–25.

Indices of Aromaticity.—Pearson[11] has demonstrated that the absolute hardness, η, which is half of the HOMO–LUMO gap in Hückel theory, represents a good measure of aromaticity. It can be calculated according to eqn. (1) where I is the

$$\eta = (I - A)/2 = (E_{LUMO} - E_{HOMO})/2 \qquad (1)$$

ionization potential, A the electron affinity, E_{LUMO} the energy of the lowest unoccupied molecular orbital and E_{HOMO} the energy of the highest occupied molecular orbital. The greater the HOMO–LUMO gap, the higher the stability of the molecule.

Very recently, Zhou and Parr[12] demonstrated that the relative hardness, η_r, also represents a good measure of aromaticity. The relative hardness can be calculated from the energies of HOMO and LUMO orbitals of the investigated conjugated molecule and the corresponding hypothetical acyclic reference structure according to eqn. (2) where η_a is given by eqn. (3).

$$\eta_r = \eta - \eta_a \qquad (2)$$

$$\eta_a = (E^{ac}_{LUMO} - E^{ac}_{HOMO})/2 \qquad (3)$$

As has been emphasized by Zhou *et al.*,[13] absolute hardness is in fair correlation with the topological resonance energy per π electron, TREPE. TREPE[14] is the quantitative descriptor of aromaticity and is defined by eqns. (4) and (5) where $N(\pi)$ and N

$$\mathrm{TREPE} = \mathrm{TRE}/N(\pi) \qquad (4)$$

$$\mathrm{TRE} = \sum_i^N a_i\,(x_i - x_i^{ac}) \qquad (5)$$

are respectively the total number of π electrons and the number of π centres in a conjugated molecule. x_i And x_i^{ac} represent, respectively, the Hückel MO energies of a given molecule and the MO energies of the corresponding acyclic reference structure, whilst a_i is the orbital occupancy number.

The above indices of aromaticity were calculated using an Atari 1040ST computer and the appropriate computer program,[15] whilst the HOMO and LUMO, which also serve as indices of reactivity, were calculated by the standard HMO method.[16] Parameters for heteroatoms were taken from the literature.[17]

Results and Discussion

In Table 2, the following properties are given: absolute hardness, relative hardness, HOMO energy, TREPE, superdelocalizability indices[18] for nucleophilic attack on position C-2 of the cationic form AH^+ of the flavylium salts, absorption maximum of the cationic form AH^+ of the flavylium salts in UV–VIS spectra and pK values.

A good linear correlation ($r = 0.883^{**}$) exists between the absolute hardness and relative hardness (Fig. 1). For alternant conjugated hydrocarbons eqn. (1) reduces to $\eta = -E_{HOMO}$, so that E_{HOMO} can also serve as a measure of aromaticity.[26] Fig. 2 shows that there is a reasonable correlation between E_{HOMO} and the relative hardness ($r = 0.837^{**}$), better than the correlations with the absolute hardness ($r = 0.799^{**}$) and with TREPE ($r = 0.748^{**}$). From the above it appears that the relative hardness can also serve as a theoretical measure of aromaticity for the flavylium salts.

According to the definition, the absolute hardness represents

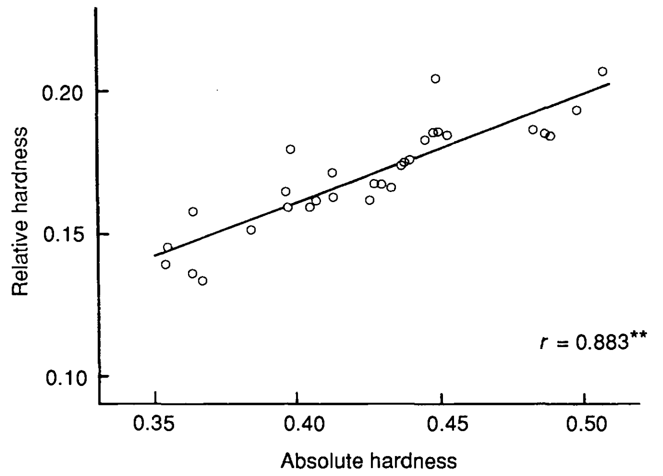


Fig. 1 Correlation of relative hardness (in $-\beta$) with absolute hardness (in $-\beta$) for a cationic form of flavylium salts. The correlation is significant at the 1% level.

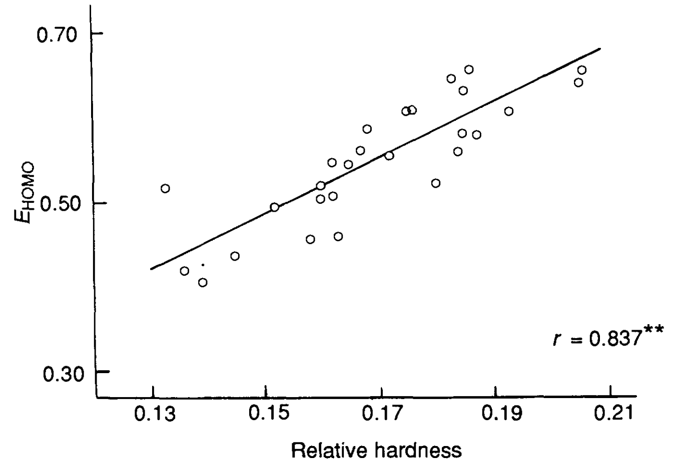


Fig. 2 Correlation of E_{HOMO} (in β) with relative hardness (in $-\beta$) for the cationic form of flavylium salts

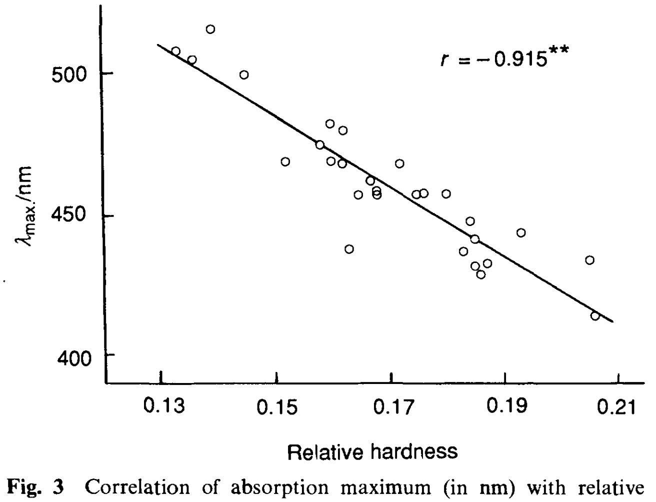


Fig. 3 Correlation of absorption maximum (in nm) with relative hardness (in $-\beta$) for a cationic form of flavylium salts

half of the energy gap between the highest occupied molecular orbital and the lowest unoccupied molecular orbital. A small energy gap means that the absorption maximum is shifted towards the red in the visible spectrum. Therefore, correlations between the aromaticity indices and UV–VIS absorption spectra could be established. There is a reasonable correlation between the absorption maximum of the cationic form AH$^+$ of flavylium salt with TREPE ($r = -0.760^{**}$), E_{HOMO} ($r = -0.808^{**}$) and absolute hardness ($r = -0.840^{**}$), but the best correlation is with the relative hardness ($r = -0.915^{**}$) (see Fig. 3).

The pK value of the overall transformation AH$^+$ $\rightleftharpoons$ (A + B + C) + H$^+$ can serve as an experimental measure of the stability of the cationic form AH$^+$ of flavylium salts. The pK values and the absolute hardness are proportional quantities, *i.e.*, the higher the pK values, the greater the absolute hardness. However, the correlation between these two quantities is not particularly good ($r = 0.682^{**}$).

Standard HMO calculations can be used to obtain the reactivity indices of flavylium salts. The superdelocalizability index, S_2^N, for nucleophilic attack on position C-2 of the flavylium chromophore is especially interesting. A higher value of the S_2^N index indicates that the hydration reactions will proceed more easily, which results in a discoloration of flavylium salt solutions. Although the correlation between the absolute hardness and the S_2^N indices for the cationic and anhydrobase form of flavylium salts is not particularly high some useful conclusions can be drawn. For example, one can see that high reactivity is correlated with low stability, and low reactivity with high stability. Generally, a cationic form can be associated with higher stability and lower reactivity. An anhydrobase form can be associated with lower stability and higher reactivity. We can arrive at the same conclusion if we apply the aromaticity criterion suggested by Zhou and Parr:[12] for aromatic molecules $\eta \geq 0.25$ and $\eta_r \geq 0.08$. It follows that the cationic forms of all investigated flavylium salts are aromatic molecules, whilst the corresponding anhydrobase forms ($\eta = 0.191$–0.266 and $\eta_r = 0.004$–0.032) are effectively nonaromatic. Based on this it can be predicted that the hydration reactions will take place with anhydrobase forms more readily than with cationic forms. This is in agreement with our recent reports.[8,10]

Pearson's assertion[27] that 'There seems to be a rule of nature that molecules arrange themselves so as to be as hard as possible. A large HOMO–LUMO gap increases stability,' has been termed the principle of maximum hardness by Zhou and Parr.[12] Is this principle also applicable to the flavylium salts? The replacement of the carbon atom in position C-1 of 2-phenylnaphthalene ($\eta = 0.3487$, $\eta_r = 0.0960$, HOMO–LUMO gap = 0.6974, TREPE = 0.0221) by oxygen causes a considerable stabilization of the resultant flavylium chromophoric system ($\eta = 0.5150$, $\eta_r = 0.2452$, HOMO–LUMO gap = 1.0300, TREPE = 0.0332). However, in slightly acidic solutions the unsubstituted flavylium chromophore is quite unstable and readily undergoes hydration reactions. The position of substitution, and the nature of the substituent are both critical in determining the effect of substitution on the stability of the flavylium chromophore. If a single H atom is replaced by an OH group then the correlation between the S_2^N index and absolute hardness of the compounds obtained is very good ($r = -0.923^{**}$) (see Fig. 4).

It can be safely predicted that the presence of an OH group in positions C-6, C-8 and C-3′, and especially in position C-3, destabilizes the flavylium chromophore, whilst in positions C-5, C-7, C-2′ and C-4′ it has a stabilizing effect. An exceptional stabilizing effect is achieved by the OH group in position C-4. However, no flavylium salts have been found in nature which have the OH group in position C-4, because it is expected that such salts will undergo hydrolysis in the acidic medium to yield the corresponding stable chalcone (see Scheme 2). If a CH_3 group is introduced into the flavylium chromophore, a relationship nearly identical to that in Fig. 4 is obtained. It can be stated therefore that the C ring of the flavylium chromophore has a dominant effect on stability. This stabilizing effect of position C-4 was recognized some time ago.[28] C-4 substitution with

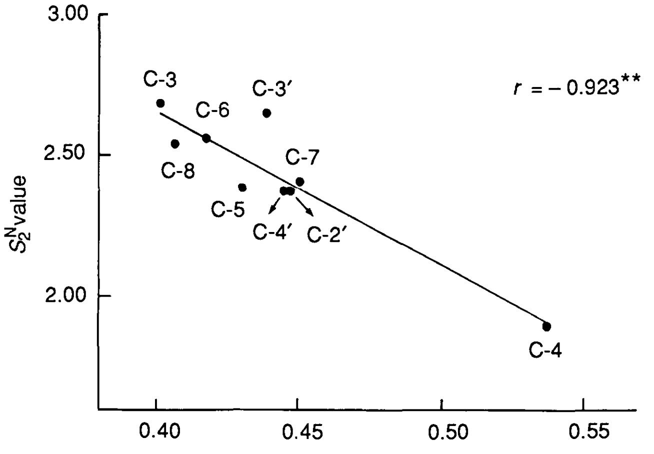


Fig. 4 Correlation of superdelocalizability (nucleophilic) (in β^{-1}) with absolute hardness (in $-\beta$) for a cationic form of flavylium chromophore substituted with one OH group in a specified position

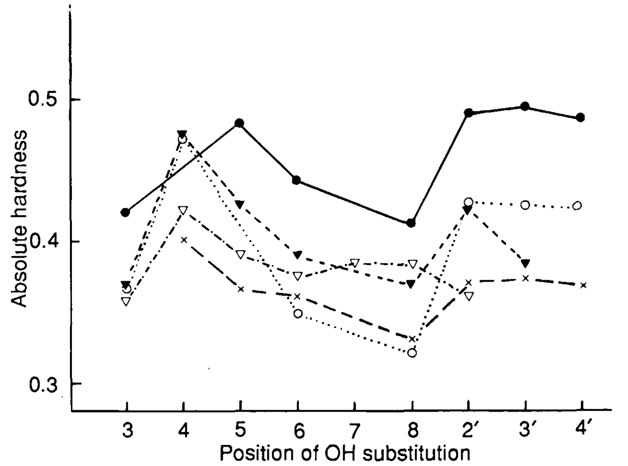
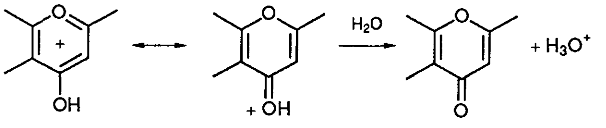


Fig. 5 Correlation of absolute hardness (in $-\beta$) with position of substitution for flavylium chromophores, substituted with one OH group. ●, 7-OH-4-CH_3; ○, 5,7-diOH; ▼, 7,4′-diOH; ▽, 3′, 4′- diOH; ×, 3,7-diOH.



Scheme 2

methyl, phenyl, methoxyphenyl or carboxyl groups provides an exceptional stability to the flavylium chromophore, but such compounds have not been found in nature. Comparatively, 3-hydroxyflavylium salts are highly unstable. Therefore it is not surprising that the majority of natural flavylium salts do not contain a free OH group in position C-3, but have sugar or acylated sugar in this position, which considerably stabilizes the flavylium chromophore.

It is well known that substituents in positions C-5 and C-7 of ring A, as well as in position C-4′ of ring B stabilize the flavylium chromophore.[9,29] The natural flavylium salts have OH or O-sugar groups in these positions. In Fig. 5 we give the absolute hardness of various substituted flavylium salts when an additional OH group is introduced in specified positions. The calculation was performed for the sequence of substituent combinations in the A, B and C rings of the flavylium chromophore. Fig. 5 exhibits some of the most interesting combinations. It is predicted that in the A ring position C-7 has a higher stabilizing effect than position C-5. The highest stability is demonstrated by 7-hydroxy-4-methylflavylium chromophore with an additional OH group in the B ring, and the lowest stability by the 3,7-dihydroxyflavylium chromophore. On the basis of the calculated S_2^N indices and the absolute hardness for the possible combinations of OH and OCH_3 substituents in the B ring it can be predicted that the highest stability and the lowest reactivity will be obtained by 7-hydroxy-4-methylflavylium chromophore substituted with OH or OCH_3 in positions C-2′, C-4′ and C-6′. In this case the OCH_3 group will yield a somewhat higher stability, which is in agreement with the experimental findings for similar flavylium salts without the C-4 substituent.[20] Therefore, it appears that 7-hydroxy-4-methyl-2′,4′,6′-trimethoxyflavylium salt represents a model compound with high stability and low reactivity. It will be interesting to see if some future experimental work supports this prediction.

Conclusion

It has been demonstrated that new indices of aromaticity (absolute hardness and relative hardness) can be used as convenient theoretical tools to predict the flavylium salts with high stability and low reactivity. This is based on a good agreement between the experimental facts and the predictions obtained by means of aromaticity and reactivity indices. The principle of maximum hardness is also useful in this respect.

The greatest contribution to the stability of the flavylium chromophore system is provided by substituents in positions C-4, C-7, C-2′, C-4′ and C-6′. It is predicted that 7-hydroxy-4-methyl-2′,4′,6′-trimethoxyflavylium salt will be very stable.

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