

Experimental and Theoretical Study of the Styrylbenzopyrylium Chromophore

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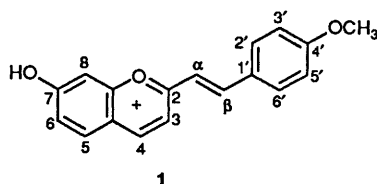
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Structural transformations of 7-hydroxy-4'-methoxystyrylbenzopyrylium chloride have been investigated in buffer solutions of pH 2.00–11.00. The reactivity and aromatic stability of this and related styrylbenzopyrylium salts are predicted using standard HMO calculations and the concept of hardness, and correlated with experimental findings. Electronic spectra are calculated using the PPP method. Several model compounds are proposed, which are expected to exhibit high stability and low reactivity, and whose solutions should be red coloured.

Because of the general unavailability of naturally occurring pigment material the widely available and easily synthesized azo dyes were used in the preparation of foods.¹ Later, recognition of undesired physiological activity in animals treated with some of these compounds resulted in the restriction in their use for food colouring purposes. For this reason it has been suggested that only natural colouring matters present in edible plant materials should be considered as colour additives for foods. Anthocyanins are the main pigments of fruits and flowers.² At present, much work is being carried out on the use of anthocyanins as food colouring materials.³ This use, however, is entirely restricted to those foods or beverages having a pH below 4; in slightly acid solutions, pH *ca.* 4–6, most anthocyanins are rapidly decolourised. It has been suggested that some yellow and orange synthetic flavylum salts have potential to be used as colour additives for fruit drinks and juices.⁴ All potential red substitutes for the synthetic azo dyes have a low tinctorial strength. Being closely related in structure to natural anthocyanins, styrylbenzopyrylium compounds may prove useful as substitutes for red dyes from coal tar. In spite of the abundance of reports dealing with flavylum salts,⁵ studies of styrylbenzopyrylium salts are rather rare in the literature.^{6–8}

This study is a continuation of our previous work,⁹ and reports an attempt to design a model styrylbenzopyrylium chromophore which would have a high stability and low reactivity, and whose slightly acidic solutions should be red coloured. To achieve this goal we combined UV–VIS absorption measurements with theoretical calculations using HMO and PPP methods. The aromatic stabilities and reactivities, as well as UV–VIS spectral properties of many postulated structures were calculated. The salt experimentally studied is 7-hydroxy-4'-methoxy-styrylbenzopyrylium chloride (compound **1**, hereafter).



Experimental

For all experiments, the required amount of pigment (analytical concentration 1.51×10^{-5} mol dm⁻³) was dissolved in Britton–Robinson buffers (pH 2.00–11.00) of constant ionic strength (0.2 mol dm⁻³) and the resultant solution allowed to equilibrate in the dark at 20 °C. UV–VIS absorption spectra of buffered

pigment solutions were recorded on a Pye Unicam SP8–100 UV–VIS spectrophotometer. The spectra were recorded immediately after preparation (10 min) and after standing for 6 days.

Indices of Aromaticity and Reactivity.—Pearson¹⁰ has demonstrated that the absolute hardness, η , which is half of the HOMO–LUMO gap in Hückel theory, represents a good measure of aromaticity and reactivity. It can be calculated according to eqn. (1) where I is the ionization potential, A the

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

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 and inertness of the molecule.

Recently, Zhou and Parr¹¹ demonstrated that the relative hardness, η_r , also represents a good measure of aromaticity and reactivity. The relative hardness can be calculated from the energies of the HOMO and LUMO of the conjugated molecule and the corresponding hypothetical acyclic reference structure¹² according to eqn. (2) where η_a is given by eqn (3).

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

$$\eta_a = (E_{\text{LUMO}}^{\text{ac}} - E_{\text{HOMO}}^{\text{ac}})/2 \quad (3)$$

Since the styrylbenzopyrylium chromophoric system is positively charged, it is susceptible to nucleophilic attack. The electron-accepting molecular orbital is, of course, the LUMO. Therefore, the reactivity of each atom for the nucleophilic attack is defined as the square of the atomic orbital coefficient (c_{LUMO}^2) in the LUMO.

Activation hardness¹³ $\Delta\eta^\ddagger$ is also a very convenient reactivity index for predicting the favoured position for nucleophilic attack.¹⁴ It can be calculated according to eqn. (4) where the

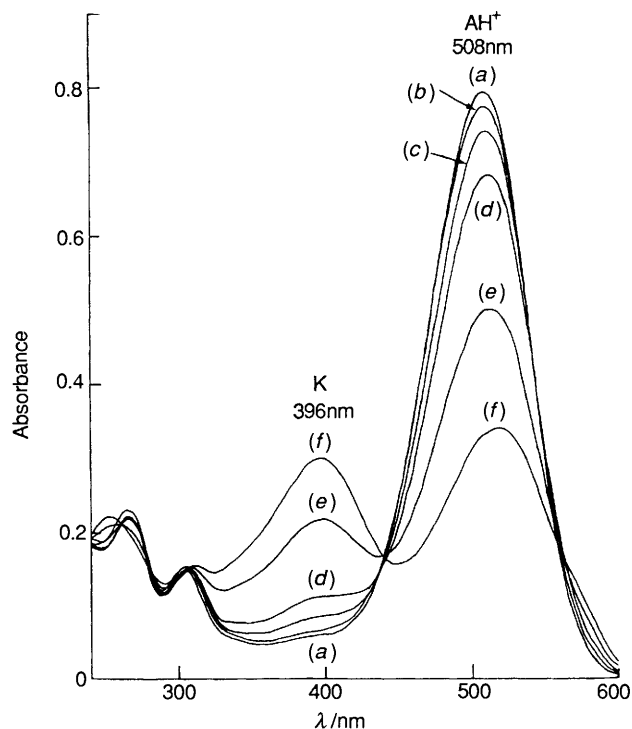
$$\Delta\eta^\ddagger = \eta^{\text{R}} - \eta^{\text{T}} = (x_{\text{LUMO}}^{\text{R}} - x_{\text{HOMO}}^{\text{R}} - x_{\text{LUMO}}^{\text{T}} + x_{\text{HOMO}}^{\text{T}})/2 \quad (4)$$

symbols η , R, T, x_{LUMO} and x_{HOMO} stand, respectively, for hardness, reactant, transition state, and Hückel energies of the LUMO and the HOMO. In the transition state a hole is localized at the carbon atom where the nucleophilic attack will take place and the conjugated fragment contains all electrons that have previously been distributed over the whole molecule. We should note that the smaller the value of activation hardness is, the faster the nucleophilic attack is.

The above indices of aromaticity and reactivity were obtained

Table 1 Parameters used in the PPP calculations

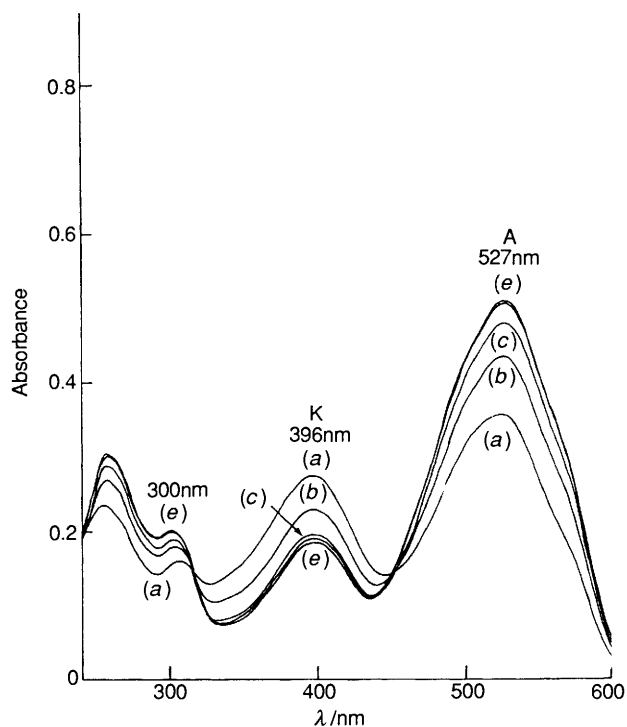
Atom, μ	I_{μ}/eV	A_{μ}/eV	$\gamma_{\mu\mu}/\text{eV}$	$\beta_{C-\mu}/\text{eV}$	n_{π}	$l_{C\mu}/\text{\AA}$	Ref.
C	11.16	0.08	11.08	-2.390	1	1.397	20
O ⁺	34.15	18.85	15.30	-2.290	1	1.360	21
-O-	35.76	17.70	18.06	-2.270	2	1.360	22
OH	26.14	7.32	18.82	-1.950	2	1.360	23
OCH ₃	33.00	11.47	21.53	-1.808	2	1.370	24
=O	17.28	2.70	14.58	-3.070	1	1.240	22
CH ₃	28.43	12.99	15.44	-1.673	2	1.500	25

**Fig. 1** Absorption spectra of **1** in solutions of pH 2.00–4.50 after standing 10 min. pH: (a), 2.00; (b), 2.50; (c), 3.00; (d), 3.50; (e), 4.00; (f), 4.50.

using appropriate computer programs.^{15,16} HMO parameters for heteroatoms were taken from the literature.¹⁷

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.^{18,19} To our knowledge the exact geometry of any styrylbenzopyrylium compound has not, so far, been reported. Idealized, planar geometries have been assumed, with the rings taken as regular hexagons. All C–C bonds within the rings were set equal to 1.397 Å. All valence angles have been taken to be equal to 120°.

We have selected a consistent set of valence state energy parameters (Table 1). This set of parameters has been developed and tested in calculations of various styrylbenzopyrylium structures. In Table 1, 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 neighbours are designated as $\gamma_{\mu\mu}$ and $\beta_{C-\mu}$, respectively, 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.²⁶ The heteroatom model of the methyl group has been used.

**Fig. 2** Absorption spectra of **1** in solutions of pH 5.00–7.00 after standing 10 min. pH: (a), 5.00; (b), 5.50; (c), 6.00; (d), 6.50; (e), 7.00.

Results and Discussion

Absorption spectra of compound **1** in buffer solutions pH 2.00–4.50 are presented in Fig. 1. Characteristic absorption of the cationic form AH⁺ of this compound, which appears at $\lambda_{\text{max}} = 508$ nm, is stable and almost unchanged up to pH 3.00. When the pH value of the solution is increased from 3.00 up to 4.50, a new absorption maximum is formed at $\lambda = 396$ nm which is associated with the ketone form K. The isosbestic point at $\lambda = 440$ nm is evidence of the equilibrium established between them. Further decreasing the acidity, already at pH 5.50, results in the formation of anhydro base A, with an absorption maximum at $\lambda_{\text{max}} = 527$ nm, together with a hypsochromic shift of the ketone K band, $\lambda = 396$ nm (Fig. 2). After standing the acidic buffer solutions for 6 days (Fig. 3) equilibrium is established between the cationic and ketone forms at $pK = 3.64 \pm 0.16$. At the same time anhydro base absorption disappears.

Behaviour of **1** in alkaline buffer solutions is presented in Fig. 4. The anhydro base form maintains its characteristic absorption up to pH 9.00. Further increase of the pH value results in a hypsochromic and hyperchromic shift of the absorption maximum. This is probably associated with formation of the ionized ketone form K⁻, $\lambda = 510$ nm. After 6 days (Fig. 5) these buffer solutions show a disappearance of anhydro base. Neutral and weakly alkaline medium pH 6.00–8.00 favours the ketone form K. In strongly alkaline medium pH 10.00–11.00 ketone form K disappears, followed by formation of ionized ketone K⁻, $\lambda = 510$ nm.

The resulting changes in acidic solutions can be explained with the following rationale. On reduction of acidity the cationic form is converted into the anhydro base form through rapid deprotonation. Under the existing experimental conditions the anhydro base form could not be detected (Fig. 1). This could be explained by the possibility that the anhydro base form is highly unstable and undergoes a hydration reaction to form an unstable pseudobase B which yields a ketone form K as the final product. Therefore, it appears that the cationic and ketone forms exist in equilibrium (Figs. 1 and 3). Scheme 1

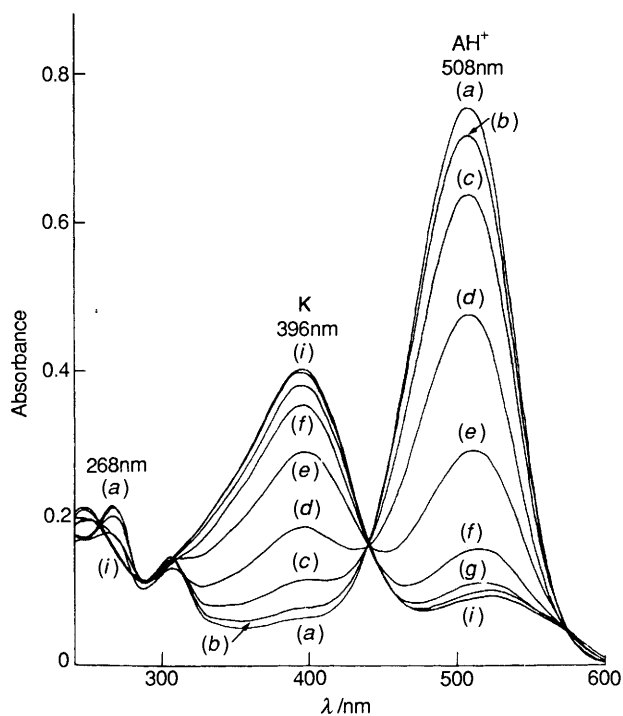
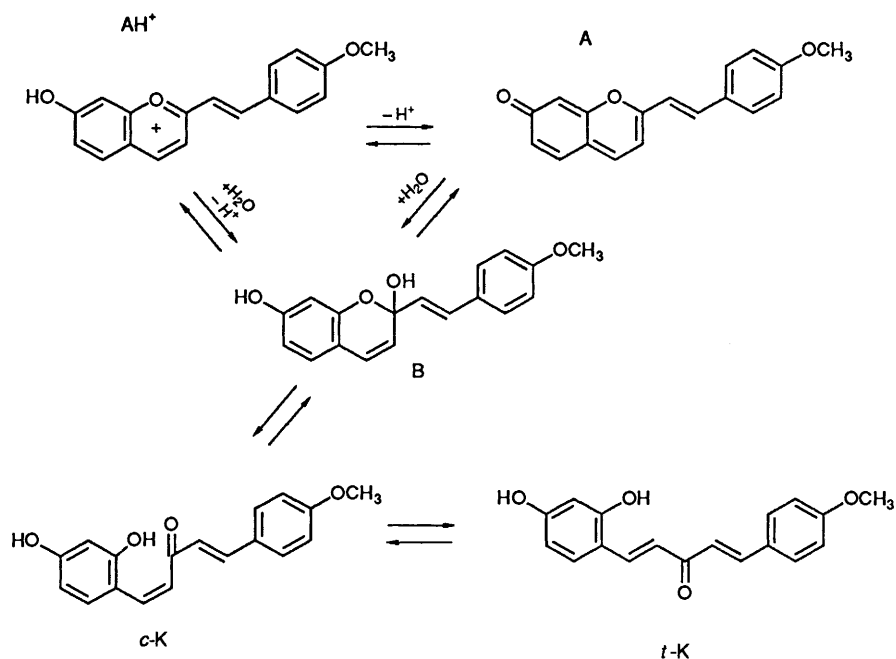


Fig. 3 Absorption spectra of **1** in acidic solutions after standing 6 days. pH: (a), 2.00; (b), 2.50; (c), 3.00; (d), 3.50; (e), 4.00; (f), 4.50; (g), 5.00; (h), 5.50; (i), 6.50

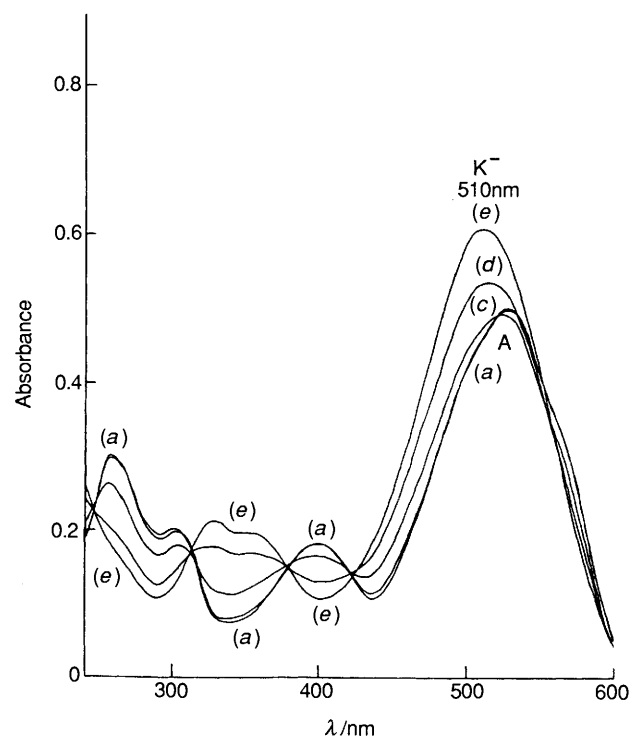


Fig. 4 Absorption spectra of **1** in alkaline solutions after standing 10 min. pH: (a), 8.00; (b), 9.00; (c), 10.00; (d), 10.50; (e), 11.00

illustrates structural transformations of **1** with pH in acidic aqueous solutions.

In approximately neutral solutions pH 6.50–9.00, measured immediately after preparation, the anhydro base form was detected successfully (Figs. 2 and 4). In these solutions hydration reactions also take place and after 6 days the only forms present are ketone **K** and the corresponding ionized ketone K^- (Fig. 5).

A general conclusion is that the transformations of styrylbenzopyrylium compounds are quite similar to the structural transformations of flavylium salts.

Using the PPP method it was possible to obtain a good

agreement between experimental data and theoretical values for the singlet–singlet ($\pi \rightarrow \pi^*$ type) transition energies for a series of flavylium salts.^{21,23,27,28} Thus, we have used the PPP method to predict the electronic absorption spectra for the cationic and anhydro base forms of styrylbenzopyrylium structures.

The long wavelength band, λ_{max} , of styrylbenzopyrylium salts in UV–VIS spectra characterized by high molecular absorption coefficients ($\log \epsilon$ ca. 4.5) is attributed to the $\pi \rightarrow \pi^*$ (HOMO–LUMO) transition. This absorption band is characteristic for a given styrylbenzopyrylium structure. In Table 2 calculated values of transition energies and oscillator

Table 2 Calculated and observed maximum absorption wavelengths and oscillator strengths

Styrylbenzopyrylium chromophore		Calculated		Observed	
		λ_{\max}	(<i>f</i>)	λ_{\max}	(<i>e</i>)
Unsubstituted 7-OH-4'-OH	AH ⁺ form	456 nm	(1.325)	—	—
	AH ⁺ form	522 nm	(1.690)	507 nm	(5.2 × 10 ⁴) ^a
	A-7 form ^b	551 nm	(1.856)	—	—
7-OH-4'-OCH ₃	AH ⁺ form	504 nm	(1.516)	508 nm	(5.2 × 10 ⁴)
	A-7 form	526 nm	(1.712)	527 nm	(3.4 × 10 ⁴)
7,4'-diOH-3'-OCH ₃	AH ⁺ form	525 nm	(1.701)	517 nm	(3.8 × 10 ⁴) ^a
	A-7 form	556 nm	(1.849)	—	—
7-OH-4'-OCH ₃ -4-CH ₃	AH ⁺ form	492 nm	(1.460)	—	—
	A-7 form	525 nm	(1.620)	—	—

^a In 1% aqueous citric acid. ^b The A-7 form is the anhydro base formed by loss of a proton from the hydroxy group at C-7.

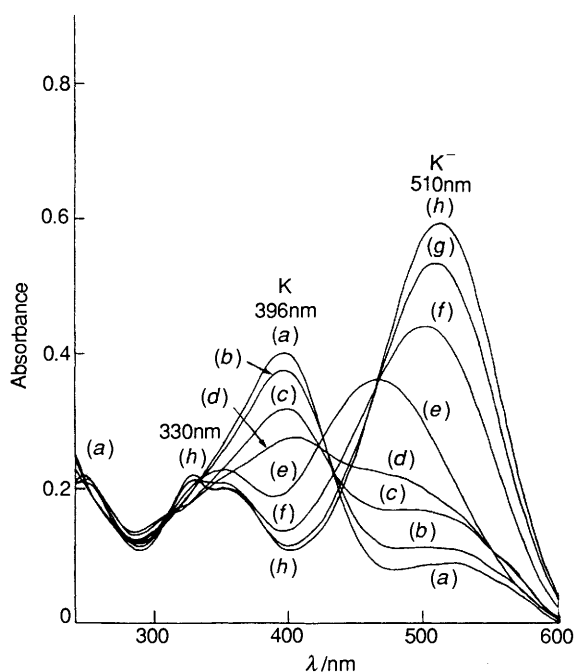


Fig. 5 Absorption spectra of **1** in solutions of pH 6.50–11.00 after standing 6 days. pH: (a), 6.50; (b), 7.00; (c), 7.50; (d), 8.00; (e), 9.00; (f), 10.00; (g), 10.50; (h), 11.00.

Table 3 Comparison of experimental data for 7-OH-4'-OCH₃ flavylium salt and 7-OH-4'-OCH₃ styrylbenzopyrylium salt (AH⁺ form)

	λ_{\max}	pK
7-OH-4'-OCH ₃ flavylium salt	458 nm	3.14 ^a
7-OH-4'-OCH ₃ styrylbenzopyrylium salt	508 nm	3.64

^a Ref. 29

strengths are given, *viz.* experimental singlet–singlet $\pi \rightarrow \pi^*$ transitions for different substitution models of the styrylbenzopyrylium chromophore. In general we have obtained a very good agreement between the calculated and experimental singlet–singlet transition energies for the structures under study.

Experimental results showed that the long wavelength absorption band of the anhydro base form is shifted towards longer wavelengths and the corresponding intensity is somewhat weaker than that of the cationic form. Methyl substitution at position 4 of the flavylium chromophore results in a hypsochromic shift of the long wavelength band. The PPP results for the studied styrylbenzopyrylium salts, except for

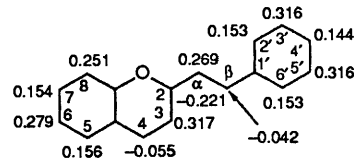


Fig. 6 The values of activation hardness (in $-\beta$) for nucleophilic attack at various positions of styrylbenzopyrylium chromophore

spectral intensities, agree quite well with these experimental facts.

Since the styrylbenzopyrylium system is positively charged, it is susceptible to nucleophilic attack. In our recent paper¹⁴ we showed that the activation hardness appears to be a very convenient reactivity index for predicting the favoured position for nucleophilic attack on aromatic substrates. Activation hardness is an index which measures the ease with which the atom *i* in an aromatic substrate reacts with nucleophiles. The smaller the value of the activation hardness, the greater the probability of nucleophilic attack at the atom *i*. The calculated value of activation hardness for nucleophilic attack on the styrylbenzopyrylium chromophore is smallest at position C-2 (Fig. 6). This leads to the prediction that this position will readily react with nucleophiles (*e.g.* nucleophilic addition of water), which results in formation of colourless forms B and K and fading of the styrylbenzopyrylium salt solution. Our experimental results (see Figs. 1 and 3 and Scheme 1) are in agreement with this prediction.

Previous studies^{6–8} on the stability of styrylbenzopyrylium compounds showed that some yellow flavylium salts are of advantage in beverage colouration. But if we compare experimental results for closely related flavylium and styrylbenzopyrylium salts (Table 3), the opposite assertion can be made.

The relevant conclusion derived from Table 3 is as follows. Insertion of a vinyl group between the benzopyrylium and phenyl moieties of the flavylium chromophore, which results in production of the styrylbenzopyrylium chromophore, shifts λ_{\max} of the AH⁺ form bathochromically by 50 nm. At the same time the stability of the AH⁺ form rises by 0.5 pK units. The PPP-calculated electronic spectra for a number of salts indicate that insertion of the vinyl group results in bathochromic shifts of *ca.* 50–60 nm.

Hydration reactions can be suppressed by the attachment of appropriate substituents to the styrylbenzopyrylium core. The position of substitution is critical in determining the effect of substitution on the reactivity of the styrylbenzopyrylium chromophore. We used simple semiempirical indices in studying this effect.

In 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 c_{LUMO}^2 index is the appropriate

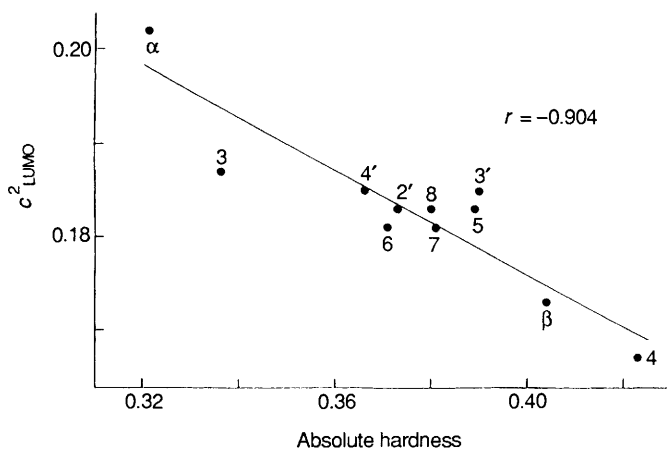


Fig. 7 Correlation of c_{LUMO}^2 with absolute hardness (in $-\beta$) for cationic forms of styrylbenzopyrylium chromophore substituted with a single OH group in a specified position

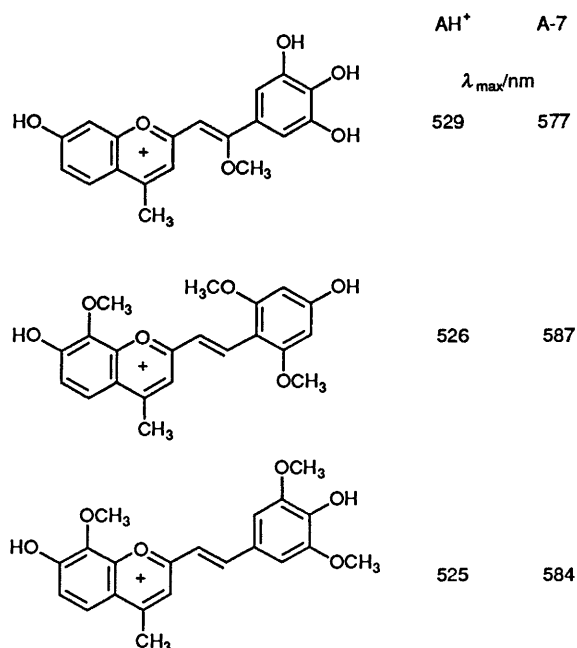


Fig. 8 Proposed stable red styrylbenzopyrylium model compounds

reactivity index. A higher value of the c_{LUMO}^2 index indicates that the nucleophilic attack will proceed more easily at a given site. If a single H atom in the styrylbenzopyrylium chromophore is replaced by an OH group then the correlation between the c_{LUMO}^2 indices and the corresponding absolute hardness of the compounds obtained is approximately linear ($r = -0.904$), see Fig. 7. If a CH_3 group is introduced instead of an OH group a relationship nearly identical to that in Fig. 7 is obtained. This confirms the empirical observation that nucleophilic attack on aromatic substrates occurs most readily at those molecules which possess smaller values of absolute hardness and higher c_{LUMO}^2 indices. It can be safely predicted that the presence of a substituent in the α and C-3 position highly destabilizes the styrylbenzopyrylium chromophore. An exceptional stabilizing effect is achieved by substituents in the C-4 position.

In our recent paper³⁰ we concluded that replacement of the hydrogen atom in position 4 of the flavylum chromophore by a methyl group causes approximately a 1.62 pK unit increase in the stability of the AH⁺ form. If we assume addition of the stabilizing effects of the vinyl and 4-methyl groups then it can be predicted that the stability of the AH⁺ form of the 4-methylstyrylbenzopyrylium chromophore will rise by more

than 2 pK units relative to the corresponding 4-unsubstituted flavylum chromophore. This leads to the prediction that the pK value of the overall transformation $AH^+ \rightleftharpoons (A + B + K) + H^+$ for 7-OH-4'-OCH₃-4-CH₃ styrylbenzopyrylium salt will be close to the value of 5.26. This means that slightly acidic solutions of this salt retain a stable red colouration.

Conclusions

The influence of various substituents (OH, OCH₃, CH₃), their positions and combinations on the reactivity of styrylbenzopyrylium chromophore were studied by calculating the absolute hardness, relative hardness and c_{LUMO}^2 indices. We have calculated these indices, as well as electronic absorption spectra for a number of postulated styrylbenzopyrylium structures, both cationic and anhydro base forms. In Fig. 8 are presented model compounds which are predicted to exhibit high stability, low reactivity and stable red colouration in slightly acidic solutions.

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