LITERATURE CITED

Amić, D.; Vukadinović, V.; Radoš, A. The Program for the HMO Calculations on Microcomputers. *Kem. Ind.* **1989,** *8,* 365–371.

Baranac, J.; Amić, D. Spectrochemical Investigations of the Influence of pH on the Mechanism of Structural Transformations of the 4-Substituted Flavylium Chromophore. *J. Serb. Chem. Soc.* **1985,** *50,* 299–305.

Bendz, G.; Martensson, O.; Nilsson, E. Studies of Flavylium Compounds. I. Some Flavylium Compounds and Their Properties. *Ark. Kemi* **1967,** *27,* 65–77.

Brouillard, R. Chemical Structure of Anthocyanins. In *Anthocyanins as Food Colors*; Markakis P., Ed.; Academic Press: New York, 1982.

Brouillard, R. Flavonoids and Flower Colour. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman and Hall: London, 1988.

Brouillard, R.; Iacobucci, G. A.; Sweeny, J. G. Chemistry of Anthocyanin Pigments. 9. UV-visible Spectrophotometric Determination of the Acidity Constants of Apigeninidin and Three Related 3-Deoxyflavylium Salts. *J. Am. Chem. Soc.* **1982,** *104,* 7585–7590.

Kurtin, W. E.; Song, P.-S. Electronic Structures and Spectra of Some Natural Products of Theoretical Interest-I. Molecular Orbital Studies of Anthocyanidins. *Tetrahedron* **1968,** *24,* 2255–2267.

Mazza, G.; Brouillard, R. Color Stability and Structural Transformations of Cyanidin 3,5-Diglucoside and Four 3-Deoxyanthocyanins in Aqueous Solutions. *J. Agric. Food Chem.* **1987,** *35,* 422–426.

Ohta, H.; Watanabe, H.; Osajima, Y. Hückel Molecular Orbital Calculations on Reactivity of Some Flavylium Compounds. *Nippon Nogeikagaku Kaishi* **1980,** *54,* 415–422.

Sweeny, J. G.; Iacobucci, G. A. Effect of Substitution on the Stability of 3-Deoxyanthocyanidins in Aqueous Solutions. *J. Agric. Food Chem.* **1983,** *31,* 531–533.

Timberlake, C. F. Improvements Relating to the Colouring of Foodstuffs. U.K. Patent No. 5477/68, 1968.

Timberlake, C. F.; Bridle, P. Flavylium Salts, Anthocyanidins and Anthocyanins. 1. Structural Transformations in Acid Solutions. *J. Sci. Food Agric.* **1967,** *18,* 473–478.

Trinajstić, N. Personal communication, 1987.

# Reactivity of Some Flavylium Cations and Corresponding Anhydrobases

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The indices of superdelocalizability were calculated for the nucleophilic attack in positions C-2 and C-4 of the cationic and anhydrobase forms of 10 synthetic flavylium salts. Compounds 1–5 have no substituent at position C-4, while compounds 6–10 are methylated at this position. The inductive model was shown to be the most appropriate for treating the methyl group. The obtained results indicate that decolorization of weakly acidic solutions of flavylium salts was caused by hydration reactions taking place at the C-2 position of the anhydrobase form of flavylium chromophore. UV-vis spectra of compounds 2 and 5 confirm the obtained results.

Flavylium (2-phenylbenzopyrylium) salts in acidic aqueous solutions attain an equilibrium between two colored forms, cationic AH+ and anhydrobase A, and two colorless forms, pseudobase B and chalcone C (Brouillard, 1982):



R = H, OH, OCH3
R′= H, Sugar
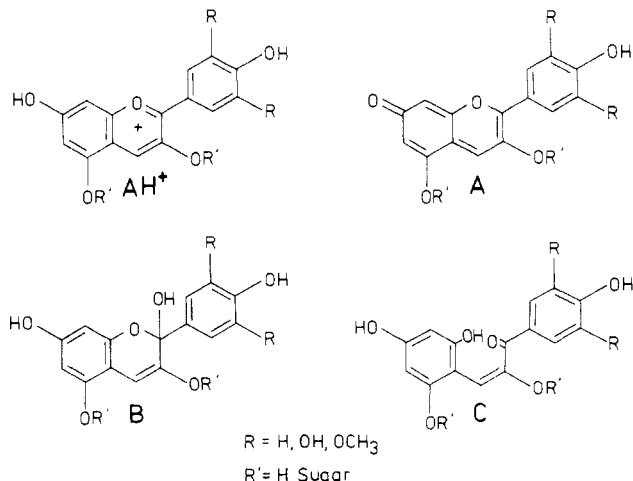
Flavylium salt glycosides—anthocyanins—are associated with the largest number of red and blue hues of flowers, fruits, and vegetables. In vivo mechanisms such as intermolecular and intramolecular copigmentation stabilize the colored forms of flavylium chromophore (Brouillard, 1983). When separated from cell fluids, most of anthocyanins (as well as their aglycons anthocyanidins) are virtually colorless within the range pH $\approx$4–6 (Asen, 1976). The increased interest for the application of flavylium compounds as food colorants induced not only isolation of some stable natural acylated anthocyanins (Brouillard, 1981) but also the syntheses of a number of 4-substituted flavylium salts, which keep stability over a wide range of pH values (Timberlake, 1968).

Flavylium salts are stable only in strongly acidic solutions. Reduction of acidity causes deprotonation and transformation of cationic form into the anhydrobase form. If the flavylium core is substituted with OH groups in several positions, a number of prototropic tautomers is formed, for example:
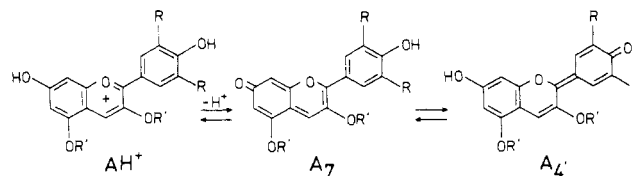


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**Table I. Parameter Values for the HMO Calculations**

| element of group (X) | Coulomb integral $(h_x)$ | resonance integral $(K_{C-x})$ | ref |
|---|---|---|---|
| carbon (C) | 0 | 1 | a |
| pyrylium oxygen (O⁺) | 2 | 0.9 | a |
| ether oxygen (Ö) | 2 | 0.34 | b |
| ketone oxygen (Ö) | 0.22 | 0.99 | b |
| hydroxyl (OH) | 1.2 | 0.8 | a |
| methoxyl (OCH₃) | 1.65 | 0.9 | c |

*a* Ohta et al., 1980. *b* Aihara, 1976. *c* Trinajstić, 1987.

The explicit evidence for the concurrent existence of more tautomeric anhydrobase forms has not been reported yet. The formation of colorless forms of pseudobase B and chalcone C in weakly acidic solutions is caused by hydration reactions. Conflicting opinions of different authors about the form of flavylium chromophore undergoing the hydration are found in literature (Jurd and Geissman, 1963; Jurd, 1972; Timberlake and Bridle, 1975; Hrazdina, 1982; McClelland and McGall, 1982; Brouillard and Dubois, 1977a; Brouillard and Delaporte, 1977b; Brouillard et al., 1978). Theoretical predictions of flavylium compound reactivities calculated by different variations of the MO method (Bendz et al., 1967; Kurtin and Song, 1968; Ohta and Osajima, 1979; Ohta et al., 1980; Debicki-Pospišil et al., 1983) did not deal with this problem to a greater extent. This paper presents the results of reactivity indices calculations for cationic and anhydrobase forms of flavylium salts 1–10 using Hückel molecular orbital method. The obtained results are discussed and compared to the experimental facts.
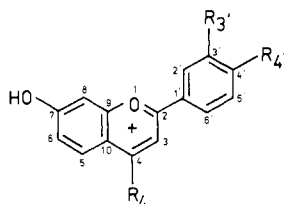
|     | R₄  | R₃′  | R₄′  |
|-----|-----|------|------|
| 1   | H   | H    | H    |
| 2   | H   | H    | OCH₃ |
| 3   | H   | OH   | OH   |
| 4   | H   | OCH₃ | OH   |
| 5   | H   | H    | OH   |
| 6   | CH₃ | H    | H    |
| 7   | CH₃ | H    | OCH₃ |
| 8   | CH₃ | OH   | OH   |
| 9   | CH₃ | OCH₃ | OH   |
| 10  | CH₃ | H    | OH   |

## EXPERIMENTAL SECTION

Flavylium salts 2 and 5 were synthesized according to the published procedures (Jurd, 1966). The electronic spectra were recorded on a Pye Unicam SP 8-100 UV–vis spectrophotometer using $10^{-5}$ mol dm⁻³ solution (MeOH–0.01% HCl) in buffers having ionic strength $I$ = 0.2 mol dm⁻³.

The HMO calculations were executed on Atari 1040ST computer by applying a Pascal program (Amić et al., 1989). The parameters used for these calculations are listed in Table I.

## RESULTS AND DISCUSSION

The electronic spectra of 7-hydroxy-4′-methoxyflavylium perchlorate (2) and 7,4′-dihydroxyflavylium perchlorate (5), which were recorded in acidic buffers imme-
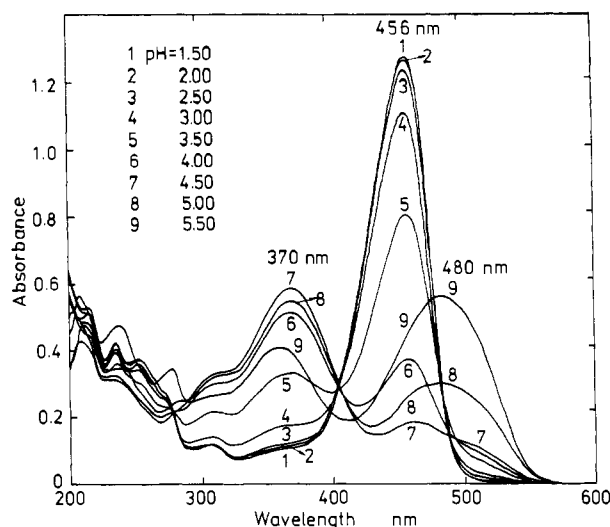


**Figure 1.** Absorption spectra of 7-hydroxy-4′-methoxyflavylium perchlorate at 20 °C, concentration $3.41 \times 10^{-5}$ mol dm⁻³, after 3 min.
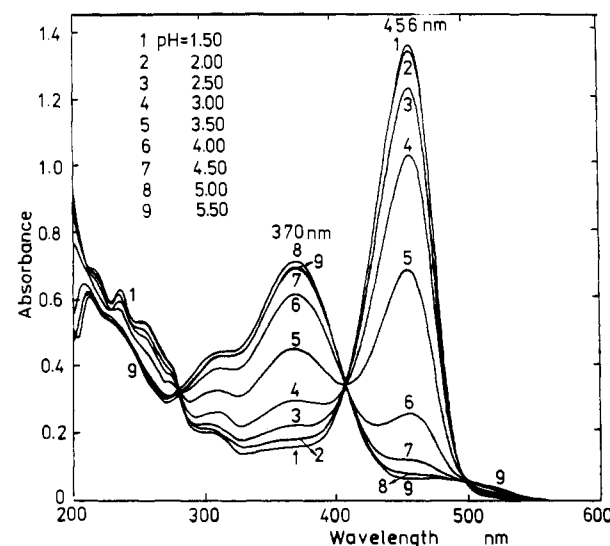


**Figure 2.** Absorption spectra of 7-hydroxy-4′-methoxyflavylium perchlorate at 20 °C, concentration $3.66 \times 10^{-5}$ mol dm⁻³, after standing 2 h.



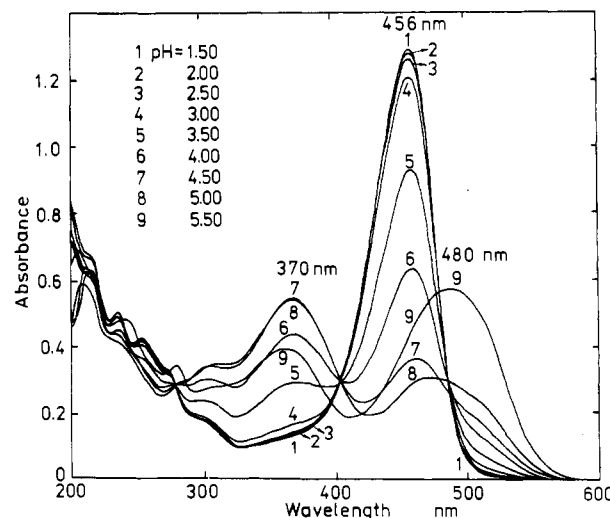**Figure 3.** Absorption spectra of 7,4′-dihydroxyflavylium perchlorate at 20 °C, concentration $3.05 \times 10^{-5}$ mol dm⁻³, after 3 min.

diately following solution preparation (after 3 min) and after 120 min, are presented in Figures 1–4. Cationic

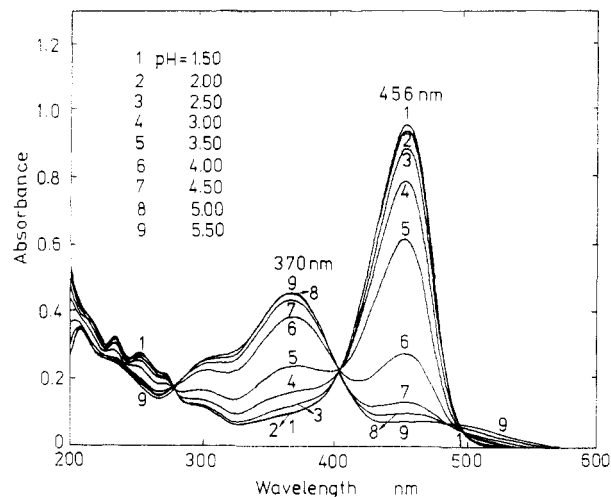938 J. Agric. Food Chem., Vol. 38, No. 4, 1990

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**Figure 4.** Absorption spectra of 7,4'-dihydroxyflavylium perchlorate at 20 °C, concentration 2.25 × 10⁻⁵ mol dm⁻³, after standing 2 h.

form of compound **2** (λ = 456 nm) and the cationic form of compound **5** (λ = 456 nm) are stable only under strongly acidic solutions (curves 1-3). The reduction of acidity causes a pronounced hypochromic effect on the absorption band of cationic form, which is accompanied by the concurrent formation of new bands at λ = 480 and 370 nm (Figures 1 and 3). Depending on the interval needed to attain equilibrium, the band at λ = 480 nm disappears and the hyperchromic effect appears for the band at λ = 370 nm. After 2 h (Figures 2 and 4) the equilibrium has been achieved between the cationic and the chalcone forms. The resulting changes can be explained with the following rationale: By reduction of acidity the cationic form is converted into the anhydrobase form through rapid deprotonation. The resulting anhydrobase is highly unstable and is converted into chalcone. On the basis of similar observations, the same conclusion was also reached by other workers (Jurd and Geissman, 1963; Jurd, 1972; Timberlake and Bridle, 1975; Hrazdina, 1982). If the methyl group is introduced into the position C-4 of compounds **2** and **5** (compounds **7** and **10**), significant stabilization of both cationic and anhydrobase forms is accomplished, resulting in negligible formation of chalcone (Brouillard et al., 1982; Baranac and Amić, 1985). The equilibrium of compounds **6, 7,** and **10** is attained very rapidly, so their absorption spectra have equal band intensities for the solutions equilibrated for 3 and 120 min (Baranac and Amić, 1985, 1986). Recently, Brouillard and co-workers published several papers (Brouillard and Dubois, 1977a; Brouillard and Delaporte, 1977b; Brouillard et al., 1978) in which they assert, on the basis of kinetic and thermodynamic measurement results, that hydration reactions take place exclusively on the cationic form of flavylium chromophore. The p$K$ value of the transformation $AH^+ \rightleftharpoons (A + B + C) + H^+$ can be taken as the measure of the stability of cationic form. On the other hand, the stability and reactivity of the cationic as well as anhydrobase form can be predicted theoretically. The standard Hückel molecular orbital method is especially suitable for this purpose. Figure 5 presents the π-electron densities and bond orders for different forms of compounds **5** and **9**. Positive charge of the flavylium core, delocalized over the entire heteroaromatic system, according to HMO calculations, has the highest partial value at positions C-2 and C-4. This causes the high reactivity of these positions toward the nucleophilic reagents (such as $H_2O$), which results in the formation of colorless forms, i.e., fading of the solution colors. Therefore,
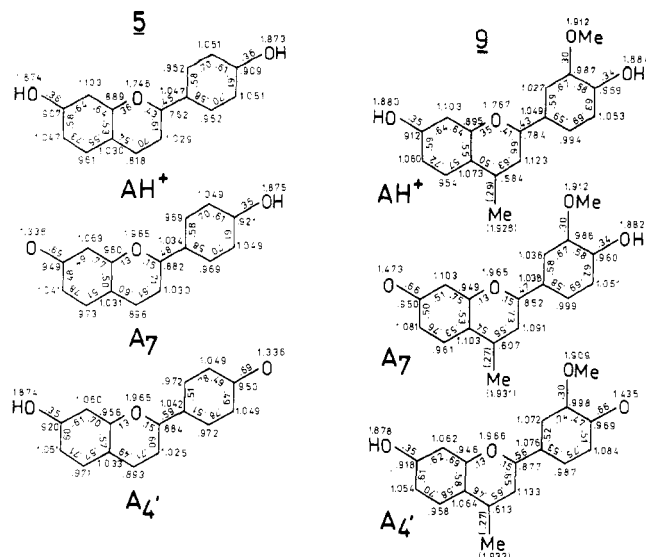


**Figure 5.** Electron densities and bond orders for compounds 5 and 9 from HMO calculations. Methyl group was treated on the basis of a inductive model $h_x = -0.6$ (heteroatom model $h_x = 2, K_{c-x} = 0.7$).
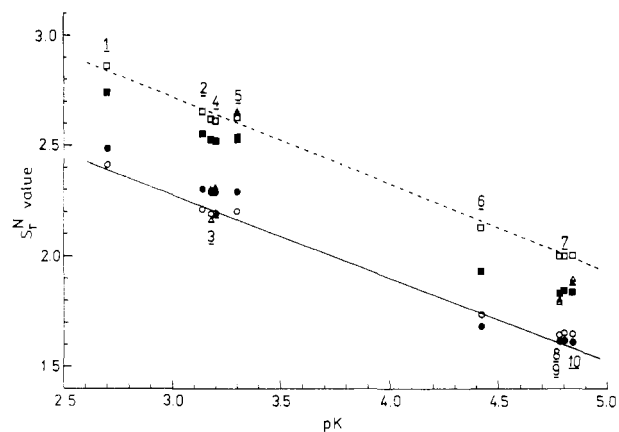


**Figure 6.** Least-squares linear regression plot of the $S_r^N$ indices versus the experimental p$K$ values for flavylium compounds 1-10. $S_r^N$ values: 2 (O) and 4 (●) positions of cationic forms; 2 (□) and 4 (■) positions of anhydrobase forms $A_7$; 2 (△) and 4 (▲) positions of anhydrobase forms $A_{4'}$.

the indices of superdelocalizability for the nucleophilic attack $(S_r^N)$ in positions C-2 and C-4 represent the relevant parameter of reactivity, i.e., the lower the value of $S_r^N$ index, the higher the stability of flavylium chromophore.

The effect of the methyl group attached to the π-electron system can be investigated within the confines of three models: conjugation, inductive, and heteroatom (Parkanyi et al., 1980). p$K$ values of investigated flavylium compounds were used as the experimental foundation for evaluation of suitability of specific methyl group models. The statistical analysis of results using the least-squares method for the regression line between the experimental p$K$ values and calculated $S_r^N$ indices gave the best correlation for the inductive model (Table II). The results of the calculations of nucleophilic superdelocalizability indices by the application of inductive model $(h_x = -0.6)$ for cationic $AH^+$ and anhydrobase $A_7$ forms of compound 1-10 are presented together with the experimental p$K$ values in Table III and Figure 6. The solid line on Figure 6 connects the points with lowest $S_r^N$ index values (O for the 4-unsubstituted salts and ● for the 4-substituted salts), and the dashed line connects the points

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**Table II. Parameters of the Methyl Group and Results of Statistical Analysis of Experimental p$K$ Values and Calculated $S_r^N$ Indices[a]**

| methyl group model | flavylium chromophore | $S_r^N$ | a | b | n | s | r |
|---|---|---|---|---|---|---|---|
| conjugation model[b] (H$_3$) | AH$^+$ | $S_2^N$ | −5.024 | 14.455 | 8 | 0.24 | −0.9528 |
| | | $S_4^N$ | −4.336 | 13.239 | 8 | 0.13 | −0.9872 |
| $h_x = -2.3$, $K_{c-x} = 3.1$ | A$_7$ | $S_2^N$ | −4.452 | 15.117 | 8 | 0.25 | −0.9489 |
| | | $S_4^N$ | −4.185 | 13.924 | 8 | 0.16 | −0.9805 |
| inductive model[b,c] | AH$^+$ | $S_2^N$ | −3.136 | 10.137 | 8 | 0.07 | −0.9960 |
| | | $S_4^N$ | −2.636 | 9.240 | 8 | 0.07 | −0.9962 |
| $h_x = -0.5$ | A$_7$ | $S_2^N$ | −2.878 | 10.813 | 8 | 0.07 | −0.9963 |
| | | $S_4^N$ | −2.630 | 9.873 | 8 | 0.06 | −0.9975 |
| | AH$^+$ | $S_2^N$ | −2.793 | 9.363 | 8 | 0.07 | −0.9965 |
| $h_x = -0.6$ | | $S_4^N$ | −2.307 | 8.470 | 8 | 0.08 | −0.9945 |
| | A$_7$ | $S_2^N$ | −2.523 | 9.856 | 8 | 0.06 | −0.9974 |
| | | $S_4^N$ | −2.267 | 8.933 | 8 | 0.07 | −0.9961 |
| heteroatom model[b,d] | AH$^+$ | $S_2^N$ | −4.947 | 14.275 | 8 | 0.23 | −0.9570 |
| | | $S_4^N$ | −4.349 | 13.268 | 8 | 0.13 | −0.9871 |
| $h_x = 2$, $K_{c-x} = 0.7$ | A$_7$ | $S_2^N$ | −4.567 | 15.422 | 8 | 0.25 | −0.9504 |
| | | $S_4^N$ | −4.344 | 14.334 | 8 | 0.16 | −0.9799 |
| | AH$^+$ | $S_2^N$ | −5.464 | 15.486 | 8 | 0.31 | −0.9219 |
| $h_x = 1.83$, $K_{c-x} = 0.6$ | | $S_4^N$ | −5.040 | 14.910 | 8 | 0.19 | −0.9707 |
| | A$_7$ | $S_2^N$ | −5.044 | 16.755 | 8 | 0.33 | −0.9109 |
| | | $S_4^N$ | −4.992 | 16.042 | 8 | 0.23 | −0.9571 |

[a] Least-squares method. p$K_{calcd} = aS_r^N{}_{calcd} + b$. The other parameters are defined as follows: a, slope; b, intercept; n, number of points; s, standard error; r, correlation coefficient. All data are significant on a 1% probability level. [b] Parkanyi et al., 1980. [c] Ohta and Osajima, 1979. [d] Ohta et al., 1980.

**Table III. Calculated $S_r^N$ Indices and p$K$ Values[a] for the Inductive Model of the Methyl Group**

| no. | compound | flavylium cation AH$^+$ | | | | | anhydrobase A$_7$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | p$K_{exptl}$ | $S_2^N$ | p$K_{calcd}$ | $S_4^N$ | p$K_{calcd}$ | $S_2^N$ | p$K_{calcd}$ | $S_4^N$ | p$K_{calcd}$ |
| 1 | 7-OH | 2.70[b] | 2.411 | 2.63 | 2.483 | 2.74 | 2.860 | 2.64 | 2.737 | 2.73 |
| 2 | 7-OH-4′-OCH$_3$ | 3.14[b] | 2.210 | 3.19 | 2.301 | 3.16 | 2.649 | 3.17 | 2.549 | 3.15 |
| 3 | 7,3′,4′-(OH)$_3$ | 3.18[b] | 2.191 | 3.24 | 2.285 | 3.20 | 2.618 | 3.25 | 2.523 | 3.21 |
| 4 | 7,4′-(OH)$_2$-3′-OCH$_3$ | 3.20[b] | 2.191 | 3.24 | 2.285 | 3.20 | 2.618 | 3.25 | 2.524 | 3.21 |
| 5 | 7,4′-(OH)$_2$ | 3.30[b] | 2.197 | 3.23 | 2.291 | 3.19 | 2.631 | 3.22 | 2.535 | 3.19 |
| 6 | 7-OH-4-CH$_3$ | 4.44[c] | 1.735 | 4.52 | 1.682 | 4.59 | 2.129 | 4.48 | 1.932 | 4.55 |
| 7 | 7-OH-4′-OCH$_3$-4-CH$_3$ | 4.80[d] | 1.648 | 4.76 | 1.615 | 4.75 | 2.015 | 4.77 | 1.841 | 4.76 |
| 8 | 7,3′,4′-(OH)$_3$-4-CH$_3$ | | 1.642 | 4.78 | 1.611 | 4.75 | 2.002 | 4.80 | 1.832 | 4.78 |
| 9 | 7,4′-(OH)$_2$-3′-OCH$_3$-4-CH$_3$ | | 1.642 | 4.78 | 1.611 | 4.75 | 2.002 | 4.80 | 1.832 | 4.78 |
| 10 | 7,4′-(OH)$_2$-4-CH$_3$ | 4.84[e] | 1.644 | 4.77 | 1.611 | 4.75 | 2.007 | 4.79 | 1.835 | 4.77 |

[a] Least-squares method. [b] Ohta et al., 1980. [c] Baranac and Amić, 1985. [d] Brouillard et al., 1982. [e] Baranac et al., 1990.

with highest $S_r^N$ values (□ for the 4-unsubstituted salts and 4-substituted salts); see also the caption with Figure 6.

The following points can be stressed on the basis of the data presented in Figure 6:

4-Unsubstituted flavylium compounds (1–5) are much more susceptible to the hydration reactions (decolorization) than 4-substituted flavylium compounds.

Cationic forms of 4-unsubstituted as well as 4-substituted flavylium salts (solid line) have much lower indices of superdelocalizability compared to the anhydrobase forms (dashed line). That means that the hydration reactions will take place more readily on anhydrobase forms than on cationic forms.

Toward the nucleophilic addition in position C-2, prototropic tautomer A$_7$ will be the most reactive form; i.e., the pseudobases of flav-3-ene type will be formed (in the case of tautomer A$_{4'}$ of compound 5, pseudobases of type flav-2-ene will be formed first).

The addition of water to the anhydrobase forms of all compounds has a lower probability in position C-4 (except for the tautomer A$_{4'}$ of compound 5).

Cationic forms are less susceptible to the nucleophilic addition: As expected, 4-unsubstituted compounds are more sensitive to the addition in position C-4 and the 4-substituted compounds to the addition in position C-2.

It can be predicted that the p$K$ value for compounds 8 and 9 will be 4.78.

## CONCLUSION

On the basis of presented data, a following conclusion can be drawn: Under weakly acidic conditions, the cationic form of flavylium chromophore forms the anhydrobase form (or forms). The anhydrobase forms (contrary to the assertation of Brouillard and co-workers), especially prototropic tautomer A$_7$, are converted through hydration reactions into colorless pseudobase and/or chalcone forms:

$$\text{AH}^+ \overset{-\text{H}^+}{\rightleftharpoons} \text{A} \overset{+\text{H}_2\text{O}}{\rightleftharpoons} \text{B and/or C}$$
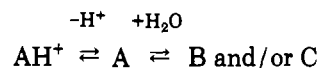
This conclusion is also in agreement with experimental observations of McClelland and McGall (1982) and with the theoretical predictions obtained with the topological resonance energy (TRE) model (Amić et al., 1990).

If a methyl group is present in position C-4, then both cationic and anhydrobase forms are significantly stabilized and reactions of hydration proceed at a largely reduced rate.

## LITERATURE CITED

Aihara, J.-i. A New Definition of Dewar-Type Resonance Energies. *J. Am. Chem. Soc.* **1976**, *98*, 2750–2758.

Amić, D.; Vukadinović, V.; Radoš, A. The Program for the HMO Calculations on Microcomputers. *Kem. Ind.* **1989,** *8,* 365–371.

Amić, D.; Jurić, A.; Trinajstić, N. Topological Resonance Energies of Some Forms of the Flavylium Chromophoric System. *Croat. Chem. Acta* **1990,** in press.

Asen, S. Known Factors Responsible for Infinite Flower Color Variations. *Acta Hortic.* **1976,** *63,* 217–223.

Baranac, J.; Amić, D. Spectrochemical Investigations of the Influence of pH on the Mechanism of Structural Transformations of the 4-Substituted Flavylium Chromophore. *J. Serb. Chem. Soc.* **1985,** *50,* 299–305.

Baranac, J.; Amić, D. Unpublished results, 1986.

Baranac, J.; Amić, D.; Vukadinović, V. Spectrophotometric Study of the Influence of Individual Substituted Positions on Flavylium Chromophore Stability. *J. Agric. Food Chem.* **1990,** preceding paper in this issue.

Bendz, G.; Martensson, O.; Nilsson, E. Studies of Flavylium Compounds. I. Some Flavylium Compounds and Their Properties. *Ark. Kemi.* **1967,** *27,* 65–77.

Brouillard, R. Origin of the Exceptional Colour Stability of the Zebrina Anthocyanin. *Phytochemistry* **1981,** *20,* 143–145.

Brouillard, R. Chemical Structure of Anthocyanins. In *Anthocyanins as a Food Colors;* Markakis, P., Ed.; Academic Press: New York, 1982.

Brouillard, R. The in vivo Expression of Anthocyanin Colour in Plants. *Phytochemistry* **1983,** *22,* 1311–1323.

Brouillard, R.; Dubois, J. E. Mechanism of the Structural Transformations of Anthocyanins in Acidic Media. *J. Am. Chem. Soc.* **1977a,** *99,* 1359–1364.

Brouillard, R.; Delaporte, B. Chemistry of Anthocyanin Pigments. 2. Kinetic and Thermodynamic Study of Proton Transfer, Hydration and Tautomeric Reactions of Malvidin 3-Glucoside. *J. Am. Chem. Soc.* **1977b,** *99,* 8461–8468.

Brouillard, R.; Delaporte, B.; Dubois, J. E. Chemistry of Anthocyanin Pigments. 3. Relaxation Amplitudes in pH-Jump Experiments. *J. Am. Chem. Soc.* **1978,** *100,* 6202–6205.

Brouillard, R.; Iacobucci, G. A.; Sweeny, J. G. Chemistry of Anthocyanin Pigments, 9. UV-visible Spectrophotometric Determination of the Acidity Constants of Apigeninidin and Three Related 3-Deoxyflavylium Salts. *J. Am. Chem. Soc.* **1982,** *104,* 7585–7590.

Debicki-Pospišil, J.; Lovrić, T.; Trinajstić, N.; Sabljić, A. Anthocyanin Degradation in the Presence of Furfural and 5-Hydroxymethylfurfural. *J. Food Sci.* **1983,** *48,* 411–416.

Hrazdina, G. Anthocyanins. In *The Flavonoids;* Harborne, J. B., Mabry, T. J., Eds.; Chapman and Hall: London, 1982.

Jurd, L. Methods of Using Flavylium Compounds for Food Coloring. U.S. Patent No. 3 266 903, 1966.

Jurd, L. Some Advances in the Chemistry of Anthocyanin-Type Plant Pigments. In *The Chemistry of Plant Pigments;* Chichester, C. O., Ed.; Academic Press: New York, 1972.

Jurd, L.; Geissman, T. A. Anthocyanins and Related Compounds. II. Structural Transformations of Some Anhydro Bases. *J. Org. Chem.* **1963,** *28,* 2394–2397.

Kurtin, W. E.; Song, P.-S. Electronic Structures and Spectra of Some Natural Products of Theoretical Interest-I. Molecular Orbital Studies of Anthocyanidins. *Tetrahedron* **1968,** *24,* 2255–2267.

McClelland, R. A.; McGall, G. H. Hydration of the Flavylium Ion. 2. The 4′-Hydroxyflavylium Ion. *J. Org. Chem.* **1982,** *47,* 3730–3736.

Ohta, H.; Osajima, Y. Report of the Association of Fruit Juice (Kajuu Kyokai Ho), No. 254, Feb 1979 (extra number).

Ohta, H.; Akuta, S.; Osajima, Y. Stability of Anthocyanin Pigments and Related Compounds in Acidic Solutions. *Nippon Shokuhin Kogyo Gakkaishi* **1980,** *27,* 81–85.

Parkanyi, C.; Herndon, W. C.; Shawali, A. S. Optimum HMO Parameters for the Three Models of the Methyl Group. *J. Org. Chem.* **1980,** *45,* 3529–3531.

Timberlake, C. F. Improvements Relating to the Colouring of Foodstuffs. U.K. Patent No. 5477/68, 1968.

Timberlake, C. F.; Bridle, P. The Anthocyanins. In *The Flavonoids;* Harborne, J. B., Mabry, T. J., Eds.; Chapman and Hall: London, 1975.

Trinajstić, N. Private communication, 1987.

# Structure of a β-Cyclodextrin–Vanillin Inclusion Complex

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Vanillin was found to form a stable complex with β-cyclodextrin. A binding constant value of $1.11 \times 10^4$ $M^{-1}$ was determined for the 1:1 complex. $^1H$ NMR chemical shift differences and circular dichroism spectra indicated that both phenolic end and aldehyde end were affected by complexation. 2D nuclear Overhauser enhancement spectroscopy indicated proximity of $OCH_3$ in vanillin to the anomeric protons of β-cyclodextrin. 1D difference NOE experiments have revealed that vanillin exists with the phenolic end nearer to the narrower end and the aldehyde end to the wider end of the torus-shaped β-cyclodextrin molecule.

Extensive literature is available since Freudenberg and Cramer (1948) discovered that cyclodextrin can form inclu- sion compounds (Saenger, 1980). β-Cyclodextrin (BCD) consists of seven glucose units in a ring with $\alpha(1\rightarrow4)$ links