

Spectrophotometric Investigations of Monohydroxylic Flavylium Compounds

D. Amić*

*Faculty of Agriculture, University of Osijek, Terjska cesta bb,
P. O. Box 117, 54001 Osijek, Croatia, Yugoslavia*

and

J. Baranac

*Institute of Physical Chemistry, Faculty of Science, University of Belgrade,
P. O. Box 550, 11001 Belgrade, Serbia, Yugoslavia*

Received October 1, 1990.

Monohydroxylic flavylium compounds in positions C-7 or C-4' were investigated in buffer solutions of pH 2.00–8.50. The direction of structural transformations of these compounds, which was absorptiometrically followed, was correlated to the influence of substituted positions. Transformation processes were quantitatively defined by the corresponding pK values.

INTRODUCTION

Interesting structure of flavylium chromophore, which represents a complex heterocyclic π -electron system, with an oxygen atom, produces characteristic absorption spectra. This enables correlation of flavylium structure changes to the corresponding absorption bands. The influence of various substituents, their positions and combinations, is manifested not only in absorption bands but also in the corresponding transformation mechanisms.

Upon dissolution of flavylium salts in acidic aqueous solutions¹, a complex equilibrium is established between two coloured forms (flavylium cation AH^+ and anhydro base A) and two colourless ones (pseudobase B, and cis chalcone cC or trans chalcone tC), Figure 1. Ionized chalcone forms (cC^- , cC^{2-} , tC^- and tC^{2-}) which can exist in basic media are also included in Figure 1.

At present, much work is being carried out on the use of flavylium salts (anthocyanins, anthocyanidins and related compounds) as food colouring materials². This

* Corresponding author

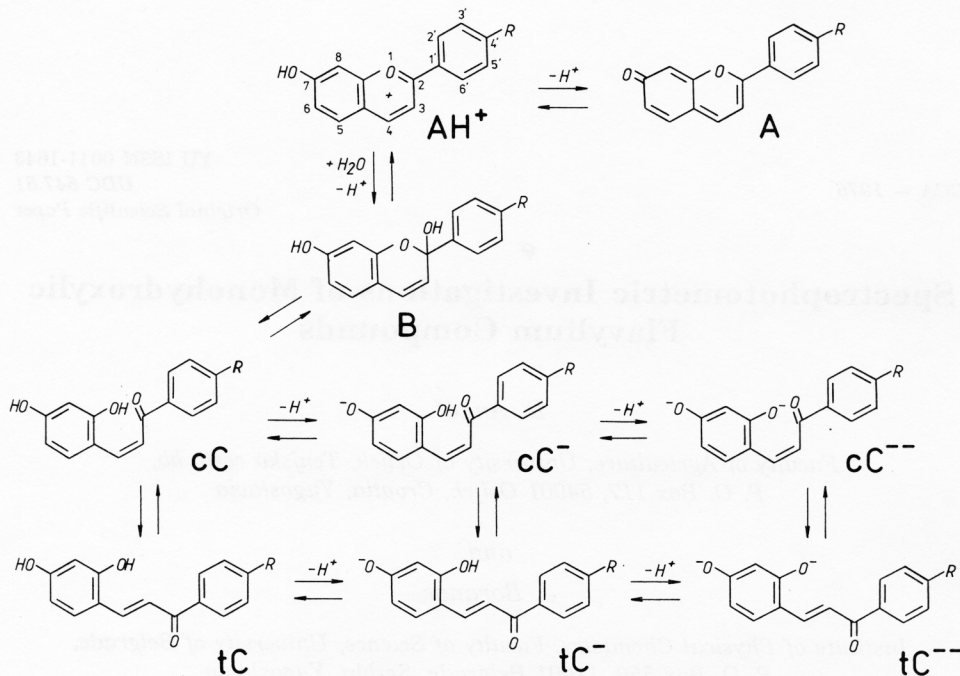


Figure 1. Structural transformations of flavylum compounds.

use, however, is entirely restricted to those foods or beverages having a pH below 4; in slightly acid solutions, $\text{pH} \approx 4\text{--}6$, most of flavylum salts are rapidly decolourized³.

Various substituents: hydroxy and methoxy groups, methyl group, sugars and acylated sugars have a marked effect upon the colour and stability of flavylum salts⁴⁻⁶. In this paper we have focused on monohydroxy flavylum salts, substituted in position C-7 or C-4', and their individual influences on possible structural transformations in solutions of varying pH value. These salts have been studied previously in some detail⁷⁻¹¹. Our approach provides a much more illustrative description of structural transformations as well as the forms which appear in solutions at different pH values. The assignment of absorption bands to AH^+ , A, B, cC, cC^- , tC and tC^- forms were performed according to the literature¹².

EXPERIMENTAL

The 7-hydroxyflavylium chloride and 4'-hydroxyflavylium chloride were supplied by G. A. Iacobucci and J. G. Sweeny (The Coca Cola Co., Atlanta, GA).

For all experiments, the required amount of pigment (analytical concentration $\approx 10^{-5}$ mol dm^{-3}) was dissolved in Britton-Robinson buffers (pH 2.00 to 8.50) of constant ionic strength ($I = 0.2$ 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 Unicam SP8-100 spectrophotometer. The spectra were recorded immediately after preparation (10 minutes) and after standing for 4 days.

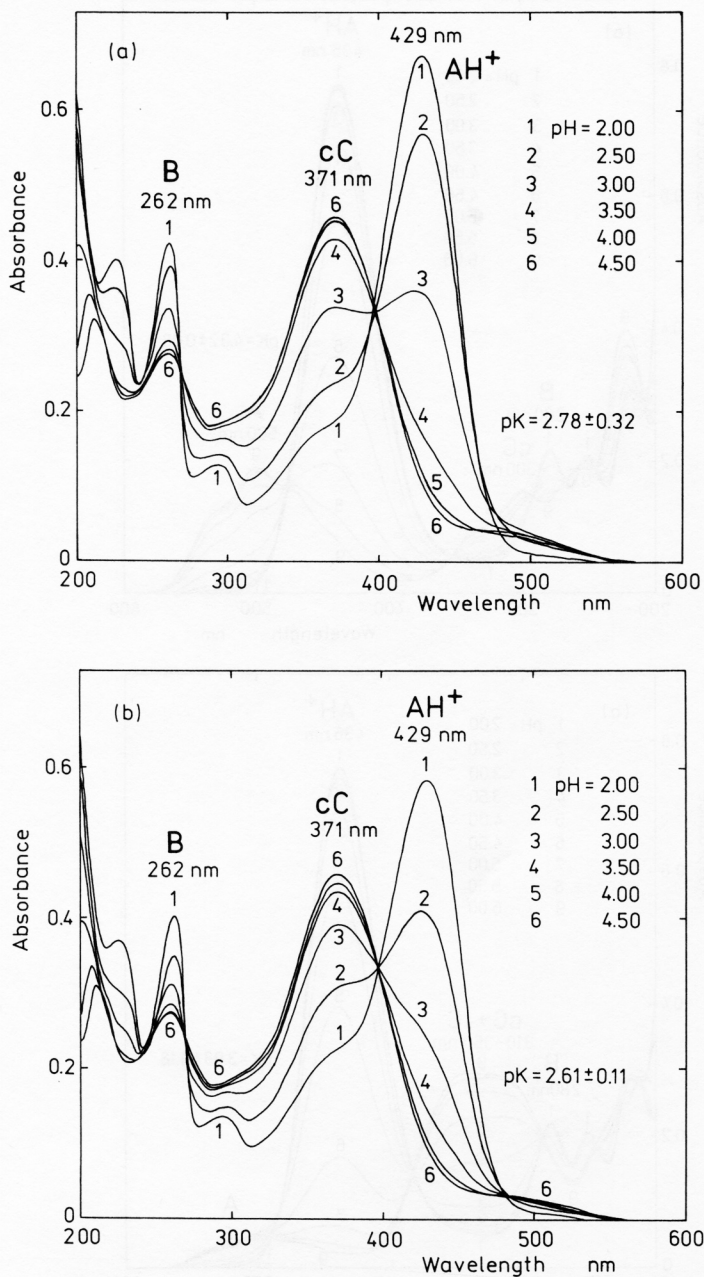


Figure 2. Absorption spectra of 7-hydroxyflavylum chloride in solutions of pH 2.00–4.50, concentration $2.40 \cdot 10^{-5} \text{ mol dm}^{-3}$: a) after standing for 10 minutes; b) after standing for 4 days.

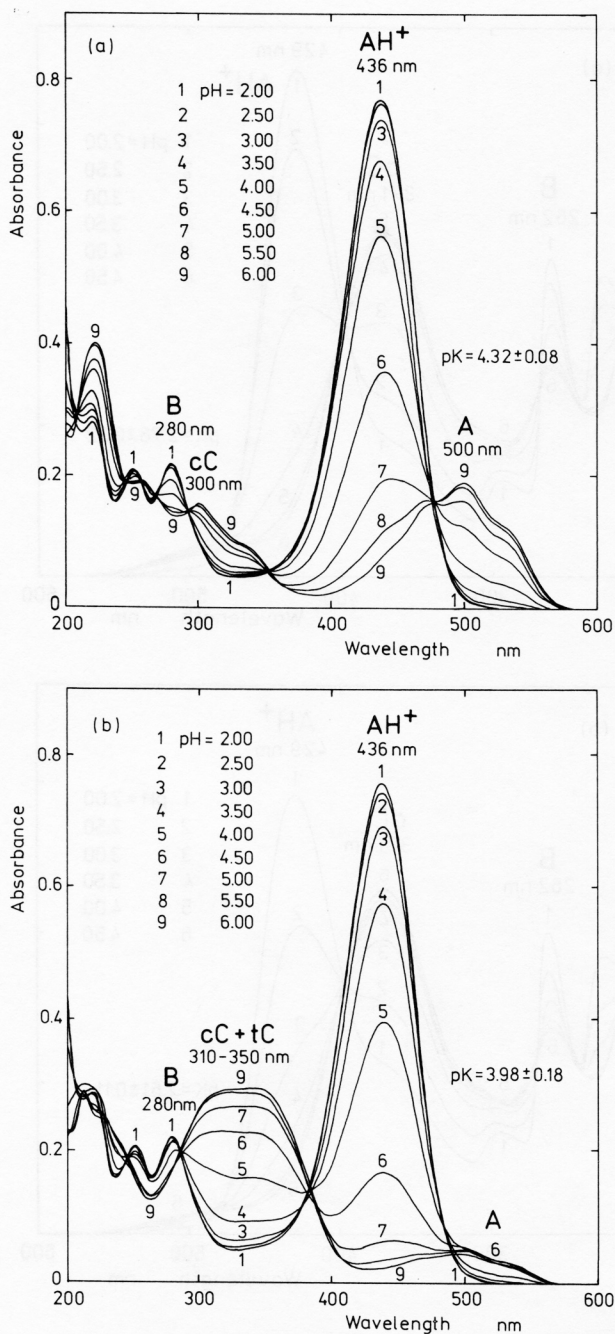


Figure 3. Absorption spectra of 4'-hydroxyflavylium chloride in solutions of pH 2.00–6.00, concentration $2.24 \cdot 10^{-5} \text{ mol dm}^{-3}$: a) after standing for 10 minutes; b) after standing for 4 days.

RESULTS

Absorption spectra of 7-hydroxyflavylium chloride, compound I, in acid buffers pH 2.00–4.50, are presented in Figure 2. Only the cationic form, with an absorption maximum at $\lambda=429$ nm, exists up to pH 2.50. Already at pH 3.00 the *cis* chalcone maximum at $\lambda=371$ nm becomes significant as a result of the transformation from cationic form. At pH 4.50, the cationic maximum disappears, while the *cis* chalcone one achieves its maximum. The isosbestic point at $\lambda_{is}=396$ nm defines the equilibrium between them. After standing of the solutions for 4 days, the equilibrium established between the cationic and *cis* chalcone form remains unchanged, except for minor changes in the intensity and pK values.

In the same buffer solutions, 4'-hydroxyflavylium chloride, compound II, has a more stable cationic form, $\lambda=436$ nm, which holds up to pH 4.50, Figure 3. At higher pH values this compound forms the anhydro base, characteristic absorption of which is mostly pronounced at pH 6.00 with $\lambda=500$ nm. The equilibrium between the cationic and the anhydro base form is expressed through the isosbestic point at $\lambda_{is}=478$ nm. After 4 days, the same solutions of compound II, show a different trend of change, i. e. they form the chalcones instead of the anhydro base, similar to compound I, which means that the primary process of deprotonation at 4'-hydroxy group is substituted by a process of hydration. Further behaviour of compound I in buffer solutions of pH values above 4.50 is presented in Figure 4. The *cis* chalcone form created stays on till pH 5.50, when a prominent maximum of the anhydro base form begins to appear at $\lambda=476$ nm, becoming more intensive with increasing pH values, accompanied by a decreasing intensity of the *cis* chalcone maximum. At pH 8.50, the *cis* chalcone maximum disappears. Equilibrium is established between these forms with an isosbestic point at $\lambda_{is}=417$ nm. After standing of the indicated buffer solutions of compound I for 4 days, equilibrium is established between the *cis* chalcone and the ionized form of same chalcone.

The anhydro base form of compound II, Figure 5, maintains its characteristic absorption in the range of pH values from 6.50 to 8.00, accompanied by a pronounced hyperchromic effect. After 4 days, these buffer solutions of compound II, show a disappearance of anhydro bases, whose formation and existence have been confirmed immediately after preparation. They form a chalcones, which are in equilibrium with the ionized *trans* chalcone form into which they are transformed.

DISCUSSION AND CONCLUSIONS

The compounds investigated represent monohydroxy flavylium salts of 3-deoxy type, with OH group in various positions. As we had investigated the flavylium chromophore substituted simultaneously in positions C-7 and C-4' with OH group¹³, we attempted, through the choice of these compounds, to determine more clearly their individual influence, as well. It was found out, as observable from the results presented, that the course of their structural transformations differs when pH values of the solutions are varied in the pH range 2.00–8.50.

Both of these compounds produce stable cationic forms in strongly acidic solutions: compound I up to pH 2.50, Figure 2, and compound II up to pH 4.50, Figure 3.

Reduction of the acidity of these solutions leads to the deprotonation of cationic forms and formation of the corresponding anhydro base forms. Under the existing experimental conditions, anhydro base form of compound I, Figure 2, could not be detected, while the anhydro base of compound II was detected successfully, Figure 3. This could be explained by the possibility that the deprotonation reaction of the 7-OH

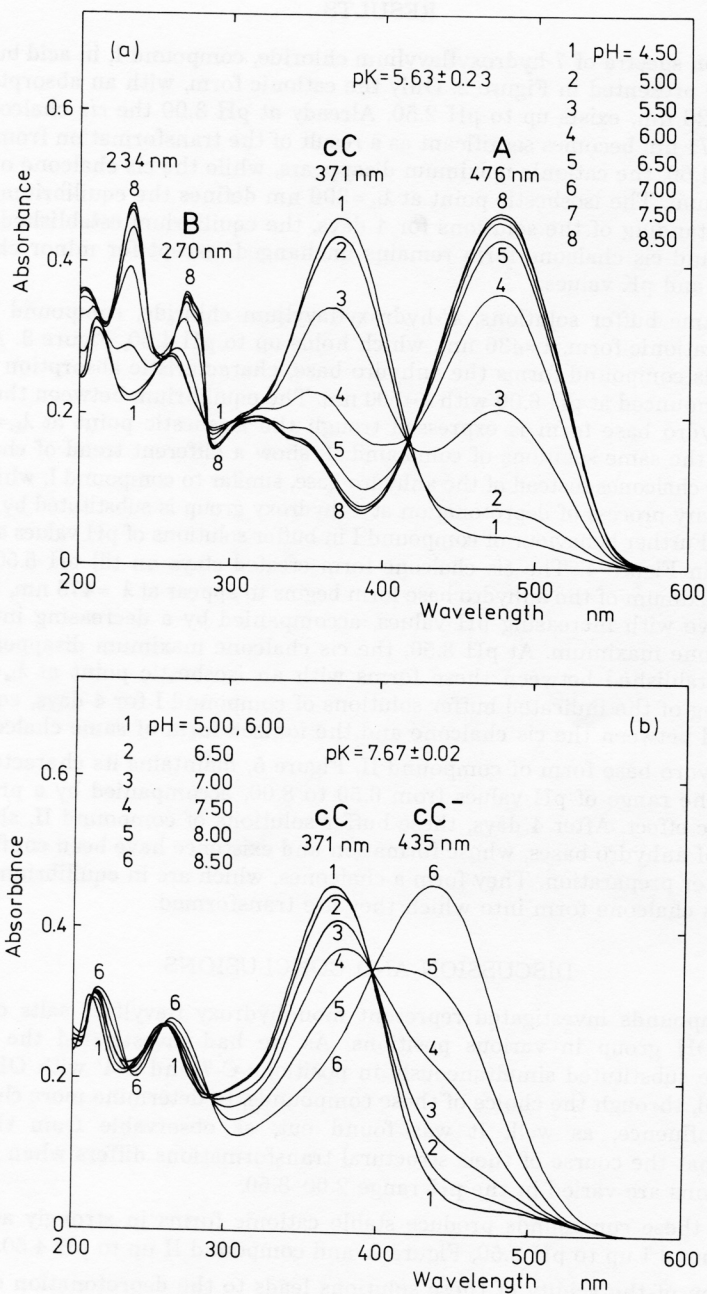


Figure 4. Absorption spectra of 7-hydroxyflavylum chloride in solutions of pH 4.50–8.50, concentration $2.40 \cdot 10^{-5}$ mol dm⁻³: a) after standing for 10 minutes; b) after standing for 4 days.

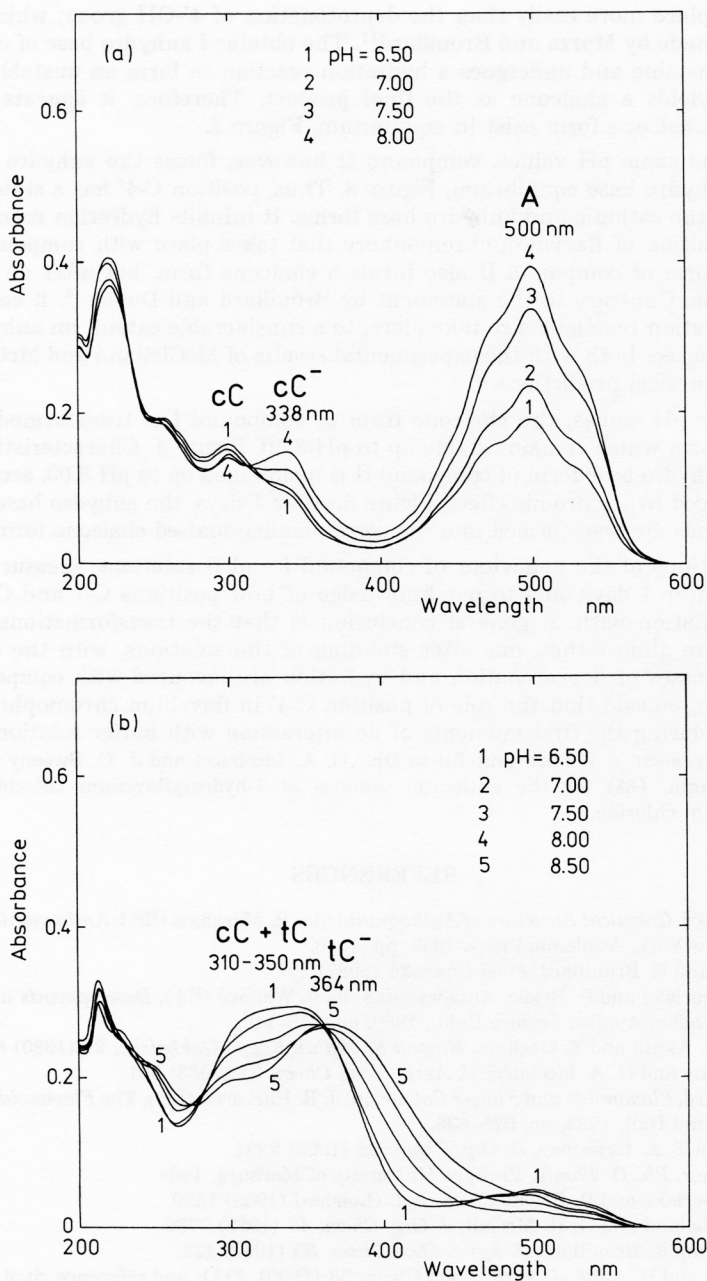


Figure 5. Absorption spectra of 4'-hydroxyflavylium chloride in solutions of pH 6.50–8.50, concentration $2.24 \cdot 10^{-5} \text{ mol dm}^{-3}$: a) after standing for 10 minutes; b) after standing for 4 days.

group takes place more easily than the deprotonation of 4'-OH group; which was an assumption made by Mazza and Brouillard¹¹. The obtained anhydro base of compound I is highly unstable and undergoes a hydration reaction to form an unstable pseudo-base which yields a chalcone as the final product. Therefore, it appears that the cationic and chalcone form exist in equilibrium, Figure 2.

Under the same pH values, compound II however, forms the anhydro base and cationic – anhydro base equilibrium, Figure 3. Thus, position C-4' has a stabilizing effect both on the cationic and anhydro base forms. It inhibits hydration reactions and the decomposition of flavylum chromophore that takes place with compound I. Anhydro base form of compound II also forms a chalcone form, but after an extended period of time. Contrary to the statement by Brouillard and Dubois¹⁴, it can be said that the hydration reactions also take place, to a considerable extent, on anhydro base form, which agrees both with the experimental results of McClelland and McGall¹⁰ and with our theoretical predictions.¹⁵

At higher pH values, the chalcone form of compound I is transformed into anhydro base form which remains stable up to pH 8.50, Figure 4. Characteristic absorption of the anhydro base form of compound II is maintained up to pH 8.00, accompanied by a pronounced hyperchromic effect, Figure 5. After 4 days, the anhydro base forms of both compounds are transformed into the corresponding ionized chalcone forms.

Investigations of the behaviour of compound I and II solutions, measured immediately and after 4 days, add to our knowledge of how positions C-7 and C-4' affect the transformation path. A general conclusion is that the transformations of these compounds are almost the same after standing of the solutions, with the exception that the processes of deprotonation and hydration are favoured with compound I. It can, therefore, be said that the role of position C-4' in flavylum chromophore is stabilizing only during the first moments of its interaction with buffer solutions.

Acknowledgement. – We are grateful to Drs. G. A. Iacobucci and J. G. Sweeny (The Coca Cola Co., Atlanta, GA) for the authentic samples of 7-hydroxyflavylium chloride and 4'-hydroxyflavylium chloride

REFERENCES

1. R. Brouillard, *Chemical Structure of Anthocyanins*, in: P. Markakis (Ed.), *Anthocyanins as Food Colors*, New York, Academic Press, 1982, pp. 1–40.
2. G. Mazza and R. Brouillard, *Food Chem.* **25** (1987) 207.
3. C. F. Timberlake and P. Bridle, *Anthocyanins*, in: J. Walford (Ed.), *Developments in Food Colours-I*, London, Applied Science Publ., 1980, pp. 115–149.
4. H. Ohta, S. Akuta and Y. Osajima, *Nippon Shokuhin Kogyo Gakkaishi*, **27** (1980) 81.
5. J. G. Sweeny and G. A. Iacobucci, *J. Agric. Food Chem.* **31** (1983) 531.
6. R. Brouillard, *Flavonoids and Flower Colour*, in: J. B. Harborne (Ed.), *The Flavonoids*, London, Chapman and Hall, 1988, pp. 525–538.
7. L. Jurd and T. A. Geissman, *J. Org. Chem.* **28** (1963) 2394.
8. F. C. Werner, *Ph. D. Thesis*, Philipps University of Marburg, 1964.
9. C. F. Timberlake and P. Bridle, *Chem. Ind. (London)* (1965) 1520.
10. R. A. McClelland and G. H. McGall, *J. Org. Chem.* **47** (1982) 3730.
11. G. Mazza and R. Brouillard, *J. Agric. Food Chem.* **35** (1987) 422.
12. J. Baranac and D. Amić, *J. Agric. Food Chem.* **38** (1990), 2111, and reference cited therein.
13. J. Baranac, D. Amić and V. Vukadinović, *J. Agric. Food Chem.* **38** (1990) 932.
14. R. Brouillard and J. E. Dubois, *J. Am. Chem. Soc.* **99** (1977) 1359.
15. a) D. Amić, J. Baranac and V. Vukadinović, *J. Agric. Food Chem.* **38** (1990) 936.
b) D. Amić, A. Jurić and N. Trinajstić, *Croat. Chem. Acta.* **63** (1990) 19.
c) D. Amić and N. Trinajstić, *J. Chem. Soc. Perkin Trans.* **2** (1990), in press

SAŽETAK**Spektrofotometrijsko ispitivanje monohidroksilnih flavilijevih spojeva***D. Amić i J. Baranac*

Monohidroksilni flavilijevi spojevi u ležajima C-7 ili C-4' ispitivani su u puferima pH = 2.00-8.50. Slijed strukturnih transformacija, praćen apsorpciometrijski, doveden je u svezu sa supstituiranim pozicijama. Transformacijski procesi kvantitativno su definirani pripadnim vrijednostima pK.