Structural Transformations of Apigeninidin-Type Flavylium Salts

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Structural transformations of apigeninidin (5,7,4'-trihydroxyflavylium chloride), 7,4'-dihydroxy-5-methoxyflavylium chloride, and 5,7,3'-trihydroxy-4'-methoxyflavylium chloride were spectroabsorptiometrically investigated in solutions of varying pH value. Transformational changes defined the stability of investigated compounds, which was, in the case of compounds I and II, correlated with the influence of position C-5 and in the case of compound III also with the influence of position C-3'.

The significant and widely spread in nature flavylium compounds attracted the attention of a number of researchers (Brouillard, 1988). The stability of the heterocyclic π-electron structure of these compounds depends on the substituted positions as well as on the substituents themselves. Various transformations of these compounds are due to the different reactivities of those positions.

Structural transformations of flavylium salts in aqueous solutions are represented by Scheme I. Transformations in acidic and neutral media (AH$^+$, A–C forms) postulated by Brouillard (1982) are well-known. For alkaline media there is still insufficient knowledge of structural transformations. Ionized forms (A$^-$, cC$^-$, cC^{2-}, tC$^-$, and tC^{2-}) that can exist in alkaline media are also included in Scheme I.

In the present work the investigation of structural transformations of apigeninidin-type flavylium salts, i.e., of the 3-deoxyflavylium compounds, has been performed. Those transformation forms as well as the forms that appear in solutions at different pH values were characterized by their electron absorption spectra.

The assignments of absorption bands to AH$^+$, A, A$^-$, B, and C forms (acidic and neutral solutions) were performed according to known facts in the flavylium salts literature (Timberlake and Bridle, 1966; Mazza and Brouillard, 1987).

Distinctions between various chalcone forms were defined on the basis of the following assumptions: generally it is known that neutral chalcone forms, cis-chalcone cC, and trans-chalcone tC, are colorless, while the corresponding ionized forms cC$^-$, cC^{2-}, tC$^-$, and tC^{2-} are yellow (Brouillard, 1986). cis-Chalcone absorbs light at shorter wavelengths than the corresponding trans-chalcone. Trans to cis isomerization is well documented (Werner, 1964; Sperling et al., 1966). The assignments of λ_{max} for various chalcone forms of flavylium salts were also performed according to the methods of Jurd and Geissman (1963), McClelland and Gedge (1980), and McClelland and McGall (1982).

EXPERIMENTAL PROCEDURES

Solutions were prepared in Britton–Robinson buffers of constant ionic strength ($I = 0.2$ mol dm^{-3}) and were not exposed to light. The electronic spectra were recorded immediately and several days after the preparation of solutions on a Pye Unicam SP8-100 UV–vis spectrophotometer. All measurements were carried out at the temperature of 20 °C.

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RESULTS

Spectroabsorptiometric measurements carried out on apigeninidin (compound I), 7,4'-dihydroxy-5-methoxyflavylium chloride (compound II), and 5,7,3'-trihydroxy-4'-methoxyflavylium chloride (compound III) reliably define

Apigeninidin chloride

7,4'-dihydroxy-5-methoxyflavylium chloride

5,7,3'-trihydroxy-4'-methoxyflavylium chloride
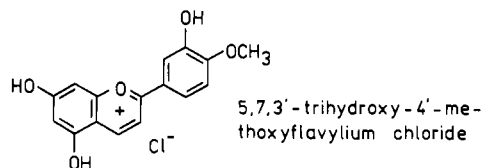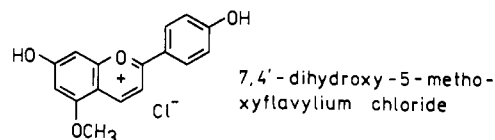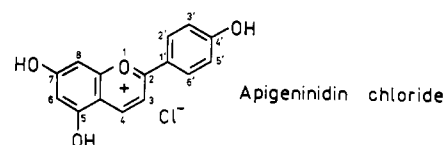
the main individual characteristics of these molecules associated with certain pH values. The cationic form AH$^+$ of compound I is stable in acidic medium (pK = 4.23 ± 0.05) in the range pH 2.00–4.00. Its fundamental maximum appears at λ = 468 nm, with a pronounced inflection on the short-wavelength side, which is characteristic of the presence of the OH group in position C-5 (Timberlake and Bridle, 1967; Baranac and Amić, 1989a,b) (see Figure 1). In the UV range of the spectrum an intensive band is observable at λ = 276 nm, which is probably associated with transient forms of pseudobase B. Increasing the pH value, already at pH 4.50, results in the formation of anhydro base A, with an absorption maximum at λ = 496 nm, together with formation of chalcone C at λ = 410 nm (curves 6–8). The anhydro base A and chalcone C formed exist up to pH 5.50. Further increase of the pH value results in a bathochromic shift of both the anhydro base A and chalcone C maximum, as shown in Figure 2, curves 1–3. Bathochromically shifted maxima correspond to the anionic form A$^-$ of apigeninidin (λ = 530 nm) and probably the ionized form of the corresponding cis-chalcone cC$^-$, which were created through the process of deprotonation. In this way, the anionic form A$^-$ of apigeninidin and
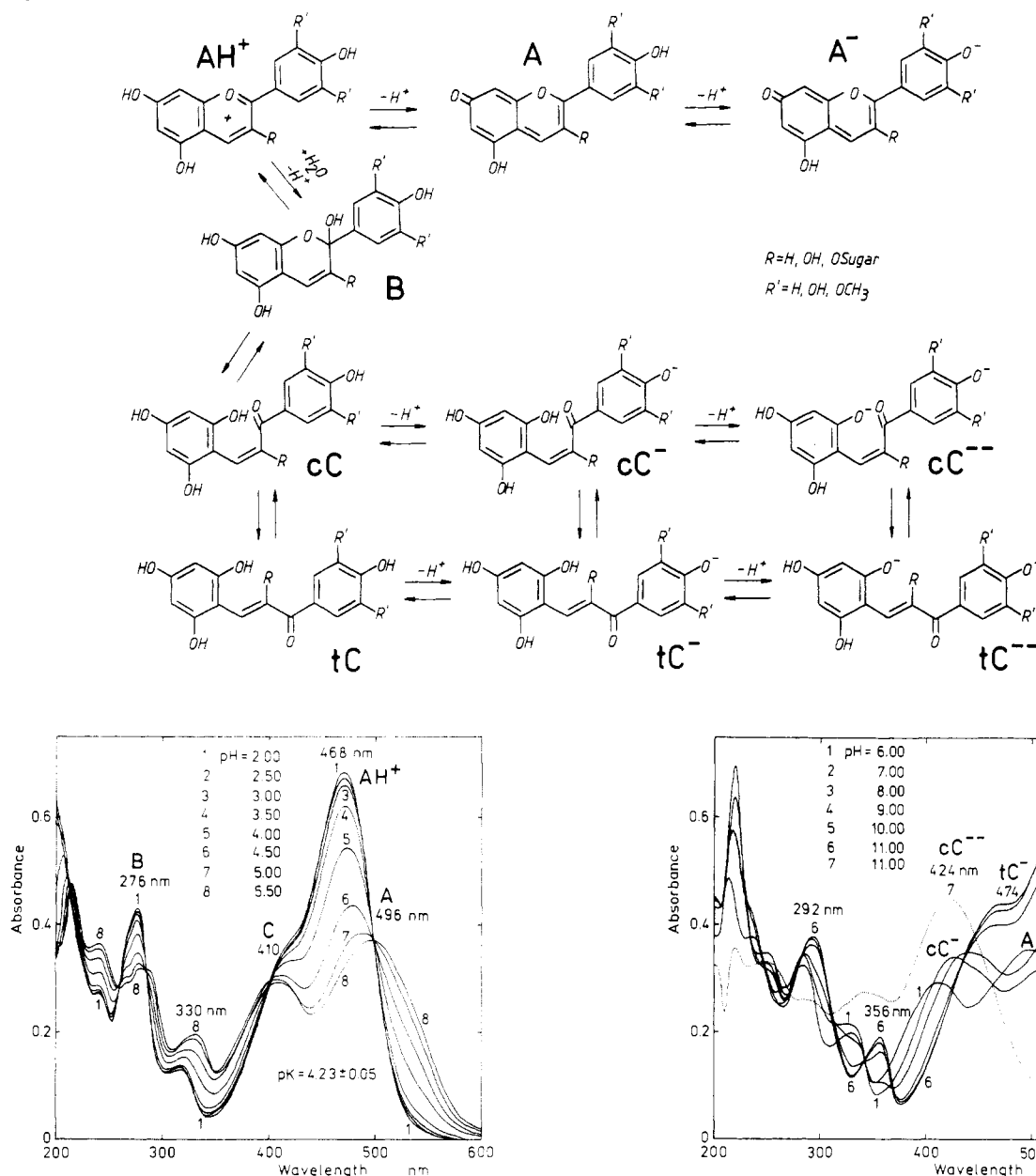
Scheme I

Figure 1. Absorption spectra of apigeninidin in solutions of pH 2.00–5.50, concentration 2.55×10^{-5} mol dm^{-3}, after standing 10 min.

Figure 2. Absorption spectra of apigeninidin in solutions of pH 6.00–11.00, concentration 2.55×10^{-5} mol dm^{-3}, after standing 10 min (curve 7 after standing 14 days).

the corresponding form of the ionized chalcone are again present together in the weakly alkaline medium. The increase in alkalinity up to pH 11.00 favors the anionic form A^- of the apigeninidin chromophore, accompanied by the ionized *trans*-chalcone form tC^-, which appears through an inflection, $\lambda_i = 474$ nm, on the short-wavelength part of the anionic A^- maximum (see Figure 2, curves 4–6).

The cationic form AH^+, $\lambda = 462$ nm, of compound II ($pK = 3.92 \pm 0.12$) does not show significantly different behavior in acidic buffer solutions than the same form of compound I. The increased pH value, i.e., weakly acidic medium, induces formation of the anhydro base A with $\lambda = 500$ nm, the chalcone C with $\lambda = 410$ nm, and the pseudobase B with $\lambda = 276$ nm. The anhydro base A is formed much more slowly than with compound I, i.e., not before pH 7.00 (Figure 3, curve 3). In the alkaline medium, pH 10.00, the anhydro base A transforms into the anionic form A^-, with $\lambda = 540$ nm. At pH 11.00 the anionic form A^- disappears, probably followed by ionized *trans*-

chalcone tC^- formation, $\lambda = 496$ nm. At the same time, the pseudobase B maximum at $\lambda = 276$ nm experiences a hypochromic effect, followed by the appearance of a chalcone C band at $\lambda = 326$ nm (see Figure 3, curve 7).

Compound III, which differs from compound I by the position of the OH group in the B-ring and the OCH_3 group in position C-4', shows an absorption spectrum not much different from the one derived from compounds I and II. The cationic form AH^+ of compound III, $\lambda = 480$ nm, is also stable in the region pH 2.00–4.00 (see Figure 4, curves 1–5) ($pK = 3.96 \pm 0.11$). In the region from pH 4.50 to pH 5.50 the anhydro base A with $\lambda = 498$ nm and the chalcone C with $\lambda = 410$ nm are also formed. Neutral and weakly alkaline medium pH 5.50–8.00 favors the formation of the ionized form of the *cis*-chalcone cC^-, which is reflected in the increase of the intensity of the chalcone maximum, $\lambda = 432$ nm, and the decrease in the intensity of the maximum belonging to the anhydro base A (see Figure 5). An equilibrium is established, expressed through
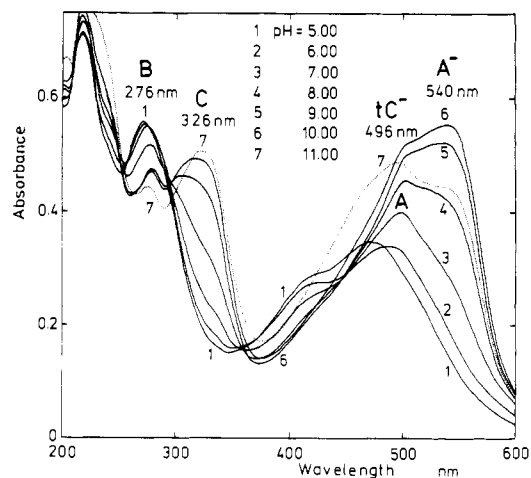
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Figure 3. Absorption spectra of 7,4'-dihydroxy-5-methoxyfla-vylium chloride in solutions of pH 5.00–11.00, concentration 5.72 × 10^{-5} mol dm^{-3}, after standing 10 min.

Figure 4. Absorption spectra of 5,7,3'-trihydroxy-4'-methoxy-flavylium chloride in solutions of pH 2.00–5.50, concentration 7.02 × 10^{-6} mol dm^{-3}, after standing 10 min.

Figure 5. Absorption spectra of 5,7,3'-trihydroxy-4'-methoxy-flavylium chloride in solutions of pH 5.50–8.00, concentration 7.02 × 10^{-6} mol dm^{-3}, after standing 10 min.

the isosbestic point at λ_i = 464 nm and pK = 6.40 ± 0.23. In the alkaline medium, pH 8.00–11.00 (see Figure 6), the ionized *cis*-chalcone form cC⁻, with λ = 432 nm, is transformed into the ionized form of the *trans*-chalcone

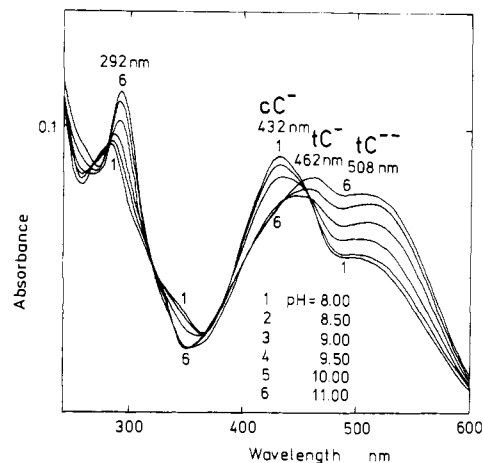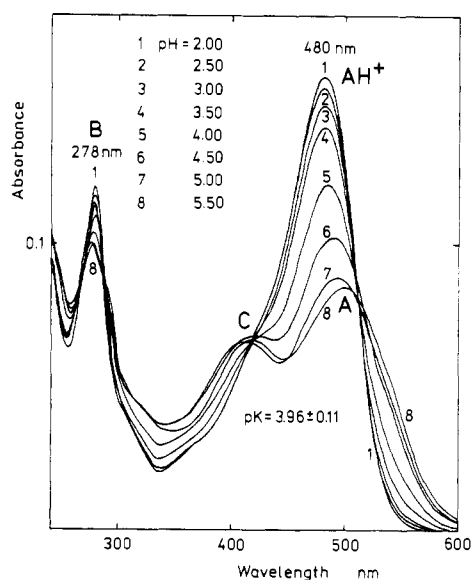**Figure 6.** Absorption spectra of 5,7,3'-trihydroxy-4'-methoxy-flavylium chloride in solutions of pH 8.00–11.00, concentration 7.02 × 10^{-6} mol dm^{-3}, after standing 10 min.
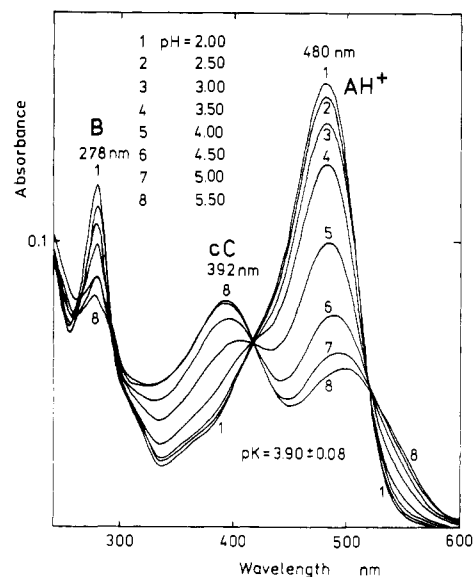

Figure 7. Absorption spectra of 5,7,3'-trihydroxy-4'-methoxy-flavylium chloride in solutions of pH 2.00–5.50, concentration 7.02 × 10^{-6} mol dm^{-3}, after standing 3 days.
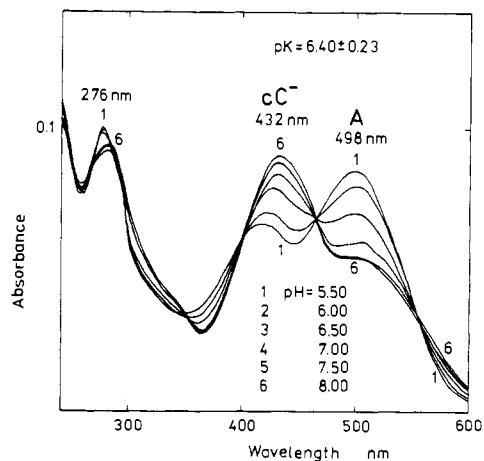
tC⁻, with λ = 462 nm and double-ionized *trans*-chalcone tC²⁻ with λ = 508 nm.

With the aim of gaining better insight into the transformation processes of these molecules, which we, up to now, followed immediately after preparation of the solution (10 min), we have also observed some of them during longer periods (compound I after 14 days and compound III after 3 days).

Absorption spectra of compound I in buffer solution of pH 2.00 (AH⁺ form, λ = 468 nm) after standing for 14 days show negligible hypochromic shift. At pH 5.50 (A form, λ = 496 nm) hypochromic shift is significant (36% loss of color), accompanied by the hyperchromic effect on the chalcone C band, λ = 390 nm. Further increase of the pH value in the range pH 6.00–10.50 causes hypochromic effect on all absorption maxima. In alkaline solution, pH 11.00, a new absorption band, λ = 424 nm, belonging to double-ionized *cis*-chalcone cC²⁻ is formed (see Figure 2, curve 7).

Compound III, presented in Figure 7, maintains its cationic form AH⁺ undisturbed up to pH 3.50 (curve 4). The anhydro base A maximum shows a hypochromic effect, while a pronounced *cis*-chalcone cC maximum appears at λ = 392 nm and pH 5.50 (curve 8). Solutions of pH 5.50–8.00, which were kept in darkness for 3 days, are shown

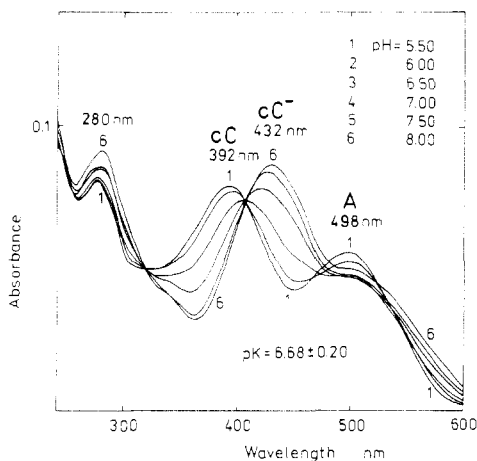2114 *J. Agric. Food Chem.*, Vol. 38, No. 12, 1990

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Figure 8. Absorption spectra of 5,7,3'-trihydroxy-4'-methoxy-flavylium chloride in solutions of pH 5.50–8.00, concentration 7.02 × 10⁻⁶ mol dm⁻³, after standing 3 days.
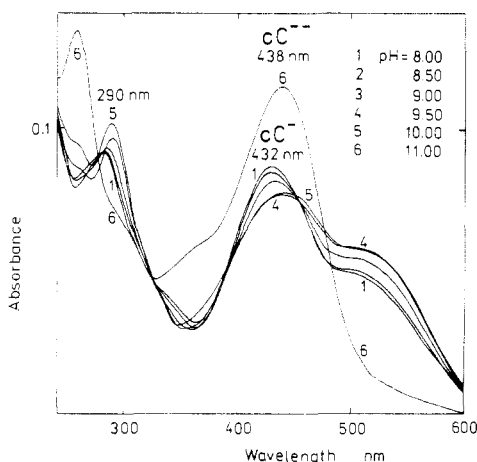

Figure 9. Absorption spectra of 5,7,3'-trihydroxy-4'-methoxy-flavylium chloride in solutions of pH 8.00–11.00, concentration 7.02 × 10⁻⁶ mol dm⁻³, after standing 3 days.

in Figure 8. The previously formed *cis*-chalcone cC band is transformed into the ionized form of the *cis*-chalcone cC⁻ (pK = 6.68 ± 0.20), with a simultaneous disappearance of the anhydro base A maximum, which becomes an inflection (curve 6). Almost identical behavior is characteristic to solutions of pH 8.00–10.00, while at pH 11.00 the characteristic absorption disappears and a new band at λ = 438 nm appears, which would probably correspond to the double-ionized *cis*-chalcone form cC²⁻ (see Figure 9, curve 6).

DISCUSSION AND CONCLUSIONS

In our recent papers (Baranac and Amić, 1989a,b; Baranac et al., 1990), which dealt with investigating the significance of each individual position in flavylium chromophore, we have studied the influence of position C-5, but in conjunction with substituted positions C-7 and C-4. As the influence of position C-4 is very dominant in the stabilization of these molecules, it has certainly masked the role of position C-5 by its own presence. This is why only in the present paper were we able to recognize more of the influence of position C-5, which is combined here with substituted positions C-7 and C-4' of compounds I and II or position C-3' of compound III.

It is known that flavylium compounds with OH groups in positions C-7 and C-4' do not form anhydro bases A from cationic forms AH⁺ when the pH value is increased. However, the presence of another OH group in position

C-5 causes formation of anhydro bases A, which stabilizes the flavylium structure. On the other hand, simultaneous chalcone C formation, as observable in Figures 1 and 4, is proof that the stabilization is insufficient. Position C-5 is not able to keep flavylium structure by directing the transformation only in the direction of deprotonation, as substituted position C-4 does, but a process of hydration also takes place here, which interferes with flavylium structure and is characteristic to substitution obtained in positions C-7 and C-4'. With investigated compounds, both of these processes take place at the same time, where one or the other dominates depending on the solution pH value. Thus, position C-5 has a certain stabilizing role (Brouillard et al., 1982; Sweeny and Iacobucci, 1983) which is probably more emphasized by Timberlake and Bridle (1967), as it causes formation of the anhydro base A but also allows formation of chalcones C through the process of hydration, which deteriorates flavylium structure. In that way it does not possess the stabilization influence of position C-4, which is able to separate deprotonation from hydration processes, especially in weakly acidic and neutral media, as observed in our previous papers (Baranac and Amić, 1985, 1989a,b; Baranac et al., 1990). In alkaline medium the process of deprotonation continues as the only one possible in the case of compound I. During this, the anhydro base A is transformed into the anionic form A⁻, and the existing chalcone takes on its ionized form tC⁻, as Figure 2 shows. Formation of the anhydro base chalcone as well as the anionic and the ionized chalcone forms is not particularly an equilibrium process. This is understandable, bearing in mind that the two processes take place simultaneously during the transformation, as we have seen. On the other hand, there are more OH groups present in this compound, making deprotonation processes possible at each of them, which interferes with equilibrium processes. Both forms are simultaneously present in alkaline medium, but with a significant dominance of the anionic form A⁻, which is more stable compared to the ionized *trans*-chalcone form tC⁻, as shown in Figure 2. After 14 days of keeping solutions of pH 11.00 in darkness, the absorption described disappears and a double-ionized *cis*-chalcone form cC²⁻ is solely present.

Compound II, which differs from compound I by an OCH₃ group in position C-5, follows mainly the transformation pattern of compound I. However, the anionic form A⁻ of this compound at pH 10.00 (Figure 3, curve 6) is much less stable than the anionic form of compound I (Figure 2, curve 6), and at pH 11.00 it transforms into the ionized chalcone tC⁻ (Figure 3, curve 7).

Compound III, which lacks the OH group in position C-4', but has an OCH₃ group there instead and also an OH group in position C-3', shows behavior almost identical with that of compound I in the range of pH from 2.00 to 5.50, as Figure 4 shows. However, in neutral and weakly alkaline medium (Figure 5) formation of chalcone is favored, accompanied by a decrease in the anhydro base, which is not the case with compound I. Behaviors of compounds I and III differ mostly in alkaline medium (Figures 2 and 6). While compound I creates a stable anionic form A⁻, formation of the same is obstructed with compound III by formation of the ionized chalcone forms cC⁻, tC⁻, tC²⁻. This behavior of compounds I and III points out the difference in the influence of positions C-3' and C-4' on formation of the anionic form A⁻ of the flavylium molecule. It can thus be observed that not only is the presence of an OH group in the B-ring needed for the formation and stability of the anionic form A⁻ (Baranac et al., 1988, 1990) but it is also important that the OH group be exactly in

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position C-4′, which is probably associated with the increased π-electron delocalization in the chromophore.

Absorption spectra of compounds I and III in acidic medium recorded immediately and several days after preparation (see Figures 4 and 7) show decrease in anhydro base absorption and increase of corresponding chalcone absorption. This fact confirms our prediction (Amić et al., 1990) that hydration reactions take place at the anhydro base forms A as well, transforming into chalcones C through transient pseudobase forms B. This, however, is not in agreement with the suggestion of Brouillard and Dubois (1977) that the hydration reactions take place exclusively at the cationic form AH⁺.

If our results are compared to those of Brouillard (Brouillard et al., 1982; Mazza and Brouillard, 1987) obtained on similar compounds, but by a pH-jump technique, priority should be given to his method with respect to kinetical defining of the transformations induced. However, our method of observing absorption spectra could more illustratively follow the development of possible transformations, accompanied by detection of the forms obtained under way, which characterize the transformation process, and that, in turn, gives it priority over the method mentioned above.

ACKNOWLEDGMENT

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