ULTRAVIOLET ABSORPTION OF FLAVONOIDS

I. Ionization Constants of Tectochrysin and Chrysin

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The widespread use of ultraviolet spectroscopy to establish the nature of the hydroxylation of the flavone skeleton is based on differences in the properties of hydroxyls differing in their position in the molecule.

As is well known, the presence of a 7-hydroxy group in the flavone molecule is shown by a bathochromic shift of the shortwave absorption band in the presence of sodium acetate [1,2]. At the same time, a 5-hydroxy group present here is not subject to the action of this alkaline reagent. It is identified by the bathochromic shifts in a medium with the complex-forming additive $AlCl_3$. This difference in the behavior of 7- and 5-hydroxy groups is based on their dissimilar acidities. However, there is no quantitative evaluation of this characteristic of the hydroxyl groups in the literature.

Consequently we have determined the ionization constants of the hydroxy group in 5-hydroxy-7-methoxyflavone (tectochrysin) and in 5,7-dihydroxyflavone (chrysin) by the spectrophotometric method. Figs. 1 and 2 give the absorption spectra of tectochrysin and chrysin in neutral (1) and alkaline (2,3) media. Making use of the features of the spectra to determine the dependence between the optical density and the pH values, the wavelengths were selected at which the greatest difference in the values of the densities for the un-ionized and ionized forms is observed. For tectochrysin these wavelengths proved to be 270, 290, and 315 mμ, and for chrysin 235, 280, and 310 mμ. The densities of solutions of both flavones and the wavelengths selected in media of different pH values are shown in Fig. 3 (a–e). The mean value of pK of the hydroxyl group in position 5 of tectochrysin determined by the graphical method [3] is 11.60. A more accurate calculation of this magnitude was carried out from the equation given by Albert and Serjeant [4]:

$$pK = pH + \lg \frac{d - d_{B^-}}{d_{HB} - d},$$

where

d is the optical density of the mixture of ion and neutral molecule at the analytical wavelength;

d_{B^-} is the optical density of the ion; and

d_{HB} is the optical density of the neutral molecule.

The results of the calculation are given in Table 1, which shows that the pK value of the 5-OH group of tectochrysin is 11.68 and, consequently, the ionization constant of this group is 2.09×10^{-12}. This value of the ionization constant shows the low acidity of the 5-OH group in techtochrysin. This can be explained by the formation of a hydrogen bond with the carbonyl group of the pyrone ring, the existence of which has been established previously by NMR spectroscopy [5]. The values of the ionization constants of two hydroxyl groups of chrysin were determined similarly. According to the graphical method (Fig. 3, c, d, e), the mean value of the constants are as follows: pK_1) 7.65; pK_2) 8.78. The calculation method gave the values: pK_1) 7.42; pK_2) 9.05 (Table 2). Consequently, the ionization constants are, respectively, $K_1 = 3.8 \times 10^{-8}$ and $K_2 = 8.91 \times 10^{-10}$.

It is quite logical to consider that the higher constant, K_1, corresponds to the 7-hydroxy group. Its acidity is comparable with that of the hydroxyl group of phloroglucinol, pyrogallol, sym-trichlorophenol, and the isomeric mononitrophenols (pK = 7.0–8.0) and considerably exceeds that of the hydroxyl groups of phenol, the isomeric cresols, and the monochlorophenols (pK = 10.20–9.40) [6]. The lower constant, K_2, is characteristic of the 5-OH group of chrysin. As can be seen, the ionization constant of this group is somewhat higher than that for the similar group in

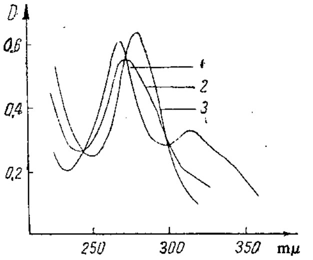

Fig. 1. Absorption of tectochrysin in aqueous ethanolic (1 : 1) solution with a concentration of 2.0 × 10^{-5} M at the following pH values: 1) 65; 2) 11.45; 3) 13.45.

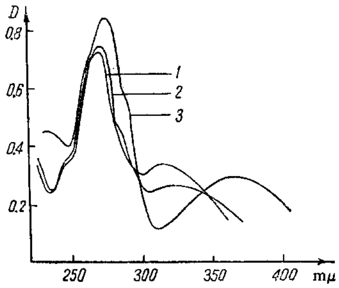

Fig. 2. Absorption of chrysin in aqueous ethanolic (1 : 1) solution with a concentration of 2.5 × 10^{-5} M at the following values: 1) 6.90; 2) 8.95; 3) 13.10.

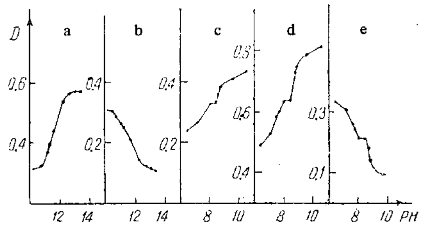

Fig. 3. Optical density as a function of the pH of solutions of tectochrysin (a, 290 mμ; b, 315 mμ) and chrysin (c, 235 mμ; d, 280 mμ; and e, 310 mμ).

tectochrysin. This fact can be explained by the mutual influence of the two hydroxyl groups in the meta position of the benzene ring. For example, it is known that the acidity of the phenolic hydroxyl group increases in the sequence phenol–m-dihydroxybenzene–1, 3, 5-trihydroxybenzene (pK = 10.0, 9.4, and 7.0, respectively) [6].

Table 1

λ, mμ	Optical density of solutions of tectochrysin (form)			pH of the solution of partially dissociated tectochrysin	pK
	dissociated	undissociated	partially dissociated		
270	0.525	0.610	0.560	11.75	11.60
290	0,570	0.300	0.440	11.75	11,72
315	0.112	0.316	0.212	11,75	11.73

Mean value of pK 7.68 ± 0.08.

EXPERIMENTAL

Flavones. Tectochrysin and chrysin were isolated by chromatography on polyamide from the extractive substances of the wood of the Siberian pine. The substances were purified by repeated chromatography on Kapron powder. After repeated recrystallization and drying in vacuum at 80° C the tectochrysin melted at 162–163° C (chloroform) and the chrysin at 275° C (methanol). The individuality of the substances was checked by one-dimensional and two-dimensional thin-layer chromatography in fixed layers of polyamide. The substances were detected by observation in UV light before and after treatment with an ethanolic solution of $AlCl_3$, and also with diazotized sulfanilic acid.

Table 2

λ, mμ	Optical density of solutions of chrysin (form)			pK_1	pH of a solution of partially dissociated chrysin	pK_2
	dissociated	undissociated	partially dissociated			
235	0.330	0.242	0.295	7.42	7.6	—
280	0.640	0,493	0.590	7.31	7.6	—
310	0.212	0.335	0,267	7.52	7.6	—
235	0,415	0.330	0,360	—	8.7	8.96
280	0,790	0.640	0,690	—	8.7	9,00
310	0.095	0,212	0.184	—	8,7	9.20

Mean value of pK_1 7.42 ± 0.1 and of pK_2 9.05 ± 0.15.

Spectrophotometric determinations. Tectochrysin and chrysin do not dissolve in water, and therefore the experiments were carried out with solutions of them in aqueous ethanol (1 : 1 by volume). The concentrations of the solutions were kept constant, 2.0×10^{-5}M for tectochrysin and 2.5×10^{-5}M for chrysin. To obtain solutions with different pH values an aqueous ethanolic solution (1 : 1 by volume) of KOH with a concentration of 0.083 M was used. A constant ionic strength was maintained by the addition of an aqueous ethanolic (1 : 1 by volume) solution of KCl with a concentration of 0.083 M (μ, 0.083). The measurements were carried out on an SF-4A spectrophotometer in a 1.0-cm cylindrical cell. The hydrogen ion concentrations were measured on a LPU-01 pH meter with a glass electrode. All the measurements were carried out at 20 ± 1° C.

CONCLUSIONS

The ionization constants of the 5-hydroxy groups in tectochrysin and chrysin have been determined spectrophotometrically, being 2.09×10^{-12} and 8.91×10^{-10}, respectively. The 7-hydroxy group of chrysin has an ionization constant of 3.8×10^{-8}.

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