ULTRAVIOLET ABSORPTION OF FLAVONOIDS

II. IONIZATION CONSTANTS OF 7- AND 4'-HYDROXY DERIVATIVES OF FLAVONE AND FLAVONOL

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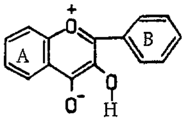
The rate of the alkylation of the hydroxy groups of flavones is determined by their acidity [1]. While 3-hydroxy groups of flavone compounds exhibit acidic properties and are capable of being methylated [1,2], the same groups in the flavanones have an aliphatic nature and do not undergo methylation [3]. However, no quantitative evaluation of the acidity of 3-OH groups of flavonoid compounds has been given in the literature.

The present paper gives the results of a spectrophotometric determination of the ionization constants of the 3-hydroxy groups in flavonol and 7- and 4'-hydroxy-substituted flavonols. The magnitude of the ionization constant of flavonol (Table 1) shows that the hydroxyl has a fairly high acidity, comparable with that for the phenolic hydroxyls in phenol, the cresols, and the monochlorophenols. The high acidity of the 3-OH group, which is present not in an aromatic but in a γ-pyrone ring can be explained by the assumption that it is just the introduction of this group into the flavone skeleton which promotes the stabilization of the pseudoaromatic (pyrylium) structure (I) of the flavonol:



From the point of view of this assumption, it is interesting to consider the results of IR spectroscopy and of a luminescence study of flavonol. The band of the stretching vibrations of the carbonyl group of flavonol is shifted sharply in the direction of low frequencies and falls into the region of the vibrations of aromatic systems: 1610 cm^{-1} (in KBr) [4] and 1613 cm^{-1} (in KBr) (our own measurements). At the same time, the frequency of the stretching vibrations of the carbonyl group of the γ-pyrone system is found at 1659 cm^{-1} (in KBr) and that of chromone at 1655 cm^{-1} (in KBr) [4]. Consequently, the considerable shift in the low-frequency direction in flavonol may be connected with an increase in the size of the conjugated system due to the introduction of an aromatic substituent into position 2 (for example, in γ-pyrone $\nu_{C=O}$ is 1680 cm^{-1}, in CCl_4, while in 2-phenyl-γ-pyrone $\nu_{C=O}$ is 1658 cm^{-1}, in CCl_4 [5]) and to the stabilization of the pyrylium structure through the protonation of the oxygen atom of the carbonyl group by the hydrogen atom of the 3-hydroxy group. In the luminescence spectrum of flavonol† the intensity of the emission band corresponding to the $\pi \rightarrow \pi^*$ transition rises tenfold as compared with the spectra of a whole series of mono-, di-, and polyhydroxyflavonoids. All this confirms the existence of a complete system of high conjugation in the flavonol molecule which, most probably, is expressed by the pyrylium structure.

The increase in the nucleophilic properties of the aromatic ring A due to the introduction of a hydroxy group in the para position with respect to the carbonyl (7-hydroxyflavonol) leads to a small decrease in the acidity of the 3-OH group (pK = 11.3 ± 0.1) as compared with flavonol (pK = 9.6 ± 0.1). With a decrease in

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† The luminescence spectra of the flavonoids were obtained in our laboratory by K. I. Lapteva; they are the subject of discussion in a separate paper.

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TABLE 1. The Constants K for a Series of Flavones Obtained by Calculation

Compound	λ, nm	pK_{av}	Mean square error, s	No. of deg. of freedom, f	$pK_{av}\pm\Delta x$	K
7-Hydroxyflavone	260	8,14	0,08	13	8,12±0,05	$9{,}44\cdot10^{-9}$
	270	8,15				
	310	8,06				
	360	8,11				
4'-Hydroxyflavone	330	9,16	0,10	11	9,14±0,06	$8{,}81\cdot10^{-10}$
	340	9,16				
	370	9,10				
	390	9,12				
3-Hydroxyflavone	270	9,62	0,17	12	9,6±0,1	$2{,}51\cdot10^{-10}$
	275	9,48				
	305	9,64				
	340	9,62				
	410	9,66				
3-Hydroxy-7-methoxyflavone	280	9,75	0,08	10	9,8±0,05	$1{,}58\cdot10^{-10}$
	325	9,86				
	345	9,83				
	400	9,77				
3,7-Dihydroxyflavone	250	8,19	0,10	6	8,3±0,1	$5{,}01\cdot10^{-9}$
	310	8,29				
	405	8,25				
	420	8,25				
	250	11,22	0,10	5	11,3±0,1	$5{,}01\cdot10^{-12}$
	405	11,32				
	420	11,23				

TABLE 2. Values of D and pH Used to Calculate the Constants K_1 and K_2 of 3,4'-Dihydroxyflavone

Expt. No.	pH	D_{270}	D_{330}	D_{420}	L_{440}	$D_{4\,0}$
1	8,66	0,109	0,269	0,073	—	0.023
2	8,84	0,120	0,256	0,120	—	0.049
3	9,77	0,166	0,180	0,361	—	–
4	9,92	0,220	0,166	0,430	—	–
5	10,0	0,218	0,137	0,475	–	0.188
6	11,09	0,245	—	—	0,419	–
7	11,6	—	—	0,530	0,486	
8	11,9	0,302	—	0,516	0,538	–
9	12,34	0,326	—	0,500	—	

the nucleophilicity of the 7-hydroxyphenyl moiety due to the replacement of the hydroxyl by the less electropositive methoxy group ($\sigma_{OH} = -0.36$; $\sigma_{OCH_3} = -0.27$ [6]) there is a slight decrease in the ionization constant of the 3-hydroxyl as compared with flavonol (see Table 1).

The bands of the stretching vibrations of the carbonyl groups in 3,7-dihydroxy- and 3-hydroxy-7-methoxyflavones are at 1620-1622 cm^{-1}, i.e., they also appear in the region of the stretching vibrations of unsaturated systems, although displaced somewhat to longer frequencies than in flavonol. A similar decrease in the acidity of the 3-OH group is observed with an enhancement of the nucleophilicity of ring B (4'-hydroxyflavone).

As was shown above, the carbonyl group in the γ-pyrone ring readily takes part in conjugation with the 2-phenyl substituent. An increase in the size of the system of conjugation through the introduction of a hydroxyl into the 4' position leads to a still greater reduction in the frequency of the vibrations of the carbonyl group (to 1603 cm^{-1} in 3,4'-dihydroxyflavone). The $\nu_{C=O}$ absorptions in 7-hydroxyflavone (1632 cm^{-1}) and in 4'-hydroxyflavone* (1633 cm^{-1}, in KBr) are at higher frequencies than in the cases where a 3-hydroxy

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* 7-Hydroxy- and 4'-hydroxyflavones were synthesized and given to us by N. G. Devyatko, of our laboratory.

TABLE 3. Values of K_1 and K_2 Calculated for 3,4'-Dihydroxyflavone by Means of the Komar' Equation and the Figures of Table 2

Expts. used for calculation	λ, nm	Constant
		$K_1 \cdot 10^{-9}$
1, 2, 3	270	1,19
1, 2, 4		0,38
1, 2, 5		0,38
1, 2, 3	330	0,619
1, 2, 4		0,509
1, 2, 5		0,403
1, 2, 3	420	0,835
1, 2, 4		0,722
1, 2, 5		0,557
1, 2, 5	450	0,957
		$K_2 \cdot 10^{-12}$
6, 8, 9	270	2,42
7, 8, 9	420	2,21
6, 7, 8	440	1,43

$K_1 = 0{,}63 \cdot 10^{-9}$ $K_2 = 1{,}99 \cdot 10^{-12}$
$pK_1 = 9{,}2 \pm 0{,}1$ $pK_2 = 11{,}7 \pm 0{,}2$
(for $P = 0{,}95$) (for $P = 0{,}95$)
$f = 9$ $s = 0{,}18$ $f = 2$ $s = 0{,}1$

group is present in the molecule, which once more indicates the specific stabilizing action of this group with respect to the pyrylium form of the flavonols.

The acidity of the 7- and 4'-hydroxy groups in the flavone and flavonol molecules is very high. The 7-hydroxy group has a greater capacity for ionization than the 4'-OH group (see Table 1).

EXPERIMENTAL

The work was performed with synthetic flavonoid compounds. The IR spectra of the compounds were taken on a UR-10 spectrophotometer (tablets with KBr).

3-Hydroxyflavone was synthesized by a modified AFO method [7], mp 169°C (ethanol).

Found %: C 75.12; H 4.23. $C_{15}H_{10}O_3$. Calculated %: C 75.64; H 4.20.

3-Hydroxy-7-methoxyflavone. This was obtained by the condensation of 2-hydroxy-4-methoxyacetophenone (8.9 g) and benzaldehyde (5.7 g) in the presence of aqueous alkali (7.3 g in 10 ml of water). The orange paste was dissolved in 500 ml of water, NaOH (5 g in 25 ml of water) was added, and the mixture was cooled to room temperature and treated with 30 ml of 15% hydrogen peroxide. After 3-4 h, the mixture was acidified and poured into a large volume of water. The 3-hydroxy-7-methoxyflavone that precipitated was then recrystallized from ethanol, mp 176°C. The yield of crude product was 36% of theoretical (5.0 g).

Found %: C 72.09; H 4.58. $C_{16}H_{12}O_4$. Calculated %: C 71.65; H 4.47.

3,7-Dihydroxyflavone. 3-Hydroxy-7-methoxyflavone (2 g) was heated with acetic anhydride (5 ml) and hydriodic acid (25 ml) in an oil bath at 150°C for 2 h. The reaction mixture was poured into water, and the precipitate was washed with water until the test for iodine was negative. The yield of crude product was 32% of theoretical (0.64 g), mp 236°C (ethanol).

Found %: C 71.25; H 3.78. $C_{16}H_{10}O_4$. Calculated %: C 70.88; H 3.93.

3-Hydroxy-4'-methoxyflavone was obtained from o-hydroxyacetophenone (10 g) and p-methoxybenzaldehyde (10 g) under conditions analogous to those for the synthesis of 3-hydroxy-7-methoxyflavone. The yield of crude product was 28% of theoretical (5.2 g), mp 232°C (ethanol).

Found %: C 71.28; H 4.55. $C_{16}H_{12}O_4$. Calculated %: C 71.65; H 4.47.

3,4'-Dihydroxyflavone was synthesized by the demethylation of 3-hydroxy-4'-methoxyflavone (2 g) in a similar manner to the preparation of 3,7-dihydroxyflavone. The yield was 82% of theoretical (1.5 g), mp 271°C (ethanol).

Found %: C 71.15; H 4.15. $C_{15}H_{10}O_4$. Calculated %: C 70.88; H 3.93.

Determination of the Ionization Constants. The ionization constants were determined by a method described previously [8] on an SF-4A spectrophotometer in 1-cm cylindrical cells at 20 ± 1°C. Aqueous ethanolic (1 : 1) solutions of the flavonoids with a concentration of $2.5 \cdot 10^{-5}$ M were used for the spectrophotometric investigations. Solutions with different pH values were obtained by means of a 0.095 M aqueous ethanolic solution of KOH. The ionic strength of the solutions, $\mu \approx 0.1$, was kept constant by the addition of a 0.095 M solution of KCl (water–ethanol).

The concentration of hydrogen ions was measured on a LPU-01 pH-meter with flow-through glass–silver chloride electrodes filled with a saturated solution of KCl. The pH-meter was calibrated by means of aqueous phthalate (pH 1.68) and borate (pH 9.18) buffers. The ionization constants for the monohydroxyflavones and for 3,7-dihydroxyflavone were obtained by calculation [9] (Table 1), and that for 3,4'-dihydroxyflavone by the Komar' method [10] (Tables 2 and 3). For each compound, the mean-square error was calculated for the values of the ionization constants obtained, and also the confidence interval of the mean for 95% probability [11] (see Tables 1 and 2).

SUMMARY

1. The ionization constants of the 7-, 4'-, and 3-hydroxy groups in mono- and dihydroxyflavones have been determined by the spectrophotometric method.

2. It has been shown that the 3-hydroxy group of flavonol possesses the fairly high acidity that is characteristic for hydroxyls of phenolic origin and is capable of stabilizing the aromatic structure of the pyrone ring of the flavonol molecule.

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