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# ULTRAVIOLET ABSORPTION OF FLAVONOIDS

## VIII. IONIZATION CONSTANTS OF KAEMPFEROL AND QUERCETIN

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We have previously studied the acid properties of a series of mono- and disubstituted flavones [1-3]. Considerable interest is presented by an investigation of the acid properties of more highly hydroxylated flavonoid derivatives, many of which are widely used as complex-forming agents in analytical chemistry. There is little information in the literature on this question. Of the large number of natural flavonoids, ionization constants have been determined only for quercetin [4] and morin [5-7], without the assignments of the values obtained to particular hydroxy groups.

The present paper gives the results of a determination of the ionization constants of tetra- and pentahydroxy-substituted natural flavones (kaempferol and quercetin) (Table 1). The assignment of the values of the constants to particular hydroxy groups was made on the basis of the results of a comparative analysis of the constants obtained for kaempferol and quercetin with similar results for mono- and dihydroxyflavones with a corresponding type of substitution. It can be seen from this that both in kaempferol and in quercetin the most highly acidic properties are possessed by the hydroxy group in position 7. The 4'-OH group will undergo dissociation next.

It is possible that the two constants in kaempferol are due to the 3-OH and 5-OH groups, in the sequence given in Table 1. But since the capacity for ionization of the 3- and 5-hydroxy groups is considerable and changes differently under the influence of substituents in positions 7 or 4', we cannot definitely affirm the assignment of the two latter constants that we have made. For this purpose the ionization constants of trihydroxyflavones with a related type of substitution must be determined.

As can be seen from Table 1, for quercetin we found the ionization only of the two hydroxy groups possessing the highest acidity. The ionization of the remaining hydroxyls takes place in a fairly strong alkaline medium (pH > 9.6) in which quercetin undergoes irreversible changes. Consequently, it did not appear possible to determine the ionization constants of these groups.

TABLE 1. Ionization Constants ($pK_a$ ± $\Delta x$) of Kaempferol and Quercetin

| Compound | $pK_{a1}$ | $pK_{a2}$ | $pK_{a3}$ | $pK_{a4}$ |
|---|---|---|---|---|
| | 7–OH | 4'–OH | 3–OH | 5–OH |
| 3,4',5,7-Tetrahydroxyflavone (kaempferol) | 8,2±0,2 | 9,5±0,1 | (10,5) | (12,5) |
| 3,3',4',5,7-Tetrahydroxyflavone (quercetin) | 7,3±0,1 | 8,4±0,1 | — | — |

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TABLE 2. Ionization Constant of the 7- and 4'-OH Groups in Some Flavonoids

| Compound | 7-OH | 4'-OH |
|---|---|---|
| 7-Hydroxyflavone | 8,12±0,05 | — |
| 3,7-Dihydroxyflavone | 8,30±0,10 | — |
| 5,7-Dihydroxyflavone | 7,30±0,30 | — |
| Kaempferol | 8,20±0,20 | — |
| Quercetin | 7,30±0,10 | — |
| 4'-Hydroxyflavone | — | 9,14±0,06 |
| 3,4'-Dihydroxyflavone | — | 9,20±0,10 |
| 4',5-Dihydroxyflavone | — | 8,74±0,05 |
| Kaempferol | — | 9,50±0,10 |
| Quercetin | — | 8,40±0,10 |

TABLE 3. Values of D and of the pH used for Calculating the Constants $K_{a1}$ and $K_{a2}$ in Kaempferol

| Exp. No. | pH | $D_{285}$ | $D_{370}$ | $D_{400}$ | $D_{420}$ |
|---|---|---|---|---|---|
| 1 | 7,67 | 0,205 | — | 0,190 | 0,048 |
| 2 | 8,2 | 0,242 | — | 0,265 | 0,112 |
| 3 | 8,55 | 0,255 | — | 0,315 | 0,160 |
| 4 | 8,70 | — | 0,400 | 0,316 | = |
| 5 | 8,78 | — | 0,402 | — | — |
| 6 | 9,25 | — | 0,370 | 0,370 | — |
| 7 | 9,52 | — | — | — | — |
| 8 | 10,20 | — | 0,315 | 0,460 | — |
| 9 | 10,24 | — | 0,292 | — | — |

TABLE 4. Values of $K_{a1}$ and $K_{a2}$ Calculated for Kaempferol from the Komar' Equation and the Figures of Table 3

| Experiments used for calculation | λ, nm | Ionization constant |
|---|---|---|
| | | $K_{a1} \cdot 10^{-9}$ |
| 1,2,3 | 285 | 6,99 |
| 1,2,3 | 400 | 5,33 |
| 1,2,3 | 420 | 5,60 |
| | | $K_{a2} \cdot 10^{-10}$ |
| 4,6,8 | 370 | 3,90 |
| 4,6,9 | — | 2,49 |
| 5,6,9 | — | 3,15 |
| 4,5,8 | 400 | 4,59 |

Mean $K_{a1} = 5,97 \cdot 10^{-9}$

$pK_{a1} = 8,2 \pm 0,2$ ($f=2$, $s=0,07$)

Mean $K_{a2} = 3,53 \cdot 10^{-10}$

$pK_{a2} = 9,5 \pm 0,1$ ($f=3$, $s=0,1$)

The results of an analysis of the experimental material in the field of the study of the acidic properties of the flavonoids permit some conclusions to be drawn. In the first place, hydroxy groups present in different positions of the flavone molecule differ considerably in their capacity for ionization. In the second place, the capacity for ionization of each hydroxy group is not constant in all types of flavonoid compounds but can vary considerably according to the nature of the substitution of the flavone skeleton. For example, it can be seen clearly from Table 2, that the acidities of 7-OH groups are different in the flavonoids listed, and the same applies to the 4'-OH groups. A general tendency can be observed to an increase in the acidities of these groups in compounds containing a hydroxyl in position 5, and also in compounds with simultaneous 5-hydroxy- and 3',4'-dihydroxy-substitution. It is very important to take this into account in the widely used method for the UV-spectroscopic identification of flavonoids based on the measurement of the absorption of ionized forms.

## EXPERIMENTAL

Kaempferol and quercetin were isolated from an acetone extract of the heartwood of the Dahurian larch by preparative chromatography on a polyamide sorbent [8]. After repeated recrystallization and drying in vacuum at 80°C, the kaempferol had mp 275-276°C (ethanol), and the quercetin mp 310-312°C (ethanol).

The individuality of the substances was checked by one-dimensional and two-dimensional chromatography in fixed layers of polyamide.

The ionization constants were determined by a method described previously [1, 2]. All the measurements were performed at 20 ± 2°C.

Since quercetin undergoes irreversible changes in media with pH > 9.6, to prepare its solutions in the required pH range we used aqueous-ethanolic (1 : 1 by vol.) universal buffer mixture. The ionic strength of the solution was kept constant ($\mu \approx 0.1$) with a 2 N aqueous ethanolic (1 : 1 by vol.) solution of KCl.

TABLE 5. Values of D and of the pH used for Calculating the Constants $K_{a_1}$ and $K_{a_2}$ for Quercetin

| Exp. No. | pH | $D_{270}$ | $D_{370}$ | $D_{400}$ |
|---|---|---|---|---|
| 1 | 6,88 | 0,324 | — | 0,270 |
| 2 | 7,32 | 0,340 | — | 0,316 |
| 3 | 7,46 | 0,345 | — | 0,336 |
| 4 | 7,80 | — | — | 0,372 |
| 5 | 8,1 | 0,356 | — | 0,402 |
| 6 | 8,3 | 0,370 | — | 0,410 |
| 7 | 8,41 | — | 0,352 | 0,432 |
| 8 | 8,78 | 0,422 | 0,330 | 0,448 |
| 9 | 9,46 | 0,458 | 0,305 | — |

TABLE 6. Values of $K_{a1}$ and $K_{a2}$ Calculated for Quercetin from the Komar' Equation and the Figures of Table 5

| Experiments used for calculation | λ, nm | Ionization constants |
|---|---|---|
|  |  | $K_{a1} \cdot 10^{-8}$ |
| 1,2,3 | 270 | 5,7 |
| 1,3,4 | 400 | 5,02 |
| 1,2,4 | — | 5,17 |
| 1,3,5 | — | 3,84 |
|  |  | $K_{a2} \cdot 10^{-9}$ |
| 5,8,9 | 270 | 4,25 |
| 6,8,9 | — | 5,6 |
| 7,8,9 | 370 | 3,3 |
| 6,7,8 | 400 | 4,0 |

Mean $K_{a1}=4,93 \cdot 10^{-8}$
$pK_{a1}=7,3 \pm 0,1 (f=3,\ s=0,08)$
Mean $K_{a2}=4,28 \cdot 10^{-9}$
$pK_{a2}=8,4 \pm 0,1 (f=3,\ s=0,09)$

The ionization constants were first estimated by a graphical method [9] and were then calculated more accurately by the method given by Komar' [10] (Tables 3-6). The constants $K_{a1}$ and $K_{a2}$ obtained for both compounds enabled the mean square error and the confidence interval of the mean at 95% probability to be obtained [11]. The values of $pK_{a3}$ and $pK_{a4}$ for kaempferol were determined only by the graphical method.

## SUMMARY

The ionization constants of the 4'- and 7-OH groups in quercetin and of the 3-, 4'-, 5'-, and 7-OH groups in kaempferol have been determined by the spectrophotometric method. The sequence of their ionization has been established.

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