

SPECTROSCOPIC INVESTIGATION OF FLAVONOIDS.
DETECTION OF FREE PHENOLIC HYDROXYL GROUPS IN VARIOUS POSITIONS

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The results of a spectroscopic investigation of flavonoid compounds in the UV region are of great importance in elucidating their structure. Many ionizing and complex-forming reagents, causing displacements of the absorption maxima depending on the nature and the position of the functional groups in the molecules of flavonoids, have been proposed [1]. For example, the ionizing influence of anhydrous sodium acetate in alcoholic solution is used to detect a free phenolic hydroxy group in position 7 [1-3].

Jurd and coworkers [1, 4, 5] state that a hydroxyl group in position 7 of flavones, flavonols, flavanones, and isoflavones must be revealed by a bathochromic displacement of the absorption maximum in the short-wave band by 10-20 m μ . However, in polyhydroxyflavonoids, bathochromic displacements of the maxima in the long-wave bands are also observed. Jurd assumes that in flavones this displacement is due to the ionization of the free hydroxyl group in position 4' and in flavonols to the ionization of the hydroxyl groups in positions 3 and 4' [1].

The experimental results that we have obtained do not agree with those reported in the literature [1]. Consequently, we have carried out an analysis of the spectra of flavonoids isolated in our laboratory and known previously in order to elucidate the possibility of determining in them a free hydroxy group in position 7 (table).

It can be seen from the table that in the flavones (nos. 13, 15, 16, 19), the flavonols (nos. 23, 24, 28, 30, and 33), the flavanones (nos. 1, 2, 4, 5), and the chalcones (nos. 9 and 10) a free 7-hydroxy group can be detected from the bathochromic displacement of the absorption maximum in the long-wave part of the spectrum. The absorption maximum of the short-wave band is scarcely affected in the case of these compounds. Where the hydroxy group in position 7 is substituted (nos. 6, 11, 12, 14, 25, 34, 39) no bathochromic displacement is observed in either band, in spite of the presence of free hydroxy groups in position 4' (nos. 3, 14, 39) or positions 3 and 4' (nos. 25 and 34). In isoflavones containing a free 7-hydroxy group, a bathochromic displacement is found in the medium-wave part of the spectrum under the influence of sodium acetate.

We assume that ionization of the 7-hydroxy group causes excitation of the π -electrons of the whole molecule. This leads to a bathochromic displacement of the maximum of the long-wave band. This assumption is in agreement with V. A. Izmail'skii's theory [13] of the possibility of conjugation through the unpaired electrons of the heteroatom in flavones, flavonols, and flavanones or along the chain including the carbonyl group [14, 15]. In the isoflavones, the phenyl substituent in the side chain is not conjugated with the remainder of the molecule.

Jurd explains the bathochromic displacement of the maximum of the short-wave band when the 7-hydroxy group is ionized by electron transitions in the benzenoid part of the molecule alone [1].

The investigation carried out has shown that when flavonoid compounds are ionized with anhydrous sodium acetate (pH 8.3), a free 7-hydroxy group is shown by a bathochromic displacement of the maximum of the long-wave band in flavones by 32-46 m μ (nos. 13, 15, 16, 19), in flavonols by 10-24 m μ (nos. 23, 24, 28, 30, 33), in flavanones by 10-30 m μ (nos. 1, 2, 4, 5), in chalcones by 10-15 m μ (nos. 9 and 10), and in isoflavones by a bathochromic displacement in the medium-wave part of the spectrum by 10-13 m μ (nos. 38, 40-42).

It is known that free hydroxy groups in positions 3 and 5 in the flavonols, in position 5 in the flavones, flavanones, and isoflavones, and in positions 2 and 6 in chalcones are revealed by bathochromic displacements caused by complex-forming reactions with aluminum chloride [1]. To distinguish a 2-hydroxy group from a 6-hydroxy group in the chalcones, aluminum chloride is supplemented with sodium acetate [16]. Hörhammer et al., [17, 18], investigating flavonol glycosides by spectroscopic methods, proposed to carry out complex formation in an acid medium. It was assumed that under these conditions the aluminum complexes with 5-hydroxy flavonoids would be decomposed and the complexes with the 3-hydroxyflavones would be stabilized. This reaction gives positive results with hydroxyflavonoids in which there is only one of the complex-forming hydroxy groups. In the majority of natural flavonols hydroxy groups are present simultaneously in positions 3 and 5. In this case, aluminum complexes in an acid medium do not make it possible to decide whether there is only one hydroxy group in position 3 or whether a 5-hydroxy group is present as well.

Because of this, the necessity has arisen of determining free hydroxy groups in positions 3 and 5 when they are present simultaneously in natural flavonoids.

For this purpose, we have tested the use of the reaction with zirconyl nitrate and citric acid proposed by Hörhammer and coworkers [19] for the qualitative detection of a 3-hydroxy group in a flavonol in the presence of a 5-

hydroxy group. The selectivity of the reaction is due to the fact that the chelate complexes with the 3- and 5-hydroxy groups and the carbonyl group in position 4 possess different stabilities to the decomposing action of citric acid. The latter takes part in a competing complex-forming reaction with the zirconyl ion already bound in the form of a chelate with 5-hydroxy flavonoids, forming a colorless complex. A six-membered complex of a flavonoid with a metal ion is decomposed while a five-membered complex, at the 3-hydroxy group, is not affected. Thus, this reaction makes it possible to detect the presence of a free hydroxy group in position 3 with sufficient certainty, but does not answer the question of a 5-hydroxy group. We have obtained more definite results in a spectroscopic study of this question (see table).

It can be seen from the table that the formation of the zirconyl complex with the participation of a 5-hydroxy group in the flavones (nos. 13-22), the flavonol 3-glycosides (nos. 26, 27, 29, 31, 32, 37), and the flavanones (nos. 5-7) leads to a bathochromic displacement of the maximum of the long-wave band by $50 \pm 10 \text{ m}\mu$. This displacement is also observed in the case of the 2-hydroxychalcones (nos. 9-12). In the flavonols containing free 5- and 3-hydroxy groups (nos. 23, 25, 28, 35, 36), the bathochromic displacement of the maximum of the long-wave band was considerably greater, amounting to $85 \pm 5 \text{ m}\mu$. This displacement is probably due to the formation of a double complex at the 3- and 5-hydroxy groups. In the presence of citric acid the bathochromic displacement of the zirconyl complex with the 5-hydroxyflavonoids is eliminated completely, and in the 3, 5-dihydroxyflavonoids it is decreased to $50 \pm 10 \text{ m}\mu$, which can be explained by the difference in the stability of the 5- and the 6-membered chelates. The 4'-rhamnoside of isorhamnetin (no. 36) forms an exception to this rule. The complex with this compound produces a bathochromic displacement of $88 \text{ m}\mu$; however, in the presence of citric acid the spectrum of the initial flavonoid in alcoholic solution is restored. This phenomenon is apparently due to a change in the glycoside of the hydroxy group in position 4' of the carbohydrate residue. However, in the free state, the 4'-hydroxy group in flavonols apparently favors stabilization of a complex at the 3-hydroxy group.

Experimental

In the investigation of the flavonoid compounds, we used concentrations in absolute ethanol of 10^{-4} M for the flavanones and $2 \times 10^{-5} \text{ M}$ for the other flavonoids. Saturated alcoholic solutions were obtained from freshly-prepared anhydrous sodium acetate. 1 ml of the initial solution of flavonoids was treated with 1 ml of 0.4% zirconyl nitrate and the resulting mixture was made up to 10 ml with absolute ethanol. The solutions of the complexes of the flavonoids containing citric acid were prepared as follows: to 1 ml of the initial solution of flavonoid were added 1 ml of 0.4% zirconyl nitrate solution, 1 ml of 4% citric acid solution, and 7 ml of absolute ethanol.

The spectra of the flavonoid compounds investigated were recorded on a SF-4 spectrophotometer.

Summary

1. Sodium acetate ionizes the 7-hydroxy group in flavones, flavonols, and flavanones, and the 4-hydroxy group in chalcones, causing a considerable bathochromic displacement of the absorption maximum of the long-wave band. In isoflavones containing a free 7-hydroxy group a bathochromic displacement of the absorption maximum of the medium-wave part of the spectrum is found.

2. A spectroscopic method using zirconyl nitrate and citric acid for detecting free hydroxy groups in positions 3 and 5 in flavonoids when they are present together and separately has been developed.

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