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Physical and Chemical Properties of Hydroxyflavones. IV. Infrared Absorption Spectra of Dihydroxyflavones Containing the 5-Hydroxyl Group (1,2)

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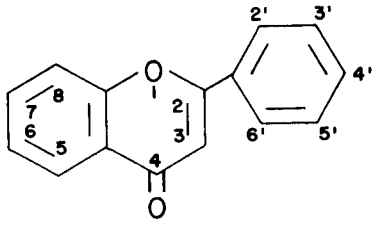
Solid state infrared curves (O-H and C-H stretching region) are given for 5,*n*-dihydroxyflavones, where *n* is 2', 3', 4', 6, 7 and 8. In chloroform solution spectra of 3,5-dihydroxyflavone and 3-hydroxy-5-methoxyflavone, the 3-OH stretching band appears at 3400 and 3334 cm^{-1}, respectively, indicative of a stronger hydrogen bond in the latter substance. Solid state and solution carbonyl bands are presented for twenty-six flavone derivatives which contain a hydroxyl, methoxyl or acetoxyl group at the 5-position. The solution spectra (dioxane or carbon tetrachloride) of fourteen flavone derivatives containing a free 5-hydroxyl group show carbonyl bands at 1655±2 cm^{-1}. Eleven flavones in which the 5-hydroxyl is blocked (carbon tetrachloride solution) give spectra with flavone carbonyl bands at 1653±3 cm^{-1}. The high resolution chloroform solution spectrum of 3,5-dihydroxyflavone possesses a multi-peaked carbonyl band with midpoint at 1641 cm^{-1}. The chloroform solution spectrum of 3-hydroxy-5-methoxyflavone has a very strong band at 1616 cm^{-1}, with shoulder at 1646 cm^{-1}. Spectral data of this and a previous paper (3) support the postulate that in 4'-hydroxyflavone the flavone carbonyl oxygen is the donor atom in an intermolecular hydrogen bond. Certain details of synthesis, and analytical data, are given for 3,5-dihydroxyflavone.

In previous work in this laboratory, the infrared spectra of all monohydroxyflavones were investigated, and interpretation reported to the extent that seemed reasonably possible (3). In the present paper, we report an extension of this study to the 5,*n*-dihydroxyflavones, where *n* is 2', 3, 3', 4', 6, 7 and 8. Emphasis in the present paper is on solid state hydroxyl stretching frequencies, and solid state and solution carbonyl bands (4).

Infrared curves for potassium bromide disks of the substances of the present study (with the exception of 3,5-dihydroxyflavone) are given in Figures 1-3. In Figure 1, it is seen that 5,6- and 4',5-dihydroxyflavone contain apparent OH stretching bands near 3300 cm^{-1}. The spectrum of 6-hydroxyflavone contains a somewhat broader band near 3300 cm^{-1} (3). In the infrared curve of 4'-hydroxyflavone, however, no such relatively high frequency peak appears. It is probable that the oxygen electrons in the intramolecularly hydrogen-bonded carbonyl of 4',5-dihydroxyflavone are less available for intermolecular hydrogen-bonding than in the carbonyl group of 4'-hydroxyflavone. Deuteration studies (5) confirm with reasonable certainty that the band near 3300 cm^{-1} in the 4',5-dihydroxyflavone spectrum is associated with a 4'-OH stretching mode. From Figures 1 and 2, it is evident that the 5,7-, 2',5-, and 3',5-dihydroxyflavone spectra contain several bands which might be assigned to OH stretching.



Deuteration studies (5) indicate that possibly bands near 2700 and 3100 cm^{-1} actually are associated with 7- and 3'-OH stretching in spectra of 5,7- and 3',5-dihydroxyflavones respectively. These results are rather interesting, since they indicate that the hydroxyl groups in these substances still are involved in rather strong hydrogen bonds. There are, of course, other groups in addition to the carbonyl, notably the hetero oxygen atom and other hydroxyl groups, which might be involved in hydrogen-bonding. The relative constancy of the carbonyl stretching frequency in solid state and solution spectra of 3',5-, 4',5- and 5,6-dihydroxyflavone would possibly indicate that the carbonyl group is not important in intermolecular hydrogen bonding in these substances. The 2'-hydroxyl stretching frequency can not be assigned with any degree of certainty in absence of deuteration studies. However, it is apparent that no distinct band above 3080 cm^{-1} is present. Hence,

it appears likely that this hydroxyl group also is involved in a rather strong hydrogen bond of some type. The spectrum of 5, 8-dihydroxyflavone (Figure 3) is rather remarkable in containing only a single distinct band near 3220 cm^{-1}. Deuteration studies (5) have not indicated which stretching mode (5-OH or 8-OH) is associated with this band. The resolution of the instrument used may have been inadequate to detect certain very weak peaks actually associated with stretching of one or the other hydroxyl group. Only in the substances for which deuteration has been effected can the 5-OH stretching band be located even tentatively. In 5, 7-, 3', 5- and 4', 5-dihydroxyflavone spectra, an apparent 5-OH stretching band occurs at 3005, 2970 and 3010 cm^{-1}, respectively. These peaks are very weak, in agreement with current viewpoints about stretching bands associated with strongly intramolecularly hydrogen-bonded hydroxyl groups (6).

Carbonyl bands in spectra of compounds of the present study (with the exception of 3, 5-dihydroxyflavone) are given in Tables I and II. All flavone derivatives in Table I contain a free 5-hydroxyl group. For the nine derivatives whose solution spectra in dioxane were determined, the flavone carbonyl stretching frequency is found at 1655±2 cm^{-1}. Those substances soluble in carbon tetrachloride (entries 8-13) also show the carbonyl peak

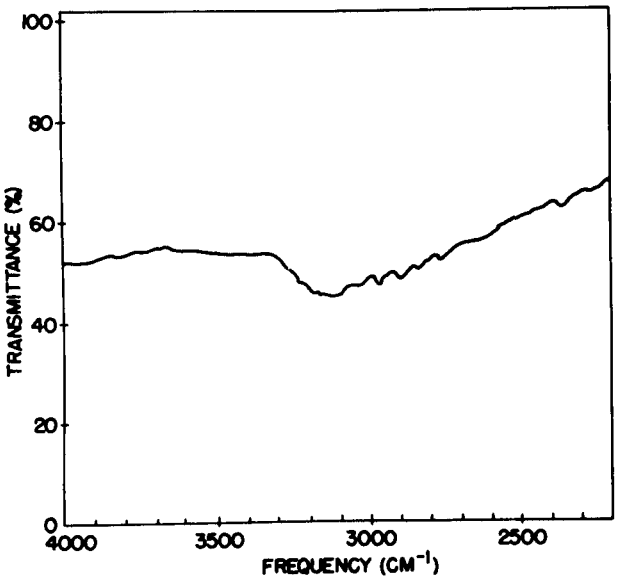


Fig. 2. Infrared Spectrum of 3', 5-Dihydroxyflavone.

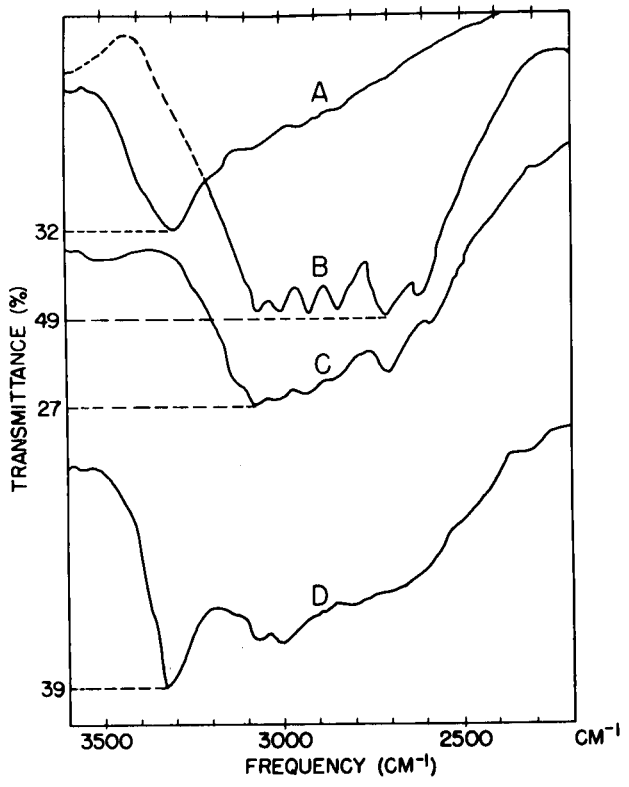


Fig. 1. Infrared Spectrum of (A) 5, 6-Dihydroxyflavone; (B) 5, 7-Dihydroxyflavone; (C) 2', 5-Dihydroxyflavone; (D) 4', 5-Dihydroxyflavone.

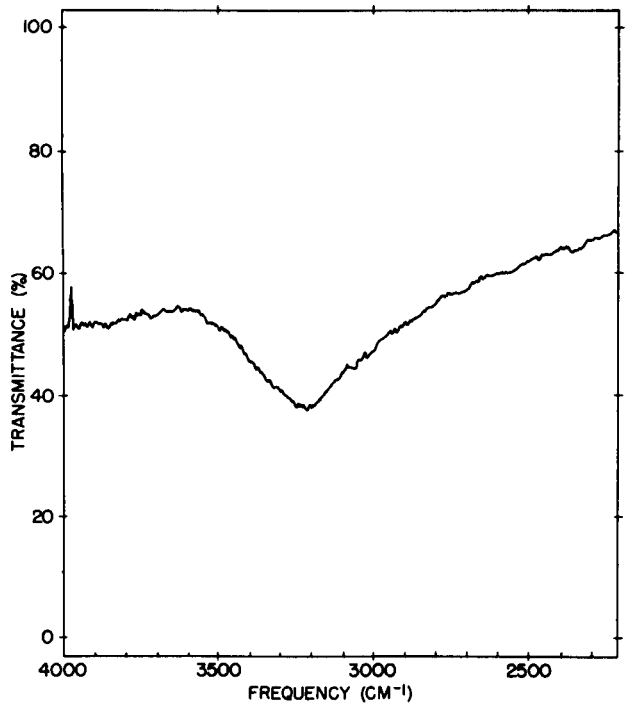


Fig. 3. Infrared Spectrum of 5, 8-Dihydroxyflavone.

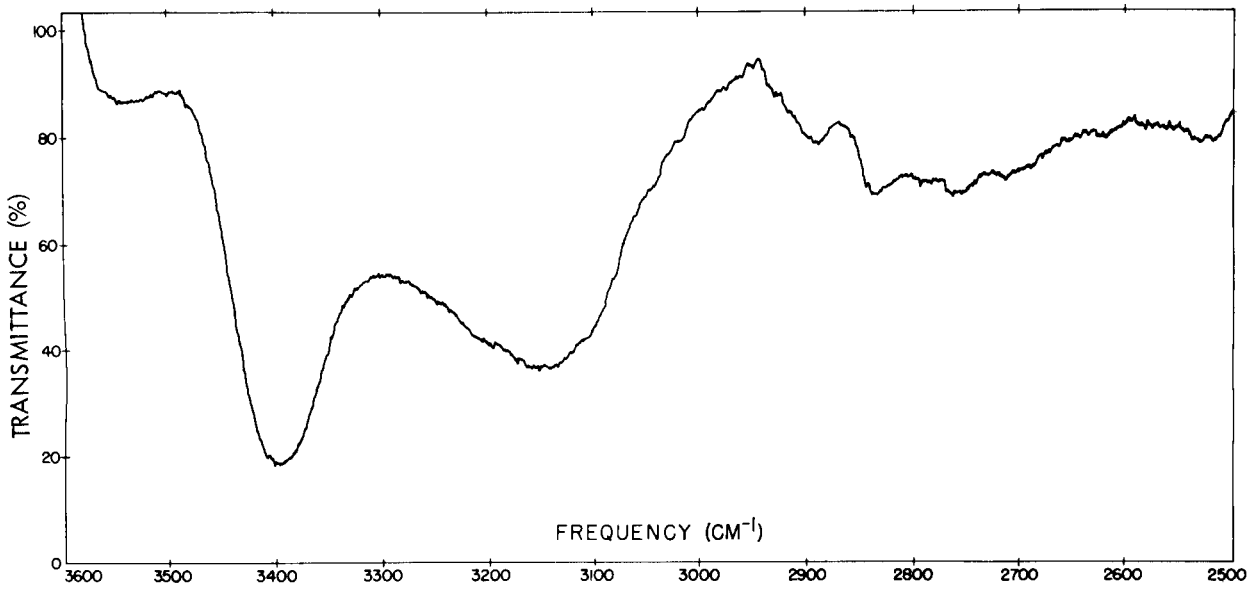


Fig. 4. Infrared Spectrum of 3,5-Dihydroxyflavone (Chloroform Solution).

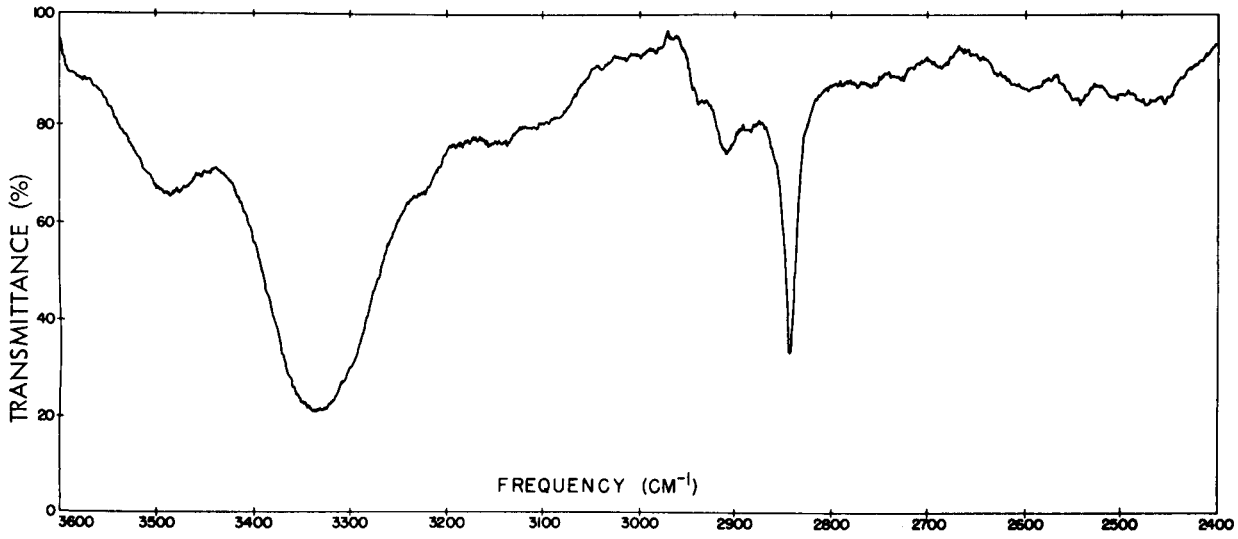


Fig. 5. Infrared Spectrum of 3-Hydroxy-5-methoxyflavone (Chloroform Solution).

at 1655±2 cm^{-1}. From Table II, the solution carbonyl band (carbon tetrachloride) for eleven of the twelve flavones studied is evident at 1653±3 cm^{-1}, with the 5,2'-dimethoxyflavone band being of slightly lower frequency. These results for solution carbonyl frequencies are in essential agreement with previous studies (7).

Solid state carbonyl values also are listed in Tables I and II. Nujol spectra were determined in order to detect possible anomalies in KBr spectra. With very few exceptions, solid state and solution carbonyl frequencies are nearly identical for substances containing a 5-hydroxyl group. This observation is reasonable in light of the intramolecular nature of the hydrogen bond in which the 5-hydroxyl group is involved. When solid state carbonyl values for flavones of Table I are compared with those in Table II (in which no substance contains a free 5-hydroxyl group), it is evident that the hydrogen-bonded carbonyl system is much less subject to polarization in the solid state. It also is apparent that differences in solid state and solution carbonyl frequencies can not be attributed solely to hydrogen-bonding, since in Table II no substance contains a hydroxyl which would contribute to hydrogen bonding.

Our earlier study (3) indicated marked differences in solid state and solution spectra (dioxane) carbonyl frequencies in spectra of 7-, 8-, 2'-, 3'-, and 4'-hydroxyflavone. These data were interpreted as indicating probable solid state intermolecular hydrogen bonds in these substances. If the postulate is accepted that the intramolecularly hydrogen-bonded carbonyl in 5-hydroxyflavones is unavailable for intermolecular hydrogen-bonding, then any hydroxyl group linked through a proton solely to the flavone carbonyl in the *n*-hydroxyflavone should be much less strongly hydrogen-bonded to the flavone carbonyl in the 5,*n*-dihydroxyflavone. This is the case with 4'-hydroxyflavone and 4',5-dihydroxyflavone, with the OH stretching frequency in the spectrum of the latter appearing near 3330 cm^{-1}, nearly 200 cm^{-1} higher than the highest frequency band in the hydroxyl stretching region of the 4'-hydroxyflavone spectrum (3). In contrast, the hydroxyl stretching bands in spectra of 2',5-, 3',5-, and 5,7-dihydroxyflavone appear at much lower frequencies than the corresponding band in the spectrum of 4',5-dihydroxyflavone, indicative of hydrogen bonding with some group other than the carbonyl group. Whether hydrogen-bonding in 2'-, 3'-, and 7-monohydroxyflavone would involve also some group other than the carbonyl can not be ascertained with certainty, but the possibility must be considered. In the spectrum of 5,8-dihydroxyflavone, a relatively high frequency band near 3220 cm^{-1} does appear, but deuteration experiments (5) did not indicate conclusively with which hydroxyl stretching mode, 5- or 8-, this band was associated. The 6-hydroxyl group does not appear to be strongly hydrogen-bonded in either 6-hydroxyflavone itself (3) or 5,6-dihydroxyflavone.

3,5-Dihydroxyflavone is sufficiently soluble in chloroform to permit study of the solution spectrum, even in the hydroxyl stretching region where such bands often are weak (8). From Figure 4, it is evident that the most prominent band is near 3400 cm^{-1}, in agreement with the previously reported value (7a). This band almost certainly is associated with the stretching mode of the 3-hydroxyl group, as previously noted (7a). It is conceivable that some of the weak bands in the 2950-2600 cm^{-1} region would be associated with stretching modes of the 5-OH group, since such bands appear in the spectrum of 5-hydroxyflavone itself. However, it also is possible that the band near 3150 cm^{-1} is due to 5-OH stretching, especially since this band is absent in the spectrum of 3-hydroxy-5-methoxyflavone (Fig. 5). Verification of this speculation would be rather interesting, since it would indicate that the 5-hydroxyl group is less strongly hydrogen-bonded in 3,5-dihydroxyflavone than in 5-hydroxyflavone itself (9, 5). There also is a remote possibility that the band near 3150 cm^{-1} is due to aromatic C-H stretching.

The spectrum of 3-hydroxy-5-methoxyflavone (Fig. 5) has a prominent band with midpoint near 3334 cm^{-1}, which is attributed to the 3-hydroxyl stretching mode. Thus, a comparison of the spectra in Figures 4 and 5 makes a 66 cm^{-1} shift of the hydroxyl band towards lower frequency evident in the spectrum of 3-hydroxy-5-methoxyflavone. It seems reasonable to conclude that the 3-hydroxyl group is more strongly hydrogen-bonded in the 5-methoxy- than in the 5-hydroxy-derivative. This observation is important as additional spectral evidence that the 5-hydroxyl group does interact with the flavone carbonyl group, even though the carbonyl frequencies of flavone and 5-hydroxyflavone have nearly identical values (3, 7a). The relatively sharp band at 2843 cm^{-1} in Fig. 5 very probably is associated with C-H stretching in the methoxyl group.

The high resolution spectrum of 3,5-dihydroxyflavone (chloroform solution) contained a strong, multipeaked carbonyl band, with the strongest and most central peak at 1641 cm^{-1}, in good agreement with the previously reported value of 1638 cm^{-1} for a carbon tetrachloride solution (7a). Other very sharp but less intense peaks were present at 1631, 1649 and 1657 cm^{-1}. The spectrum of 3-hydroxy-5-methoxyflavone contains an extremely strong, sharp band at 1616 cm^{-1}, with a medium weak shoulder extending over approximately 15 cm^{-1} midpoint 1646 cm^{-1}. If the shoulder is assumed to be unimportant and the band at 1616 cm^{-1} is assigned to the carbonyl group, then the data are interpreted reasonably as indicating that the 3-hydroxyl group is more strongly hydrogen-bonded in the 5-methoxyl derivative than in 3,5-dihydroxyflavone. There is considerable uncertainty involved in assigning bands in this region to a carbonyl group, however, since benzene in-plane skeletal modes often occur near 1600 cm^{-1}, and occasionally as high as 1624 cm^{-1} (10). As noted elsewhere (5), oxygen isotope studies may be necessary before the carbonyl band can be located with certainty.

TABLE I

Carbonyl Absorption of Flavones Containing the 5-Hydroxyl Group

Substituent(s) in Addition to 5-OH	Infrared Absorption Max, cm^{-1} KBr	Nujol	CCl_4	Dioxane
None	1653	1651	1652	1654
6-OH	1651	1652	----	1655
7-OH	1649	1648	----	1657
8-OH	1653	----	----	1657
2'-OH	1643	1644	----	1654
3'-OH	1653	1649	----	1654
4'-OH	1651	1650	----	1654
2'-OCH_3	1648	1649	1654	----
3'-OCH_3	1652	1653	1655	----
4'-OCH_3	1655	1653	1654	----
2'-OAc	1653	1652	1654	----
3'-OAc	1647	1650	1655	----
7-OAc	1665	1664	1657	----
3,7-di-OH	1650	1649	----	1655
3-OCH_3-7-OH	1647	1648	----	1653

TABLE II

Carbonyl Absorption of Dimethoxyflavones, Methoxyacetoxyflavones and Diacetoxyflavones.

Substituents	Infrared Absorption Max, cm^{-1} KBr	Nujol	CCl_4	Dioxane
5,2'-Di-OCH_3	1628	1630	1648	----
5,3'-Di-OCH_3	1645	1646	1653	1651
5,4'-Di-OCH_3	1649	1648	1650	----
7,3'-Di-OCH_3	----	----	1650	----
5-OAc-3'-OCH_3	1640	1637	1652	----
5-OAc-4'-OCH_3	1638	1640	1650	----
5,7-Di-OAc-3-OCH_3	1627	1628	1650	----
5,6-Di-OAc	1645	1647	1656	----
5,7-Di-OAc	1639	1640	1656	----
5,2'-Di-OAc	1647	1648	1654	----
5,3'-Di-OAc	1642	1640	1653	----
5,4'-Di-OAc	1642	1643	1653	----

EXPERIMENTAL (11)

Infrared Spectral Measurements.

The curves in Figures 1 and 3 were recorded with a Perkin-Elmer Model 21 spectrophotometer, sodium chloride optics, for the flavones in potassium bromide disks. The curve in Figure 3 was obtained with a Perkin-Elmer Model 237 grating spectrophotometer for a potassium bromide disk of the flavone. The curves in Figures 4 and 5 were recorded with a Perkin-Elmer Model 21 spectrophotometer, lithium fluoride optics, for chloroform solutions, 3 mm. cells. In Figure 4, the reference was polystyrene; in Figure 5, the reference was the 3336 cm^{-1} band of ammonia vapor. The data of Tables I and II were obtained with a Perkin-Elmer Model 21 spectrophotometer, sodium chloride optics. The carbonyl region of 3,5-dihydroxyflavone in chloroform solution (3 mm. cell) was investigated with a Perkin-Elmer Model 21 spectrophotometer, lithium fluoride optics, polystyrene reference. The carbonyl region of the spectrum of 3-hydroxy-5-methoxyflavone in chloroform solution (1 mm. cell) was studied with a Perkin-Elmer Model 237 grating spectrophotometer, polystryene reference.

5,*n*-Dihydroxyflavones.

All 5,*n*-dihydroxyflavones employed were prepared in this laboratory by known methods, or modifications of known methods. 2',5-, 3',5-, And 4',5-dihydroxyflavones were obtained as previously described (12). 5,7-Dihydroxyflavone (chrysin), m.p. 286-287°, was prepared by the method of Mentzer and Pillon (13). 5,6-Dihydroxyflavone was prepared by the procedure of Iyer and Venkataraman (14). 5,8-Dihydroxyflavone (primetin) was prepared by the general procedure of Seshadri and co-workers (15), with chromatography of the crude primetin thus obtained in ethyl acetate solution on Florex-Celite (5:1 wt.). Primetin was obtained as the leading yellow band, which was collected in the effluent. Pure primetin, m.p. 232-233°, was obtained by deacetylation of the diacetate with methanolic hydrochloric acid (70 mg. of diacetate heated with 10 ml. methanol and 2 drops of concentrated hydrochloric acid for 90 minutes).

3,5-Dihydroxyflavone.

This substance was prepared by the general method of Seshadri and Venkateswarlu (16). Inasmuch as the procedure employed in this laboratory differed in several respects and certain results, essential details are presented.

2-Hydroxy-6-methoxyacetophenone was prepared by the procedure of Baker (17). 2-Hydroxy-6-methoxyacetophenone (3 g.) and benzaldehyde (11 ml.) were dissolved in 95% ethanol (60 ml.) and 50% aqueous potassium hydroxide (60 ml.) added. The resulting mixture was warmed on a steam bath for 1 to 2 minutes and shaken vigorously.

The reaction mixture became homogeneous and formed a clear red solution. The reaction mixture was permitted to stand at room temperature for 72 hours, and then was diluted with water and extracted with ether to remove excess benzaldehyde. The aqueous phase was acidified with hydrochloric acid and again extracted with ether. The ether extract was washed with sodium bicarbonate solution (*ca.* 5%) and with water, and then dried over anhydrous sodium sulfate. Removal of the solvent by evaporation resulted in a residue (presumably the intermediate chalcone), which was dissolved in aqueous ethanol (prepared by adding 140 ml. of 95% ethanol to 100 ml. of water) and concentrated sulfuric acid (6 ml.) added. The acidic mixture was heated under reflux for 30 hours. Alcohol was removed by distillation *in vacuo*, and water added to the residual solution. Upon keeping, no crystallization occurred. Hence, the mixture was extracted with ether, and the ether extract washed with aqueous sodium hydroxide until it was colorless. Then the ethereal solution was washed with additional water and dried over anhydrous sodium sulfate. Solvent removal gave a residue, which was recrystallized from ethyl acetate containing a trace of petroleum ether, b.p. 40-60°. There was obtained, in 2.1 g. yield, 5-methoxyflavanone as colorless needles, m.p. 140° [lit. m.p. (16), 148-150°].

5-Methoxyflavanone (1.5 g.) was dissolved in 95% ethanol (45 ml.) and brought to gentle reflux. Then 90 ml. of concentrated hydrochloric acid (d. 1.19) and freshly prepared isoamyl nitrite (11 ml.) were added in small portions over 10-15 minutes. The reaction mixture was maintained at gentle reflux during nitrite addition. After the reaction mixture stood for two hours, its color changed from clear dark red to turbid orange. The mixture was diluted with approximately 300 ml. of water and extracted with ether. The ether extract, upon treatment with 1 *N* sodium hydroxide, gave a solid (presumably the sodium salt), which was collected by filtration, acidified, and the resulting solid crystallized from ethyl acetate to give the crystalline 3-hydroxy-5-methoxyflavone, m.p. 164° [lit. m.p. (16), 170-171°].

3-Hydroxy-5-methoxyflavone (1.5 g.), acetic anhydride (25 ml.) and 25 ml. of hydriodic acid (d. 1.7) were heated together for one hour, and then the mixture was poured into 50% aqueous sodium bisulfite. The resulting solid was collected, dried, and recrystallized from ethanol to give 3,5-dihydroxyflavone, m.p. 144° [lit. m.p. (16), 145-146°].

Anal. Calcd. for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 71.02, 71.21; H, 3.72, 3.88.

In the previous report (16) for preparation of this compound, analytical data are presented which support the molecular formula, $C_{15}H_{10}O_3 \cdot H_2O$. This formula does not correspond to a hydrate of 3,5-dihydroxyflavone, but rather to the hydrate of a monohydroxyflavone, or to 3,5-dihydroxyflavanone, $C_{15}H_{12}O_4$.

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(7a) B. L. Shaw and T. H. Simpson, *J. Chem. Soc.*, 655 (1955). (b) A recent review of several investigations is given by H. Wagner in "Methods in Polyphenol Chemistry", J. B. Pridham, Ed., Pergamon Press, London (1964), pp. 37-41.

(8) Inasmuch as chloroform transmits less than 25% of incident infrared radiation in the 2980-3100 cm^{-1} region [R. N. Jones and C. Sandorfy in "Techniques of Organic Chemistry", A. Weissberger, Ed., Vol. IX, Interscience, New York, N. Y., (1956), p. 299], absorption in this region can not be evaluated reliably in Figures 4 and 5.

(9) See, however, reference (4a), in which it is reported that the solid state spectrum of 5,7-dihydroxy-3,4'-dimethoxyflavone (in which the 3-hydroxyl to 4-carbonyl hydrogen bond obviously is impossible) contains a band at 3150 cm^{-1}. The interesting observation also is made that the spectrum of 7-hydroxy-3,4'-dimethoxyflavone contains no band at 3150 cm^{-1}.

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(11) Melting points are uncorrected. Analysis is by A. Bernhardt, Mülheim, Germany.

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