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Physical and Chemical Properties of Hydroxyflavones. V. Deuteroxyflavones from Acetoxyflavones by Imidazole-Catalyzed Deacetylation (1)

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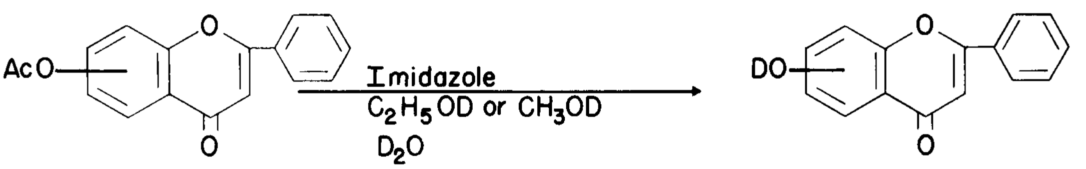
A recently described procedure for imidazole-catalyzed deacetylation of acetoxyflavones has been modified by effecting hydrolysis in a mixture of deuterium oxide and deuterioethanol (C_2H_5OD), or in deuterium oxide and deuteriomethanol (CH_3OD). Application of the new procedure to monoacetoxyflavones has resulted in the monodeuteroxyflavone. 5-Deuteroxyflavone has been obtained also by hydrolysis of a rubidium salt in deuterium oxide. Imidazole-catalyzed hydrolysis of four diacetoxyflavones in a mixture of deuteriomethanol and deuterium oxide is described. Infrared spectral data are presented for the deuteration products, with emphasis on OD stretching and vibrational modes. Comparison of spectra of appropriate monohydroxyflavones and monodeuteroxyflavones permits assignment of δ (OH) bands in four hydroxyflavone spectra.

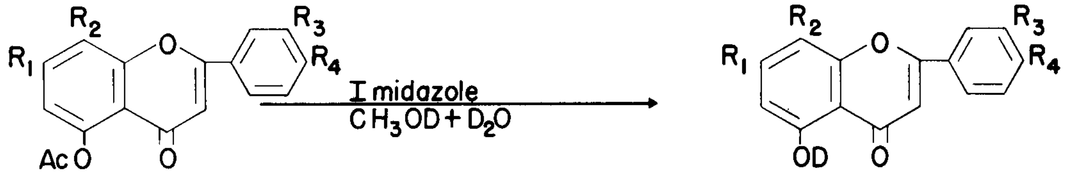
Recently we described a purification method for hydroxyflavones, in which the acetate was hydrolyzed in aqueous ethanol or methanol in the presence of catalytic amounts of imidazole (2). In the present paper we report a modification of this procedure which results in formation of a mono- or bis-deuteroxyflavone.

Hydrolysis of monoacetoxyflavones has been carried out either in deuterioethanol-deuterium oxide or deuteriomethanol-deuterium oxide mixtures at reflux temperature. Experimental details are given in Table III. The monodeuteroxyflavone resulted in all instances. Because of our previous unsuccessful attempt (3) at preparation of 5-deuteroxyflavone, this substance has been subjected to detailed study. 7-Deuteroxyflavone, prepared from interaction of 7-acetoxyflavone with deuterioethanol-deuterium oxide, gives a deuterium analysis in reasonable agreement with theory. In addition, the infrared spectrum (Figure 3) contains bands associated with the OD stretching mode, but is virtually transparent in the 2500-2900 cm^{-1} region. Infrared spectra of the other monodeuteroxyflavones (Figures 1, 2, 4-7) also are virtually transparent in the OH-stretching region, but contain bands which reasonably can be assigned to the OD-stretching mode. All infrared spectra are devoid of ester carbonyl stretching bands. Satisfactory results are obtained in preparing the four 5,n-bis-deuteroxyflavones (II, IV, VI, VIII) only if the hydrolysis of the diacetate is effected in deuteriomethanol-deuterium oxide. When deuterioethanol-deuterium oxide is used, the hydrolysis products have wide melting ranges.

Infrared curves (1700-4000 cm^{-1} region) are given in Figures 1-9 for the deuteroxyflavones prepared in this study. From Figures 1-7, it is evident that all monodeuteroxyflavones contain bands, presumably associated with O-D stretching modes, in the region 1980-2500 cm^{-1}. In Figures 2 and 3, the infrared curves for 6-hydroxy- and 7-hydroxyflavone are shown for comparison. The ratio, ν(OH)/ν(OD), for the 6-substituted flavones is 1.34, and for the two bands of the 7-substituted flavones 1.25 and 1.26. These latter values are lower than predicted from theoretical relationships (4), but are in agreement with experimentally determined ratios in the hydroxyanthraquinone series (5). From Figures 2 and 3, it is evident that the solid state O-D bands are somewhat sharper than solid state O-H bands. In spectra of the other monodeuteroxyflavones, O-D bands are readily located, in contrast to O-H bands in the complex spectra previously reported (3). If a ratio of ν(OH)/ν(OD) of 1.30 is arbitrarily assumed, then from the O-D peak values, ν(OH) in 2'-, 4'-, and 8-hydroxyflavones would be expected in the region 2574-2925 cm^{-1}. Peaks in this region have been observed previously (3), but could not definitely be assigned to O-H stretching modes. It is now possible to consider with greater certainty that these relatively low frequency peaks actually are associated with the O-H stretching vibration. The 3'-O-H peaks, however, would be expected near 3060 and 2970 cm^{-1}, as found in our previous study (3). The ν(OH)/ν(OD) ratio for the 3-substituted flavones appears to be approximately 1.35; however, the very broad O-H peak makes more exact ratio computation difficult.

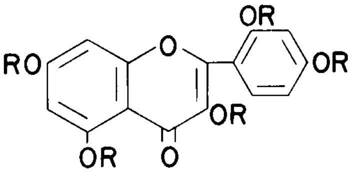
Comparison of appropriate hydroxy- and deuteroxyflavone spectra in the 900-1400 cm^{-1} region has led to assignment of O-H in-plane vibrational modes as listed in Table I. These bands lie in the fingerprint region, and would be difficult to locate without





I: $R_1 = -OAc$; $R_2 = R_3 = R_4 = H$
III: $R_2 = -OAc$; $R_1 = R_3 = R_4 = H$
V: $R_3 = -OAc$; $R_1 = R_2 = R_4 = H$
VII: $R_4 = -OAc$; $R_1 = R_2 = R_3 = H$

II: $R_1 = -OD$; $R_2 = R_3 = R_4 = H$
IV: $R_2 = -OD$; $R_1 = R_3 = R_4 = H$
VI: $R_3 = -OD$; $R_1 = R_2 = R_4 = H$
VIII: $R_4 = -OD$; $R_1 = R_2 = R_3 = H$



IX: R = Ac
X: R = D

deuteration studies. The δ (OD) bands in Table I do not appear in the spectra of the corresponding hydroxyflavone, nor, with one exception, does the δ (OH) band appear in the deuteroxyflavone spectra. The exception is 7-deuteroxyflavone, the spectrum of which contains a band near 1260 cm^{-1}, which, however, is of only medium intensity. In contrast, the corresponding band in the 7-hydroxyflavone curve is the strongest in the entire spectrum. The assignments in Table I are in reasonable agreement with those made for phenol (6), the solid state spectrum of which contains bands at 1230 and 1370 cm^{-1}, assigned to δ (OH) with ring stretch character and ring stretch with δ (OH) character respectively.

Comparison of spectra of 2'-, 3'-, and 4'-hydroxy- and deuteroxyflavones does not result in O-H in-plane vibrational band assignments because of marked similarity of spectra, or complexity of spectra in the 900-1400 cm^{-1} region.

A recent study has shown that the γ (OH) band in solution spectra of intramolecularly hydrogen-bonded phenols is uniquely broad, and occurs in the 300-860 cm^{-1} region (7). The equipment available in the present investigation, however, does not permit investigation of infrared absorption below 625 cm^{-1}. In the accessible region 625-860 cm^{-1}, the spectra of 2'-, 4'-, 3-, 6-, and 7-hydroxy- and deuteroxyflavones show no significant differences. In contrast, the spectra of 8-hydroxy- and 8-deuteroxyflavone are not identical in the 625-860 cm^{-1} region. The spectrum of 8-deuteroxyflavone contains bands at 682 and 755 cm^{-1}, which are absent from the spectrum of 8-hydroxyflavone. In the spectra of both 8-hydroxy- and 8-deuteroxyflavone, there appears a strong band at 805 cm^{-1}. However, in the 8-deuteroxyflavone curve this band is very sharp, whereas in the 8-hydroxyflavone spectrum it is multishouldered, with the most prominent shoulder appearing at 815 cm^{-1}.

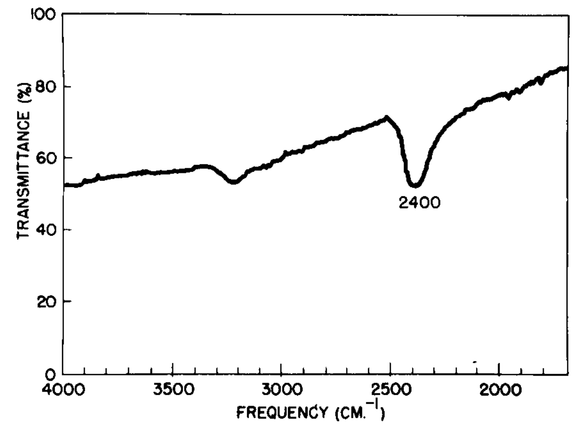


Fig. 1. Infrared Spectrum of 3-Deuteroxyflavone.

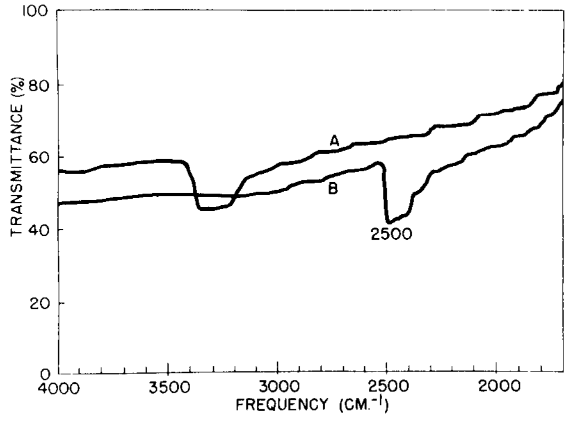


Fig. 2. Infrared Spectrum of (A) 6-Hydroxyflavone; (B) 6-Deuteroxyflavone.

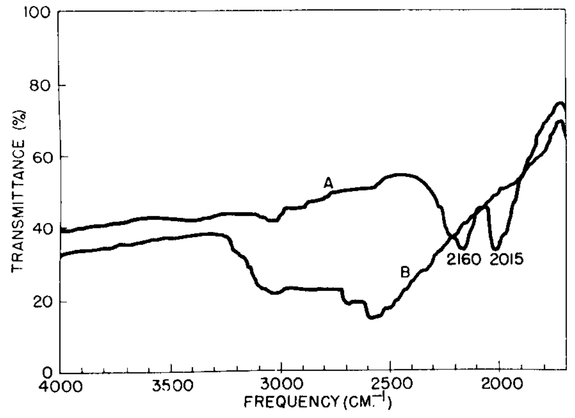


Fig. 3. Infrared Spectrum of (A) 7-Deuteroxyflavone; (B) 7-Hydroxyflavone.

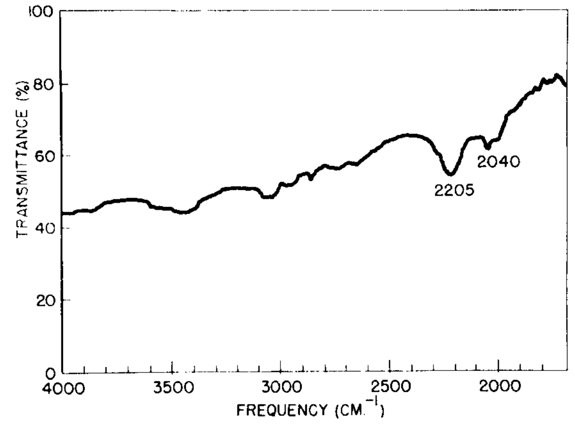


Fig. 4. Infrared Spectrum of 8-Deuteroxyflavone.

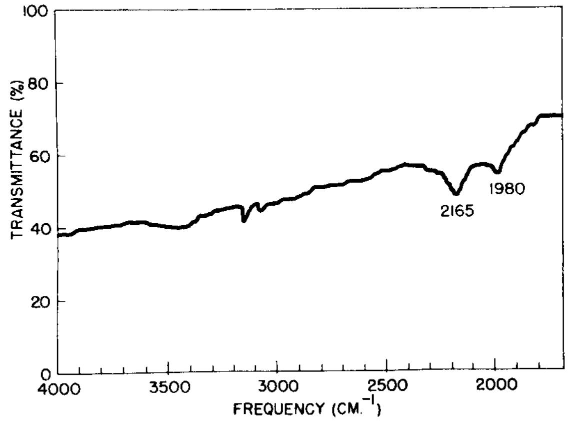


Fig. 5. Infrared Spectrum of 2'-Deuteroxyflavone.

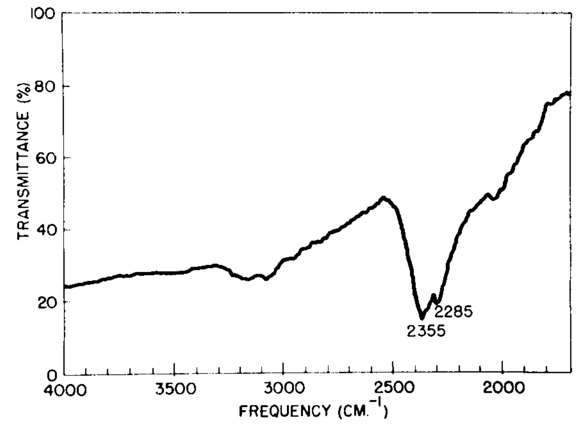


Fig. 6. Infrared Spectrum of 3'-Deuteroxyflavone.

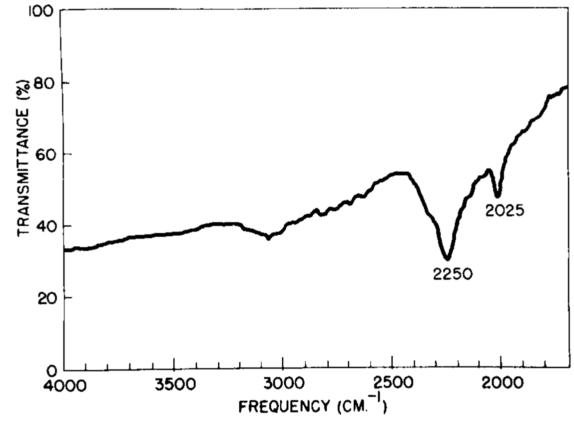


Fig. 7. Infrared Spectrum of 4'-Deuteroxyflavone.

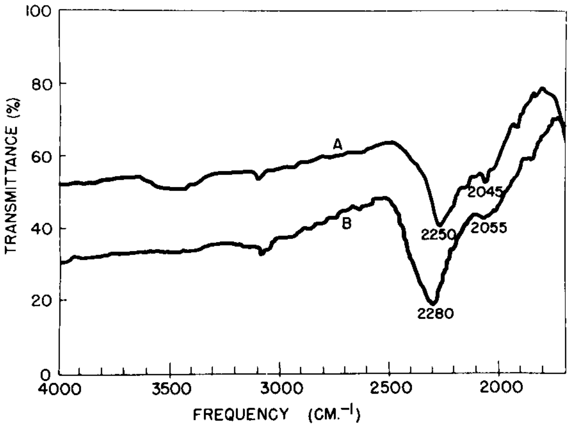


Fig. 8. Infrared Spectrum of (A) 5,7-bis-Deuteroxyflavone; (B) 5, 8-bis-Deuteroxyflavone.

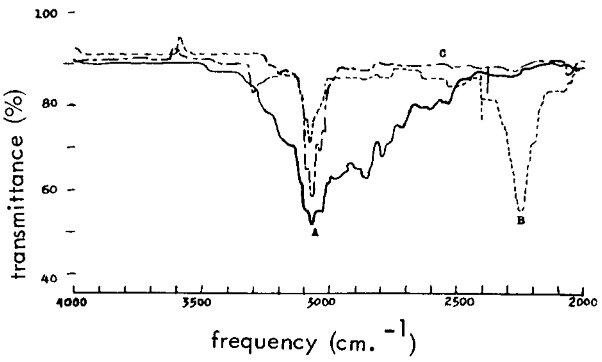


Fig. 11. Infrared Spectra in Tetrachloroethylene Solution of (A) 5-Hydroxyflavone; (B) 5-Deuteroxyflavone; (C) Flavone.

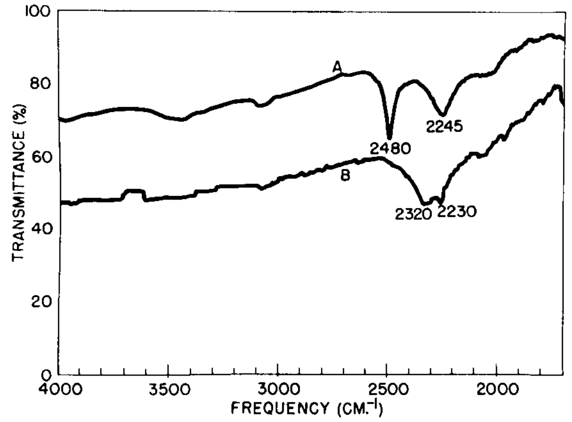


Fig. 9. Infrared Spectrum of (A) 5, 4'-bis-Deuteroxyflavone; (B) 5, 3'-bis-Deuteroxyflavone.

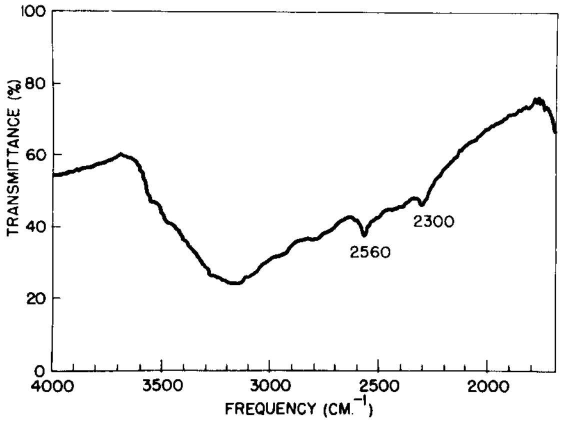


Fig. 10. Infrared Spectrum of Partially Deuterated Morin.

Hydrolysis of the appropriate diacetate (I, III, V, or VII) in deuteriomethanol-deuterium oxide has resulted in the bisdeuteroxyflavone (II, IV, VI, or VIII). Infrared curves for these deuteration products are given in Figures 8 and 9. In these cases also, the curve for the deuteroxyflavone is somewhat simpler than that for the corresponding hydroxyflavone. With the exception of 5, 8-bisdeuteroxyflavone, the spectra are reasonably interpreted on the basis that the band in the region 2240±10 cm^{-1} is associated with the 5-OD stretching vibration, whereas the other band is attributable to the other deuteroxyl group. If one selects the appropriate band in the 5,*n*-dihydroxyflavone spectrum (8), and the presumably corresponding band in the 5,*n*-deuteroxyflavone spectrum and computes the ν(OH)/ν(OD) ratio, then the following values are obtained: for 5,3'-derivatives, 1.34 for the 3-substituent and 1.33 for the 5-substituent; for 5, 4'-derivatives, 1.34 for both the 4'- and 5-substituents; and for 5, 7-derivatives, 1.34 and 1.33 for 5- and 7-substituents, respectively. Although O-D stretching bands are fairly readily located, the complexity of absorption in the OH region of the spectra of 5, 3'- and 5, 7-dihydroxyflavone (8) makes selection of one single stretching peak necessarily rather arbitrary. Bands at 2970, 3010, and 3005 cm^{-1} were chosen for the 5-OH stretching band in 5, 3'-, 5, 4'- and 5, 7-dihydroxyflavones, respectively. In the same flavones, bands at 3100, 3330, and 2716 cm^{-1} were selected for the 3'-, 4'-, and 7-OH stretching bands, respectively.

From Figure 8B, it is evident that either the 5- or 8-deuteroxyl group results in a rather strong band at 2280 cm^{-1}. In contrast, the 2055 cm^{-1} band is seen to be broad and much weaker. The band at 2280 cm^{-1} possibly is associated with a stretching mode of the 5-OD group, inasmuch as it is close to values found in other 5,*n*-bis-deuteroxyflavones, and to the stretching frequency of the

5-OD group in 5-deuteroxyflavone itself (sequel). The 2055 cm^{-1} band, and possibly also one of the shoulders on the 2280 cm^{-1} band, then would be associated with 8-OD stretching vibrations. The solid state spectrum of 5, 8-dihydroxyflavone shows only one distinct band, near 3220 cm^{-1} (8). Inasmuch as the ν(OH)/ν(OD) ratio (assuming 5-OH and 5-OD bands to be at 3220 and 2280 cm^{-1} respectively) is 1.41, it can not be stated with certainty that the 3220 cm^{-1} band is associated with an O-H stretching vibration, since such ratios usually have values of 1.35 or less. Possibly resolution with sodium chloride optics is not adequate to permit observation of the weak bands actually associated with O-H stretching vibrations in 5, 8-dihydroxyflavone.

Attempts to extend the deacetylation procedure to morin pentaacetate (IX) have not led to pure 2', 3, 4', 5,7-pentakis-deuteroxyflavone (X). The spectrum of the deacetylation product is given in Figure 10. Although the band at 2300 cm^{-1} probably is an O-D stretching band, and possibly also the one at 2560 cm^{-1}, strong absorption in the O-H stretching region still is very much in evidence. Completely O-deuterated morin may be extremely labile in moist air.

In Table II are listed flavone carbonyl bands for deuteroxyflavones of this study. In a solid state monodeuteroxyflavone spectrum, the carbonyl band is of somewhat higher frequency than the one in the corresponding solid state monohydroxyflavone spectrum (3). 4'- And 7-deuteroxyflavone are exceptional. From comparison of the value of approximately 1650 cm^{-1}, observed for the flavone carbonyl band in solution spectra of several hydroxyflavones (9), with the relatively lower frequency bands in Table II, it is assumed that these lower frequencies probably indicate hydrogen bonding between O-D and flavone carbonyl groups. The hydrogen bond involving the 6-OD group appears very weak. In contrast, the solid state hydrogen bond in 4'- and 7-derivatives appears quite strong, regardless of whether protium or deuterium participates in hydrogen bond fromation. In general, the monodeuteroxyflavones having relatively high O-D stretching frequencies have also relatively high carbonyl frequencies, a reasonable correlation if less association through hydrogen bonding in the crystal lattice is assumed. 2'-Deuteroxyflavone is quite exceptional, but there is the obvious possibility of hydrogen-bonding involving the 2'-OD group and the hetero-oxygen atom, either intra- or intermolecularly, in addition to hydrogen bonding involving the carbonyl group. The spectrum of 3-deuteroxyflavone is reasonably interpreted in light of the correlation if the shoulder near 1644 cm^{-1} is accepted as the carbonyl band. The two shoulders in the 3-deuteroxyflavone spectrum (Table II) appear on a strong band at 1610 cm^{-1}. Isotope studies with oxygen-18 may be necessary to determine conclusively which band in the 3-hydroxy- and 3-deuteroxyflavone spectra is the actual carbonyl band.

Application of the imidazole-deuterium oxide hydrolysis procedure to 5-acetoxyflavone gives a product which is assigned the structure 5-deuteroxyflavone on the basis of its spectrum (Figure 11B), melting point virtually identical with that of 5-hydroxyflavone, deuterium analysis, and ready protium exchange in ethanol and moist air. An alternative procedure for its preparation involves a rubidium salt of 5-hydroxyflavone. In a study of the alkali metal salts of the latter substance, it has been observed that the lithium, sodium and potassium salts are insoluble and stable in water, but the rubidium and cesium salts undergo hydrolytic decomposition to yield 5-hydroxyflavone. The rubidium salt is prepared by addition of aqueous rubidium hydroxide to a dioxane solution of 5-hydroxyflavone. The dry salt upon hydrolysis in deuterium oxide gives 5-deuteroxyflavone, but not in spectroscopically pure condition, since weak O-H stretching bands still are evident in the infrared spectrum. Deuterium analysis indicates a purity of approximately 80%.

In view of data in this paper supporting the 5-deuteroxyflavone structure obtained in two different ways, there arises the problem of structure of the previously reported deuteration product (3). Almost certainly, C-D bonds were present. Also, the method could have yielded inorganic phosphate contaminants, which might account for the unexpectedly high melting point. The cause of the broad infrared band in the 2740-2900 cm^{-1} region remains obscure.

In Figure 11 are presented infrared curves for tetrachloroethylene solutions of flavone, 5-hydroxyflavone and 5-deuteroxyflavone. The sharp band near 3065 cm^{-1}, common to all spectra, is assigned to an aromatic C-H stretching vibration. The spectrum of 5-deuteroxyflavone is considerably simpler than that of 5-hydroxyflavone, and the O-D stretching band near 2250 cm^{-1} is clearly evident. The presence of several bands in the 2600-3050 cm^{-1} region of the spectrum of 5-hydroxyflavone, and their absence from the corresponding spectra of flavone and 5-deuteroxyflavone, is strongly suggestive that these peaks are associated in some way with 5-hydroxyl stretching vibrations. The band near 3030 cm^{-1}, which appears as a shoulder on the 3065 cm^{-1}, is the strongest in this region. If this band is accepted as ν (OH), and the ν(OH)/ν(OD) ratio computed, a value of 1.35 is obtained, in good agreement with values obtained in considering hydroxyl groups at other positions on the flavone nucleus. Regardless of which band or group of bands is associated with the 5-hydroxyl vibration(s), it is evident that a satisfactory solvent and adequate spectrophotometer permit even strongly intramolecularly hydrogen-bonded hydroxyl groups to be detected spectrally in the 2500-3500 cm^{-1} region.

Carbonyl bands for 5-deuteroxyflavone are present at 1645 and 1639 cm^{-1}, tetrachloroethylene solution and potassium bromide disk, respectively. For 5-hydroxyflavone, the carbonyl bands in both media occur at 1654 cm^{-1}. If our previous value (3) of 1652 cm^{-1} (carbon tetrachloride solution) is employed for comparison purposes, a small influence by the deuterium bond on the carbonyl stretching frequency is evident, somewhat greater in magnitude than the experimental error.

TABLE I

δ (OH) Bands of Monohydroxyflavones (a)

Position of OH or OD	δ (OH), cm^{-1}	δ (OD), cm^{-1}	δ (OH)/δ (OD)
3	1280	1000	1.28
6	1226	983	1.25
7	1400	1037	1.35
	1260	946	1.33
8	1385	1045	1.33
	1265	948	1.33

(a) In potassium bromide disk.

TABLE II

Flavone Carbonyl Stretching Bands of Deuteroxyflavones (a)

Position of OD Substituent(s)	Flavone CO Band, cm^{-1}
2'	1642
3'	1640
4'	1632
3	1644 (shoulder) or 1619 (shoulder)
6	1648
7	1626
8	1637
5, 3'	1641
5, 4'	1641
5, 7	1639
5, 8	1646

(a) In potassium bromide disk.

TABLE III

Position -OCOCH$_3$ Group(s)	Acetoxy-flavone (mg.)	Solvent (ml.)	D$_2$O, ml.	Imidazole, mg.
6	125	CH_3OD (5)	1	25
8	80	CH_3OD (4)	2	10
3	125	C_2H_5OD (5)	1	20
2'	125	C_2H_5OD (5)	1	15
3'	40	C_2H_5OD (3)	1	10
4'	125	C_2H_5OD (5)	1	25
5, 3'	30	CH_3OD (3)	1	10
5, 4'	40	CH_3OD (4)	2	10
5, 7	40	CH_3OD (5)	2	10
5, 8	40	CH_3OD (3)	2	10

EXPERIMENTAL (10)

7-Deuteroxyflavone.

7-Acetoxyflavone (250 mg.) was dissolved at reflux in a solvent system prepared by adding 1.6 ml. of deuterium oxide to 5 ml. of ethanol-d. Imidazole (25 mg.) was added, and reflux continued for approximately 24 hours. After the reaction mixture was cooled, the crystalline 7-deuteroxyflavone was collected by filtration, washed with a small quantity of deuterium oxide and dried in a vacuum desiccator; m.p. 242-244°.

Anal. Calcd. for $C_{15}H_9O_3D$: D, 10.0 atom % excess. Found: D, 8.96 atom % excess.

Experimental details for preparation of other deuteroxyflavones are outlined in Table III.

5-Deuteroxyflavone.

A. By Hydrolysis of 5-Acetoxyflavone in Deuterium Oxide-Deuterioethanol.

5-Acetoxyflavone (250 mg.) was dissolved at reflux in a solvent system prepared by adding 3 ml. of deuterium oxide to 5 ml. of ethanol-d. Imidazole (25 mg.) was added, and reflux continued for 24 hours. After the reaction mixture cooled to room temperature, the crystalline 5-deuteroxyflavone, m.p. 156-157°, was collected by filtration, washed with 2 ml. of ethanol-d and dried at 100° *in vacuo*. Experimental operations were carried out rapidly to minimize exposure of the deuteration product to moist air.

Anal. Calcd. for $C_{15}H_9O_3D$: D, 10.0 atom % excess. Found: D, 7.66 atom % excess.

B. By Hydrolysis of Rubidium Salt in Deuterium Oxide.

Freshly prepared, dry rubidium salt of 5-hydroxyflavone (0.58 g., apparent purity 96.8%) was reacted with 11 ml. of deuterium oxide in a flask fitted with a ground-glass stopper. After standing overnight, the solution assumed a light yellow color. The precipitated 5-deuteroxyflavone was collected on a fritted glass funnel (protected from atmospheric moisture by a drying tube containing indicator Drierite), washed twice with 5 ml. portions of deuterium oxide, and dried briefly. The product, slightly moist with deuterium oxide, was extracted overnight in a Soxhlet apparatus (protected from atmospheric moisture) with 20 g. of methanol-d. Cooling of the yellow methanol-d solution resulted in the yellow 5-deuteroxyflavone, which was collected by filtration. After being dried *in vacuo* at 80° over phosphorus pentoxide, 5-deuteroxyflavone, m.p. 156-157°, was obtained.

Anal. Calcd. for $C_{15}H_9O_3D$: D, 10.0 atom % excess. Found: D, 7.64 atom % excess.

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(1) This investigation was supported by a research grant (AI-01703) from the National Institute of Allergy and Infectious Diseases, Public Health Service.

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(4) From the equation, $\nu(XD)/\nu(XH) = \left(\frac{2 + M}{2 + 2M}\right)^{1/2}$, where M is the atomic mass of atom X, it is apparent that the $\nu(OH)/\nu(OD)$ ratio would be $(1.89)^{1/2}$, or approximately 1.37: *cf.* R. N. Jones and C. Sandorfy in "Techniques of Organic Chemistry", A. Weissberger, Ed., Vol. IX, Interscience, New York, N. Y., (1956), pp. 333-334.

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(9) *Cf.* reference (3) and other works cited therein, especially B. L. Shaw and T. H. Simpson, *J. Chem. Soc.*, 655 (1955). As this paper was nearing completion, our attention was directed to the work of L. Henry and D. Molho, *Colloq. intern. centre natl. recherche sci.* (Paris), No. 64, 341 (1955) (Published 1957); *Chem. Abstr.*, 54, 10516 (1960), who deuterated 8-hydroxyflavone and chrysin (5,7-dihydroxyflavone) in dioxane with deuterium oxide at 80°. Although the deuteration procedure of Henry and Molho is markedly different from that of the present paper, there is essential agreement in the two studies on OD stretching frequencies of 8-deuteroxyflavone and 5,7-bis-deuteroxyflavone.

(10) Deuterium analyses were determined by the Analytica Corporation, New York, N. Y. Infrared spectral measurements were made with a Perkin-Elmer Model 237 grating spectrophotometer (with exception of solid state spectral data for 5-deuteroxyflavone which were recorded with a Perkin-Elmer Model 21 spectrophotometer). Solution spectra in Figure 11 were determined for 20 mg. of substance per ml. of solution, cell thickness 1 mm.

Received December 14, 1965 Lincoln, Nebraska 68508