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Absorption Spectra of Metal Complexes of Flavonoid Compounds

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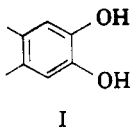
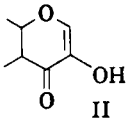
The absorption spectra of a number of flavonoid and related compounds in the presence of aluminum chloride in alcohol, with and without added sodium acetate, have been measured. Interpretations of the spectral changes have been made in terms of probable (approximate) structures of the complexes formed under these various conditions. The effects of *p*H alteration upon the ability of chelatogenic groupings of several kinds to form complexes, and the supporting influences of non-complexing substituent hydroxyl groups, have been demonstrated by these measurements.

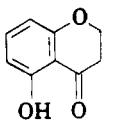
The importance of spectrophotometric measurements in the identification and structure analysis of flavonoid compounds,[1] particularly in those cases in which chromatographic separations afford solutions of pure substances in micro quantities, emphasizes the desirability of defining the conditions under which the combination of metallic ions with the chelatogenic groupings of these compounds will bring about changes in their ultraviolet absorption spectra. Most of the naturally-occurring compounds of the flavonoid class are polyphenols that contain one or more of the following structural features, which present knowledge indicates are involved in complex formation with metals:



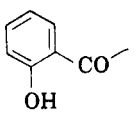
I



II



III



IV

o-Dihydroxy phenols (I) are represented by the catechins, certain anthocyanins, leucoanthocyanins and flavanones; 3-hydroxychromones (II) by the flavonols; 5-hydroxy-chromones and -chromanones (III) by many flavones, isoflavones, flavanones and non-flavonoid (*e.g.*, 2-methyl-) chromones; and *o*-hydroxycarbonyl derivatives (IV) by chalcones, dihydrochalcones, and aurones. Certain of these groupings may occur together in a single substance. For example, quercetin combines I, II, and III; butein, I and IV; luteolin, I and III; etc.

While numerous studies[2-10] have been devoted to the stability of metal complexes of flavonoid compounds, the stoichiometery of the reactions, the visible color changes accompanying their formation, and the absorption spectra of complexes of naturally-occurring flavonoid compounds, there has remained a need for a description of the experimental conditions under which complex formation can best be utilized in detecting and distinguishing between the chelatogenic groupings shown in I–IV.

The present paper presents the results of an examination of some flavonoid substances containing one or a selected combination of the groupings I–IV, and describes the effects of altering the *pH* and the proportion of metal (aluminum) ion to chelatogen upon the absorption spectra of the substances studied. The results confirm and extend some of the observations of Detty, Heston, and Wender[8] on the influence of *pH* in determining whether complex formation will occur, and indicate the importance of the experimental conditions in determining whether spectral alterations can be produced by the addition of metallic salts.

The following compounds were used:

1. catechol; pyrogallol; *d*-catechin; gallic acid
2. naringenin; homoeriodictyol
3. chalcones: 2′-hydroxy; 3,4-dihydroxy; 2′,3,4-trihydroxy; 2′,4-dihydroxy-3-methoxy
4. flavones: 5-hydroxy; 3-hydroxy; 3′,4′-dihydroxy; 3,3′,4′-trihydroxy; apigenin; luteolin; kampferol; quercetin
5. aurones: leptosin; sulfuretin

(1) T. A. Geissman, in *Modern Methods of Plant Analysis*, K. Paech and M. V. Tracey, Eds., Springer, Heidelberg (1955).

(2) T. B. Gage and S. H. Wender, *Anal. Chem.*, **22**, 708 (1950).

(3) T. A. Geissman, *Eleventh Organic Symposium, Madison, Wis.*, June, 1948.

(4) (a) L. Hörhammer, R. Hänsel, and R. Strasser, *Arch. Pharm.*, **285**, 286, 438 (1952); (b) L. Hörhammer and R. Hänsel, *Arch. Pharm.*, **286**, 425, 447 (1953).

(5) T. A. Geissman, J. B. Harborne, and E. C. Jorgensen, *Chemistry & Industry*, 1389 (1953).

(6) T. A. Geissman and L. Jurd, *Arch. Biochem. Biophys.*, **56**, 259 (1955).

(7) T. A. Geissman and J. B. Harborne, *J. Am. Chem. Soc.*, in press (1955).

(8) W. E. Detty, B. O. Heston, and S. H. Wender, *J. Am. Chem. Soc.*, **77**, 162 (1955).

(9) (a) M. Shimizu and G. Ohta, *J. Pharm. Soc. Japan*, **71**, 879 (1951). (b) M. Shimizu, *J. Pharm. Soc. Japan*, **71**, 882 (1951).

(10) T. Swain, *Chemistry & Industry*, 1480 (1954).

EXPERIMENTAL PROCEDURE

Stock solutions of the twenty compounds were prepared with 95% ethanol. The concentrations were such that after the addition of the reagents (ethanolic aluminum chloride, or sodium ethoxide in ethanol) and dilution, the final solutions used for measurement of the absorption spectra were 1.5–2.0 $\times$ 10^{-5} *M*. Sodium acetate (anhydrous) was added as the solid and allowed to settle in the cuvette before measurements were made. Under these conditions each compound was measured at the same concentration in alcohol and in alcoholic solutions of aluminum chloride, sodium ethoxide, and sodium acetate. The Beckman Model DU spectrophotometer was used. Exact temperature control was not maintained, the measurements being carried out in a room in which temperature changes of 1–2° occurred from day to day.

In Table I is shown the composition of the various solutions used for the spectrophotometric measurements.

TABLE I

COMPOSITION OF SOLUTIONS USED FOR SPECTROPHOTOMETRIC MEASUREMENTS

Solution	Stock*	ml. used 95% EtOH	EtOH-0.001 *M* $AlCl_3$	EtOH-0.01 *M* $AlCl_3$	12 *N* Aq. HCl	Anhydrous NaOAc
Abs. EtOH	2.0	8.0	—	—	—	—
EtOH-NaOAc	2.0	8.0	—	—	—	Excess
EtOH-$AlCl_3$(10)	2.0	6.0	2.0	—	—	—
EtOH-$AlCl_3$(10)-NaOAc	2.0	6.0	2.0	—	—	Excess
EtOH-$AlCl_3$(100)	2.0	6.0	—	2.0	—	—
EtOH-$AlCl_3$(100)-NaOAc	2.0	6.0	—	2.0	—	Excess
EtOH-$AlCl_3$-HCl	2.0	6.0	(2.0)	(2.0)	0.05	—

* Apigenin stock solution: 7.04 $\times$ 10^{-5} *M*. 5-Hydroxyflavone stock solution: 7.56 $\times$ 10^{-5} *M*. All other stock solutions: 10 $\times$ 10^{-5} *M*.

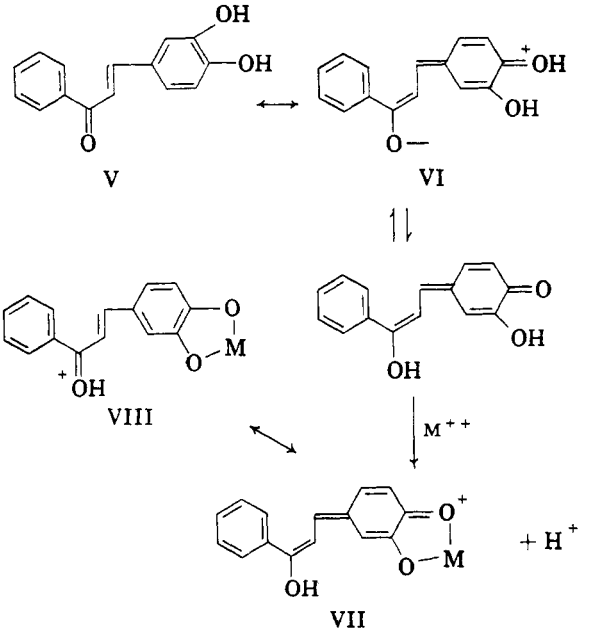
Results. The catechol grouping (I) shows little tendency to form aluminum complexes under neutral or slightly acidic conditions. The data for the first four compounds of Table II show the effects of adding alcoholic aluminum chloride to *o*-dihydroxy compounds that contain no other chelatogenic grouping.

TABLE II

EFFECTS OF ADDING ALCOHOLIC $AlCl_3$ TO *o*-DIHYDROXY COMPOUNDS

Compound	$\lambda_{max.}$, mμ EtOH	EtOH-NaOAc	EtOH-$AlCl_3$	EtOH-$AlCl_3$-NaOAc	Δλ
Catechol	278	278	278	282	4
Pyrogallol	267	267	268	273	6
Gallic acid	272.5	258	276	288	15.5
d-Catechin	279	279	279	290	11
3′,4′-DiOH flavone	342.5	406.5	344	399	56.5
	310 infl.	355 infl.	310	304	
	244	305	244		
3,4-DiOH chalcone	368	374	370.5	440	72
	264.5	264	265	263	

3,4-Dihydroxychalcone (V) *and 3′,4′-dihydroxyflavone* (IX). It is to be noted that, unlike the simple catechol derivatives (catechol, pyrogallol, catechin), those that possess a carbonyl group in a position conjugated with one of the hydroxyl groups show large bathochromic shifts with aluminum chloride when sodium acetate is present (Table II). Taking the case of 3,4-dihydroxychalcone (V) as an example, it is seen that the addition of sodium acetate has a small bathochromic effect, aluminum chloride alone a smaller (almost negligible) effect, but sodium acetate and aluminum chloride together change the spectrum completely, with the production of a new and intense peak 72 mμ nearer the visible. The fact that sodium acetate produces a large shift in the spectrum when aluminum chloride is present indicates that the formation of the metal complex is associated with the ionization of at least one of the hydroxyl groups, and that stabilization of the complex as in VII ⟷ VIII results in the loss of a proton from VI in the chelation step.* The spectrum of 3′,4′-hydroxyflavone (IX) shows no change with aluminum chloride alone, but with both sodium acetate alone and with sodium acetate-aluminum chloride a bathochromic shift of 56–64 mμ is observed. This indicates a greater acidity for the flavone than for 3,4-dihydroxychalcone, and further that the spectra of the species X and XI are nearly the same:**



* In the formulations, the symbol M^{++} will be used for the metal ion since it is very probable that only two donor positions of a given molecule of the organic compound are involved with each metal ion. In the case of the trivalent aluminum ion, the reacting species might be regarded as AlQ^{++}, where Q is a solvating molecule or ion.

** There can be no doubt that the more readily ionized of the two protons of 3′,4′-dihydroxyflavone is the one at 4′, the position conjugated to the carbonyl group.

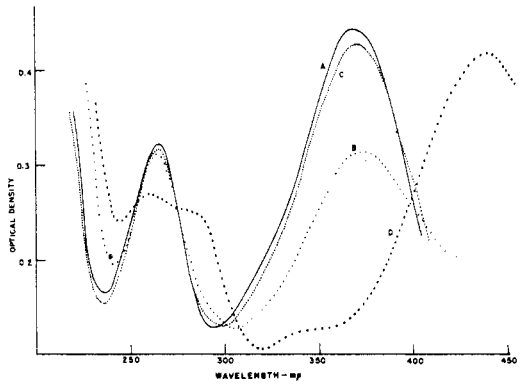


FIG. 1. 3,4-DIHYDROXYCHALCONE: A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with aluminum chloride and sodium acetate.

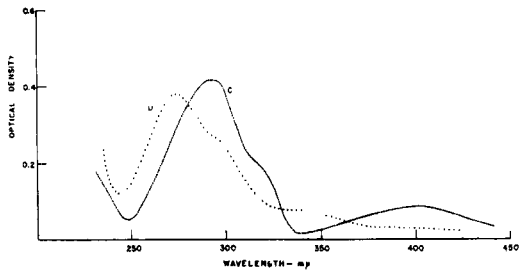


FIG. 3. 5-HYDROXYFLAVONE: C, in ethanol with aluminum chloride (132 moles per mole of flavone); D, in ethanol with aluminum chloride (132:1) and sodium acetate.

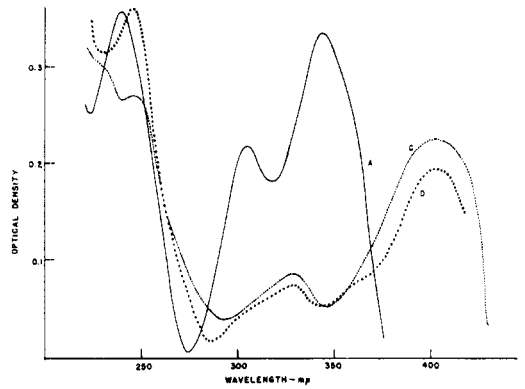


FIG. 5. 3-HYDROXYFLAVONE: A, in ethanol; C, with aluminum chloride (10:1) and trace of hydrochloric acid; D, with aluminum chloride and sodium acetate.

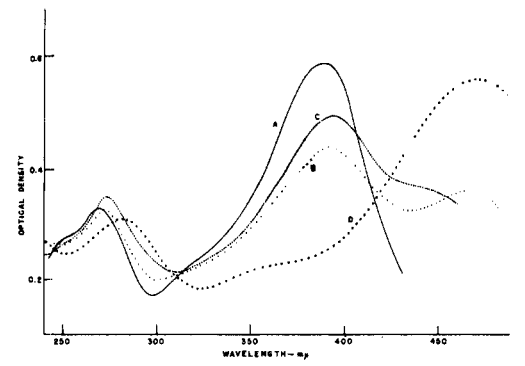


FIG. 7. 2',3,4-TRIHYDROXYCHALCONE: A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with aluminum chloride and sodium acetate.

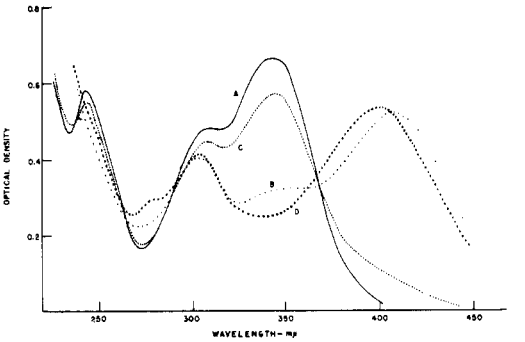


FIG. 2. 3',4'-DIHYDROXYFLAVONE: A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with aluminum chloride and sodium acetate.

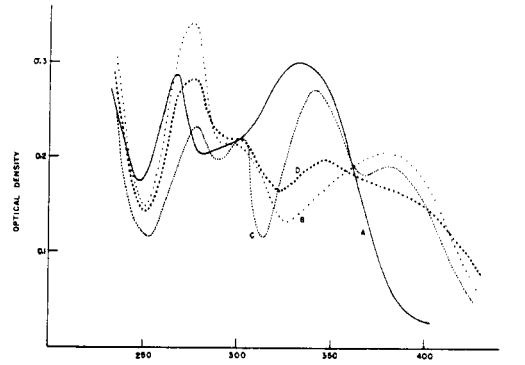


FIG. 4. APIGENIN (4',5,7-TRIHYDROXYFLAVONE): A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with aluminum chloride and sodium acetate.

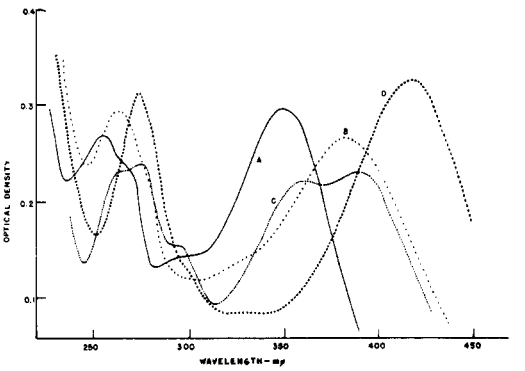


FIG. 6. LUTEOLIN (3',4',5,7-TETRAHYDROXYFLAVONE): A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with aluminum chloride and sodium acetate.

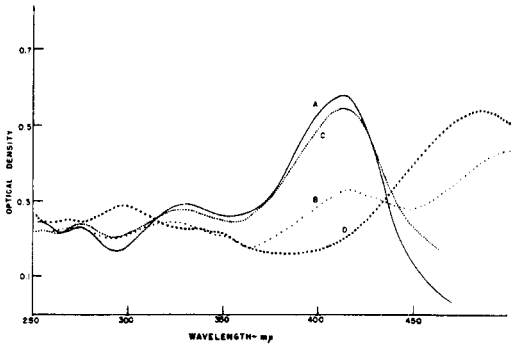


FIG. 8. LEPTOSIN (3',4'-DIHYDROXY-7-METHOXY-6-GLUCOSIDOXYBENZALCOUMARANONE): A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with aluminum chloride and sodium acetate.

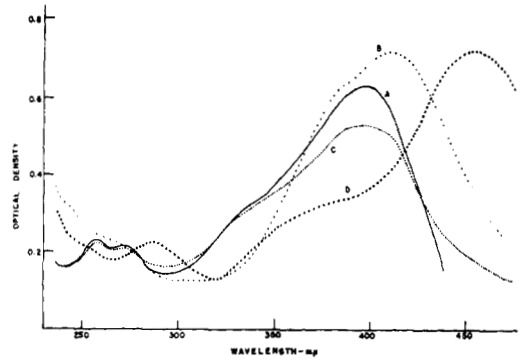
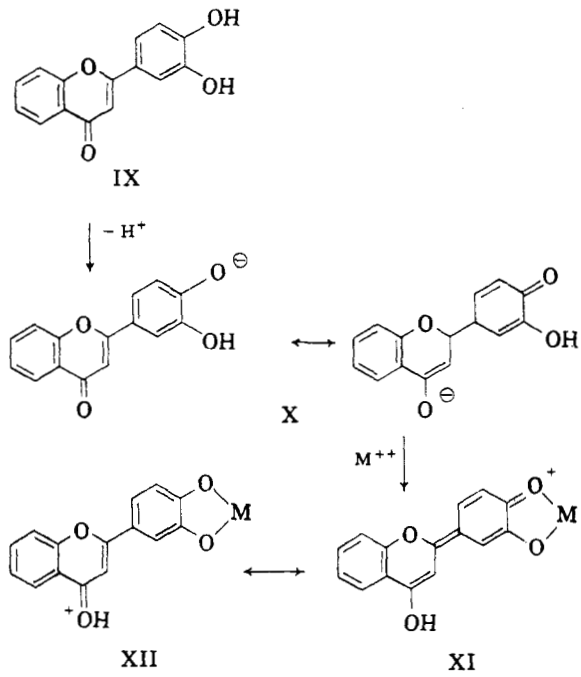
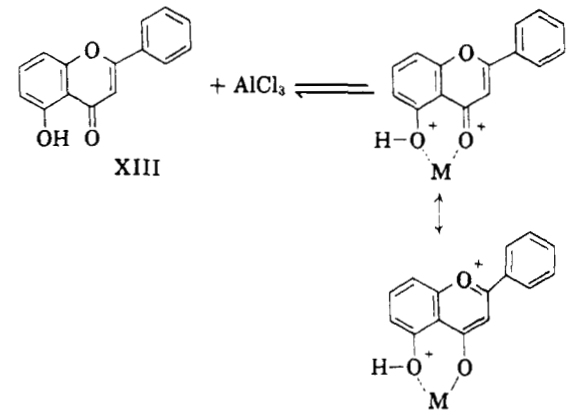


FIG. 9. SULFURETIN (3′,4′,6-TRIHYDROXYBENZALCOUMARANONE): A, in ethanol; B, with sodium acetate; C, with aluminum chloride (10:1); D, with sodium acetate and aluminum chloride.

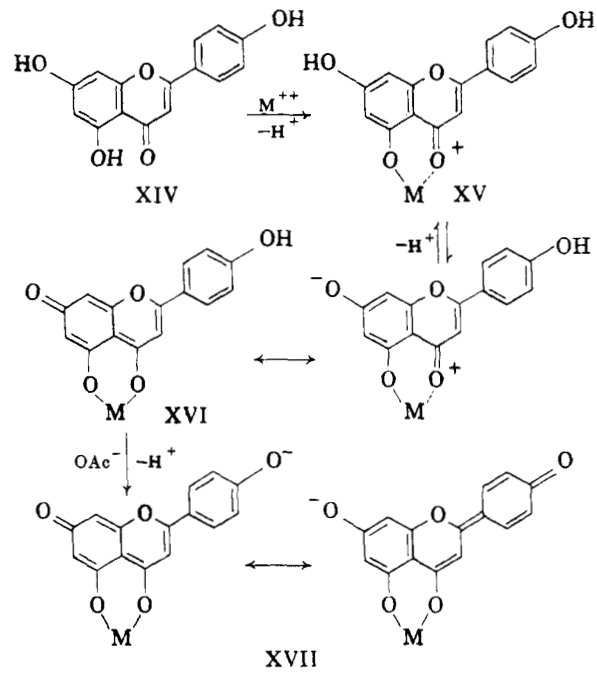


5-Hydroxyflavone (XIII). 5-Hydroxyflavone (XIII) appears to form a complex with aluminum chloride in alcohol, although the spectral effect is not large. Complex formation is incomplete at a ratio of 10 $AlCl_3$:1 flavone, but at a ratio of 132:1 a new high intensity peak at 293 mμ replaces that of the flavone (in alcohol at 272.5 mμ), and a low intensity maximum at 400 mμ appears.*** The effect of sodium acetate, in the presence of aluminum chloride, is to restore the spectrum to that observed in alcohol alone. Evidently the sodium acetate effectively competes with the weakly chelating flavone for the metal ion, but is not sufficiently basic to aid chelation by ionizing the weakly acidic hydroxyl group.

Apigenin (XIV). Apigenin (XIV), in contrast to 5-hydroxyflavone, forms a spectrally distinct complex with



alcoholic aluminum chloride, and the addition of sodium acetate to this does not restore the spectrum to what it is in alcohol, but alters it to a new form (XVII). It is probable in this case that the stability of the metal complex in alcohol alone is enhanced by ionization of the hydroxyl at 7 (or 4′) to give the species XVI:



The first ionization—that of the 5-hydroxyl group—is one that does not occur in the case of 5-hydroxyflavone itself. In the case of apigenin, the complex XV is stabilized by contributions from such forms as XVIII, a stabilization enhanced by the subsequent ionization of the second proton to give XVI:

2′-Hydroxychalcone. In excellent agreement with the behavior of 5-hydroxyflavone, 2′-hydroxychalcone shows some evidence of complex formation with aluminum chloride at a ratio of 10 $AlCl_3$:1 chalcone, and at a ratio of 100:1 a distinct new peak at 343 mμ (a bathochromic shift of 27 mμ) and a less intense peak at 425 mμ appear. The addition of sodium acetate to the aluminum chloride solution causes the reappearance of the spectrum of an alcoholic solution (λ_{max} 316 mμ).

The behavior of 2′,4-dihydroxy-3-methoxychalcone requires no discussion; the spectral data are given in Table III, where the above data are recapitulated:

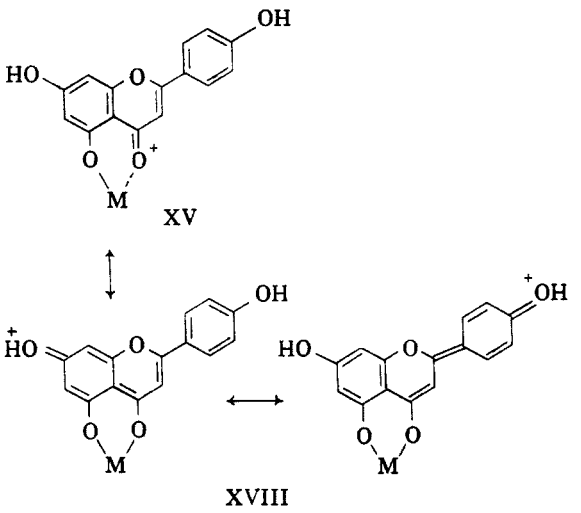
*** It is of particular interest to note that the spectrum in the $AlCl_3$-containing (132:1 molar ratio) solution is not altered by the addition of hydrochloric acid (1 drop of 12 *N* HCl per 3-ml. cuvette). This observation supports the suggestion that the species present in the presence of $AlCl_3$ is fully protonated.

TABLE III

RECAPITULATION OF SPECTRAL DATA

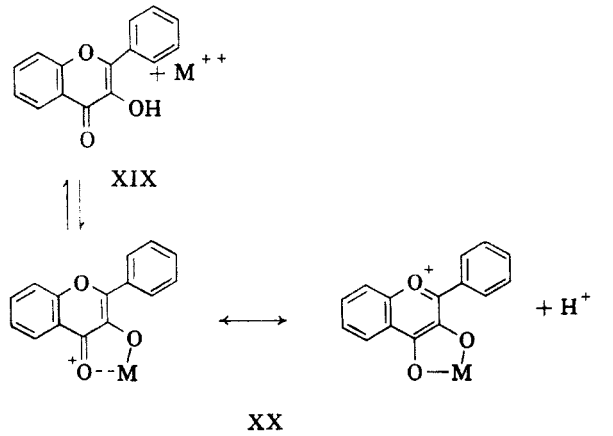
Compound	EtOH	EtOH-NaOAc	EtOH-$AlCl_3$ 10:1	EtOH-$AlCl_3$ 100:1	EtOH-$AlCl_3$-NaOAc
5-Hydroxyflavone	(337)*	(337)*			(335)*
	272.5	272	276.5	293(401)*	272.5
2′-Hydroxychalcone	316	315	(~340)**		
	221	223	320.5	343	316
2′,4-Dihydroxy-3-methoxychalcone	383	389	390		383
	266	270	270		267
Apigenin	336	379	382		(~380)**
	269	276.5	342.5		347
			303		(303)**
			278		278
Homoeriodictyol	288	328	289		328
		289			288

* Low intensity. ** Shoulder.



XV XVIII

Homoeriodictyol (Table III) shows no evidence of appreciable complex formation with alcoholic aluminum chloride. With sodium acetate, which probably ionizes the 7-hydroxyl group, a marked bathochromic shift occurs, but aluminum chloride and sodium acetate together produce a spectrum very much like that observed with sodium acetate alone. The absence of the 2,3-unsaturation, found in flavones (*e.g.*, apigenin), makes it impossible for the 4′-hydroxyl group to aid in stabilizing a 4-carbonyl-5-hydroxyl complex.

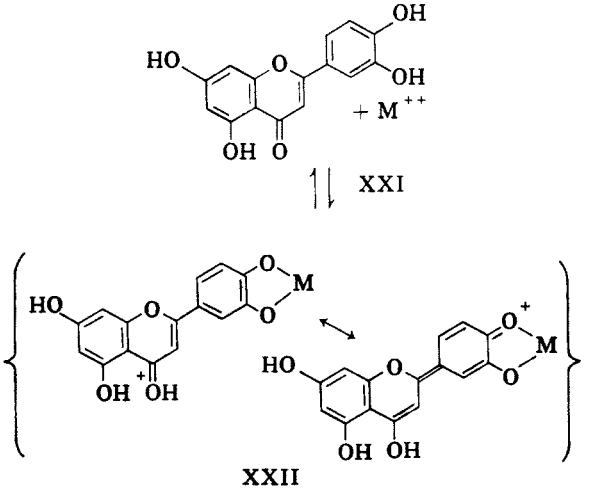


XIX XX

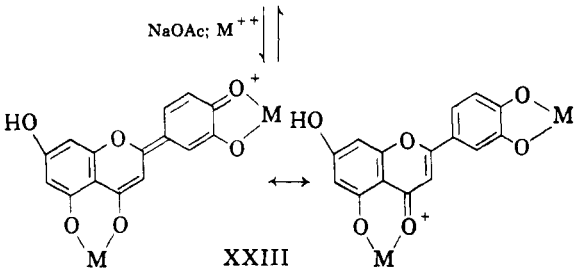
3-Hydroxyflavone (*Flavonol*) (XIX). 3-Hydroxyflavone (XIX) forms a stable complex with aluminum chloride, and this is unaltered even in the presence of 0.06 *N* hydrochloric acid. This is in harmony with the results of Hörhammer and his co-workers,[4] who have used the stability of such complexes towards acid as a means for distinguishing between 3-hydroxy and 3-glycosidoxyflavones. The result of complex formation is the formation of a flavylium structure (XX), which is greatly stabilized by its quasi-aromatic character.

This ready chelation occurs with the loss of a proton from a hydroxyl group not acidic enough to be appreciably ionized by sodium acetate alone: in the presence of sodium acetate the main maxima are unchanged and a very low intensity peak at ~410 mμ is observed. The absorption maxima in alcoholic aluminum chloride and aluminum chloride-sodium acetate are very nearly the same; this suggests that the same species is present in each case and that sodium acetate is not needed for the formation of the deprotonated species XX.

Luteolin (XXI). Luteolin (XXI) contains the weakly complexing 5-OH-4-carbonyl system and the weakly complexing 3′,4′-dihydroxy system. Like apigenin, in which the 5-OH-4-carbonyl system is reinforced by ionizable hydroxyl groups, luteolin forms a complex with aluminum chloride (XXII); but, unlike apigenin, the addition of sodium acetate to the aluminum chloride solution produces a further large bathochromic shift. Because the aluminum chloride-sodium acetate spectrum does not have an isosbestic point with the alcoholic and aluminum chloride spectra, it is suggested that a second metal ion is involved in the second shift (XXIII):



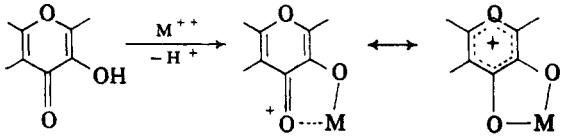
XXI XXII



XXIII

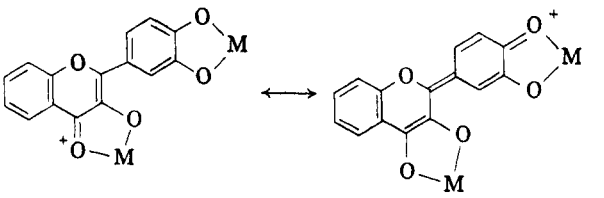
These formulations are consistent with the stoichiometric observations that have been made by Detty, *et al.*,[3] and by Hörhammer, *et al.*[4] Detty and his co-workers found, for example, that luteolin-7-glucoside formed a 2-flavone:1 metal complex with copper^{2+} at *pH* 6.5 and a 1:1 complex at *pH* 10.0. While the above formulation (XXIII) for the chelate with aluminum in the presence of sodium acetate seems to be a 1 flavone:2 metal complex, the possible (and probable) involvement of more than one flavone molecule (as shown by the formation of the 2:1 complexes) can account for the stoichiometry observed in comparable cases. A polymeric complex of flavone and metal involving two chelating groups per flavone molecule would produce a 1:1 ratio (—[M-F-M]-F-M-F-M-F-M-F-M-F-M-F—).

Other polyhydroxyflavones. Other flavones, containing both the strongly chelating 3-OH-4-carbonyl groupings and 3′,4′-dihydroxy and/or 5-OH-4-carbonyl groupings show complex formation with alcoholic aluminum chloride. In the case of the simplest of these—3,3′,4′-trihydroxyflavone—the main absorption maximum (369.5 mμ in alcohol) is shifted to 432 mμ in the presence of aluminum chloride and to 465 mμ in the presence of aluminum chloride-sodium acetate. Sodium acetate alone, however, does not cause an appreciable shift. It is significant that the shift of 62.5 mμ with alcoholic aluminum chloride is almost the same as that (61.5 mμ) observed with 3-hydroxyflavone, and thus may be attributed to the change (XXIV):



XXIV

The second bathochromic shift, in the presence of both aluminum chloride and sodium acetate, must be due to the involvement of the 3′,4′-dihydroxyl grouping, probably giving rise to the complex XXV:



XXV

In Table IV are listed the spectral data for the flavones discussed above.

2′,3,4-Trihydroxychalcone (XXVI). 2′,3,4-Trihydroxychalcone (XXVI) does not appear, from spectral evidence, to form an aluminum complex with alcoholic aluminum chloride alone. With aluminum chloride-sodium acetate, however, a complex is formed having an absorption maximum at 470 mμ, compared to the 388 mμ maximum of the chalcone itself. Again, sodium acetate alone causes the appearance of a maximum at 471 mμ, along with (the original?) maximum at 391 mμ. This is an indication that the anionic species XXVII

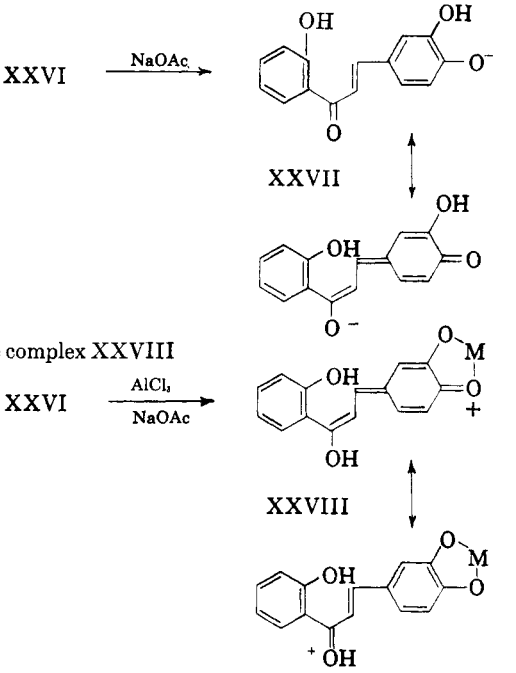


TABLE IV

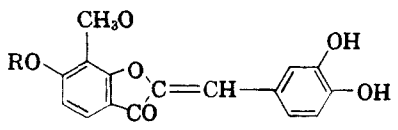
SPECTRAL DATA FOR FLAVONES

Compound	EtOH	EtOH-NaOAc	EtOH-$AlCl_3$		EtOH-$AlCl_3$-NaOAc	Δλ***
3-Hydroxyflavone	343.5	345.5	405	403*	404	61.5
	305	305	326	329	330	
	239	239	248	246	246	
Luteolin	350	382	389		418	39
	255	264.5	361		329	
			275		274	
3,3′,4′-Trihydroxyflavone	369.5	372.5	432		465	62.5
	250	249	237		277.5	
			262.5			
			232			
Kampferol	367.5	383	426		429	58.5
	326**	305	349		355	
	266	274.5	302**		273	
			270			
Quercetin	373	384	431		445	58
	301	329	309		336	
	257	259.5	268		272	

* 0.06 *N* in HCl. ** Inflection. *** Shift in long wavelength maximum with $AlCl_3$ alone.

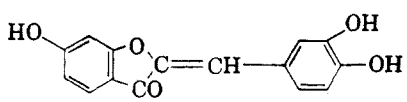
have the structures shown, in which nearly identical chromophoric systems—differing only in that one carries a positive, the other a negative, charge—give rise to nearly the same absorption maximum in each case.

Leptosin (XXIX) *and Sulfuretin* (XXX). Except for the non-conjugated 8-methoxyl group in XXIX the chromophoric systems of the two aurones are the same, and their absorption spectra are similar. In the presence of sodium acetate it appears that sulfuretin undergoes ionization at 6-OH and leptosin ionizes (incompletely) at 4′-OH.



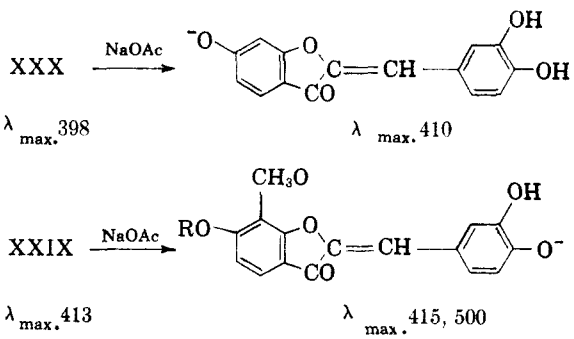
XXIX

(R = glucosyl)



XXX

Neither sulfuretin nor leptosin forms a complex with aluminum chloride in alcohol, but both complex with aluminum when sodium acetate is present. In this respect, the aurones resemble 3′,4′-dihydroxyflavone and 3,4-dihydroxychalcone, and the reactions of complex formation may be written as are V–VIII, above. The similarity in the behavior of leptosin and sulfuretin in aluminum complex formation in the presence of sodium acetate is an indication that the 6-hydroxyl group of sulfuretin is not directly concerned in the formation of the complex.



Absorption spectra. Absorption spectra of compounds V, IX, XIII, XIV, XIX, XXI, XXV, XXIX, and XXX are shown in Figs. 1–9. In each of these, except XIII, the spectra (a) in ethanol alone and in the presence of (b) sodium acetate, (c) aluminum chloride, and (d) sodium acetate plus aluminum chloride, are shown. For compound XIII (Fig. 3), the spectra in the presence of aluminum chloride (132:1) and aluminum chloride-sodium acetate only are shown.

Los Angeles 24, California