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ALUMINUM COMPLEXES OF PHENOLIC FLAVONES. SPECTRAL AND STRUCTURAL CORRELATIONS

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Abstract—A detailed re-investigation of the diagnostic specificity of aluminum chloride as a spectral reagent for locating hydroxyl groups on a flavone nucleus has been undertaken. Examination of the aluminum chloride shifts of one hundred and forty flavones indicates that in dilute solutions in ethanol the reagent complexes specifically with 3-hydroxy- and 5-hydroxy-4-carbonyl groupings. With few exceptions shifts of characteristic magnitudes thereby result. In methanol solutions, on the other hand, complex formation also occurs with 3′-4′-dihydroxy groups and flavones with this group consistently give larger bathochromic shifts in this solvent.

THE U.V. spectral shifts induced by chelatogenic and basic reagents facilitate the structural identification of members of most classes of natural flavonoid compounds. At least three groups of workers were involved in early attempts to correlate the magnitudes of various spectral shifts with the presence of specific structural features and these correlations were reviewed and systematized in a description of flavonoid spectra in 1962.[1] During the last decade phytochemical surveys and the isolation of an increasing number of new compounds has resulted in greatly expanded spectral data on a wider variety of hydroxylated flavonoid compounds. A cursory examination of the literature suggests that the claims originally made for the diagnostic specificity of various reagents are largely substantiated. However, in a number of cases expected shifts have not been obtained, e.g. on addition of basic reagents to highly hydroxylated flavones with a 6-methoxy-7-hydroxy grouping,[2–4] or the reported magnitudes of the shifts differed appreciably from those which would have been predicted on the basis of structure. Thus, in view of the extensive reliance in structural analysis on those spectral methods, it seems appropriate at this time to critically re-evaluate the specificity of each of the reagents on the basis of the new data to determine whether earlier ideas need to be modified, and particularly to draw attention to those types of structures which appear to give genuinely exceptional spectral shifts under the standard conditions.

This communication is concerned with a detailed re-appraisal of the reliability of aluminum chloride as a diagnostic reagent for hydroxyl groups located at positions 3 and 5 on a flavone nucleus. In the 1962 review it was proposed that: (1) aluminum chloride in *ethanol* solutions forms complexes only with those flavones which contain a free hydroxyl group in the 3 and/or 5 positions; (2) as a result of complex formation the long wavelength band (Band I) of flavones with a free 3-hydroxyl undergoes a characteristic bathochromic shift of

[1] L. JURD, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 107, Macmillan, New York (1962).
[2] H. H. LEE and C. H. TAN, *J. Chem. Soc.* 2743 (1965).
[3] L. FARKAS, M. NOGRADI, V. SUDARSANAM and W. HERZ, *J. Org. Chem.* **21**, 3228 (1966).
[4] L. FARKAS, M. NOGRADI, V. SUDARSANAM and W. HERZ, *Tetrahedron* **23**, 3557 (1967).

TABLE 1. ETHANOLIC ALUMINUM CHLORIDE SPECTRA OF 3-HYDROXYFLAVONES

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
1. 3-Hydroxy-	342	402	60	23
2. 3,4′,7-Trihydroxy-	357	418	61	
3. 3,3′,4′,7-Tetrahydroxy-	364	425	61	
4. 3,5,7,4′-Tetrahydroxy-	367	427	60	
(kaempferol)	368	428	60	24
5. 7-*O*-Methylkaempferol	370	429	59	25
6. 6-*C*-Glycosyl-7-*O*-methyl-	371	431	60	25
kaempferol (keyakinin)				
7. Kaempferol 7-glucuronide	368	425	57	26
8. 3,5,7,3′,4′-Pentahydroxy-	370	427	57	27
(quercetin)	371	431	60	28
	373	432	59	29
	373	431	58	
	370	440	70	14
9. 7-*O*-Methylquercetin	373	432	59	
10. 7-*O*-Allylquercetin	372	433	61	28
11. 6-*C*-Glycosyl-7-*O*-methylquercetin	376	439	63	25
12. 5-*O*-Methylquercetin	365	427	62	
13. 4′-*O*-Methylquercetin	369	429	60	30
	371	431	60	
14. Quercetin 4′-glucoside	367	427	55	31
	365	423	57	27
15. Quercetin 3′-glucoside	367	429	62	1
16. 3′-*O*-Methylquercetin	372	432	60	32
	372	431	59	
	368	433	65	14
17. 4′,7-Di-*O*-methylquercetin	369	427	58	30
18. 4′,7-Di-*O*-allylquercetin	371	429	58	28
	370	427	57	30
19. Quercetin 4′,7-diglucoside	358	415	57	27
20. 3′,4′-Di-*O*-methylquercetin	367	428	61	
21. 7,3′,4′-Tri-*O*-methylquercetin	367	427	60	30
22. 7-*O*-Methyl-3′,4′-di-*O*-benzylquercetin	368	427	59	30
23. 5,7,4′-Tri-*O*-methylquercetin	361	419	58	
24. 5,7,3′,4′-Tetra-*O*-methylquercetin			61	29
25. 3,5,7,3′,4′,5′-Hexahydroxy-(myricetin)	378	435	57	33
	377	437	60	29
	378	438	60	
Gossypetin and related flavonols				
26. 3,5,7-Trihydroxy-8-methoxy-	375	430	55	34
27. 3,5,7-Trihydroxy-4′,8-dimethoxy-	375	430	55	35
28. 3,5,7,8,3′,4′-Hexahydroxy-(gossypetin)	383	442	59	36
	386	446	60	33
29. Gossypetin 7-glucoside	388	448	60	33
30. Gossypetin 7-methyl ether	381	440	59	36
31. 3,5,7,4′-Tetrahydroxy-3′,8-dimethoxy	378	442	64	37
(limocitrin)	382	442	60	
32. 5-*O*-Methyl-limocitrin	370	445	75	37
33. 3,7-Dihydroxy-5,7,3′-trimethoxy-4′-	368	432	64	37
benzyloxy-				
Quercetagetin and related flavonols				
34. 3,5,6,7,3′,4′-Hexahydroxy-	364	425	61	36
(quercetagetin)				

TABLE 1—*continued*

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
35. Quercetagetin 7-glucoside	362	388	26	38
36. 3,5,7,3′,4′,Pentahydroxy-6-methoxy-(patuletin)	373	443	70	36
37. 3,5,7,4′-Tetrahydroxy-6,3′-dimethoxy-(spinacetin)	368	435	67	39
38. 3,5,7,4′-Tetrahydroxy-6,8,3′-trimethoxy-(limocitrol)	378 380	441 442	63 62	14
39. 3,5,7,3′-Tetrahydroxy-6,8,4′-trimethoxy-(isolimocitrol)	375	441	66	15

TABLE 2. ETHANOLIC ALUMINUM CHLORIDE SPECTRA OF 5-HYDROXYFLAVONES

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
Apigenin derivatives				
40. 4′,5,7-Trihydroxy-(apigenin)	334	382	48	14
	337	380	43	40
	336	381	45	
41. Apigenin 7-glucoside	341	386	45	41
42. Apigenin 7-rhamnoglucoside	337	383	46	
43. 7-*O*-Methylapigenin	336	378	42	
44. 4′-*O*-Methylapigenin	328	~380	52	
45. 8-*C*-Glucosylapigenin (vitexin)	334	380	46	7
	337	382	45	40
	334	382	48	42
46. Apigenin 4′,7-diglucuronide	320	332	12	43
47. 4′,7-Di-*O*-methylvitexin	~328	383	55	
48. 6,8-Dimethyl-7-*O*-methylapigenin	332	≃400, 351	68	44
Luteolin derivatives				
49. 5,7,3′,4′-Tetrahydroxy-(luteolin)	350	390	40	45
	351	390	39	
	351	390	39	16
	352	392	40	46
	350	389	39	14
50. 4′-*O*-Methyl-luteolin	345	385	40	45
	343	385	42	
51. Luteolin 4′-glucoside	337	382	45	46
52. 3′-*O*-Methyl-luteolin	349	385	36	1
	346	385	39	14
	350	385	35	45
53. 8-*C*-Glucosyl-luteolin (orientin)	350	380	30	7
	355	390	35	8
54. Homo orientin (lutonaretin)	357	390	33	8
55. Epi-orientin	352	394	42	47
56. 3′-*O*-Methyl-lutonaretin	349	384	35	48
57. 3′-*O*-Methyl-lutexin	349	385	36	48
58. 3′-*O*-Methyl-lutonarin	349	384	35	48
59. Luteolin 7-glucoside	353	398	45	46

TABLE 2—*continued*

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
	353	393	40	49
	352	378	26	50
	350	390	40	38
	353	405	42	41
60. 3′,7-Di-*O*-methyl-luteolin	348	381	33	51
61. Luteolin 7-glucuronide	353	373	20	43
62. Luteolin 7-glucosyl glucuronide	353	387	34	43
63. 3′-*O*-Methyl-luteolin 7-glucuronide	351	~380	29	17
Tricetin derivatives				
64. 5,7,3′,4′,5′-Pentahydroxy-(tricetin)	356	398	42	17
65. Tricin (3′,5′-di-*O*-methyl-tricetin)	351	356	5	16
	355	379	24	17
	350	380	30	
66. Tricin 5-glucoside	351	400	49	17
67. 5′-*O*-Methyltricetin	355	395	40	17
Kaempferol derivatives				
68. Kaempferol 3-glycoside	351	388	37	49
69. Kaempferol 3-rhamnoglucoside	348	390	42	49
	353	400	47	44
70. Kaempferol 3-rhamnoside	348	390	42	44
71. Kaempferol 3-diglucoside	350	388	38	49
	349	395	46	52
72. Kaempferol 3-triglucoside	350	385	35	53
	350	393	43	52
73. 3-*O*-Methylkaempferol	352	399	47	11
74. Robinin	353	398	45	
75. Kaempferol 7-rhamnoside 3-diglucoside	350	388	38	49
76. Kaempferol 7-glucoside 3-rhamnoglucoside	349	398	49	54
77. Kaempferol 3,7-diglucoside	351	396	45	43
78. 3-*O*-Methylkaempferol 7-glucoside (manicatin)	352	401	49	11
79. Kaempferol 3-glucuronide	352	380	28	26
80. Kaempferol 3-rutinoside 7-glucuronide	350	384	34	26
Quercetin derivatives				
81. Quercetin 3-glucoside	363	402	39	27
	364	410	46	44
82. Quercetin 3-galactoside	362	403	41	29
83. Quercetin 3-rutinoside	362	408	46	
84. Quercetin 3-triglucoside	361	410	49	52
	355	394	39	53
85. Quercetin 3-diglucoside	360	388	28	52
	355	398	43	49
86. Quercetin 3-arabino-pyranoside	357	399	42	29
	360	389	29	55
87. Quercetin 3-rhamnoside	352	398	46	29
	353	401	48	44
	352	401	49	
88. 3-*O*-Methylquercetin	360	403	43	
89. Xanthorhamnin	364	405	41	
90. Quercetin 3,7-diglucoside	363	402	39	31
91. 3,7-Di-*O*-methylquercetin	362	406	44	

TABLE 2—*continued*

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
92. Quercetin 3-glucuronide	364	390	26	26
93. Quercetin 3-rutinoside 7-glucuronide	360	400	40	26
94. Quercetin 3,4′-diglucoside	354	388	34	27
	350	397	47	26
95. 3,7,4′-Tri-*O*-methylquercetin	354	397	43	30
	347	392	45	
96. 3,7,3′,4′-Tetra-*O*-methyl quercetin	351	399	48	30
97. 3,7,3′,4′-Tetra-*O*-allyl-6-*C*-allyl quercetin	361	411	50	28
98. 7-*O*-Methylquercetin 3,3′,4′-triacetate	340	392	52	56
99. Quercetin 3,7,3′,4′-tetra-acetate	335	385	50	56
Myricetin derivatives				
100. 3,7,3′,4′,5′-Penta-*O*-methyl-myricetin	345	352	7	18
(combretol)	345	395	50	33
101. 3,3′,4′,5′-Tetra-*O*-methylmyricetin	351	402	51	
102. Myricetin 3-rhamnoglucoside	365	403	38	49
103. Myricetin 3-glucoside	362	398	36	52
104. Myricetin 3-arabinoside	360	402	42	29
105. Myricetin 3-digalactoside	368	409	41	29
3,5,7,8-*Tetrahydroxyflavone derivatives*				
106. 5,7-Dihydroxy-3,8,4′-trimethoxy-	358	410	52	57
107. 5,7,4′-Trihydroxy-8,3′-dimethoxy-3-	361	418	57	15
glucosidoxy- (limocitrin 3-glucoside)	360	415	48	
108. 5-Hydroxy-3,7,8,3′,4′-pentamethoxy-	360	420	60	33
109. Gossypetin 3-galactoside	352	375	23	36
5,6,7-*Trihydroxyflavone derivatives*				
110. 5,7,4′-Trihydroxy-6-methoxy- (hispidulin)	338	363	25	58
111. 7-*O*-Methylhispidulin (cirsimaritin)	336	353	17	59
112. 5,7,4′-Trihydroxy-6,3′-dimethoxy-	344	358	14	59
113. 5,7,3′-Trihydroxy-6,4′-dimethoxy	342	370	28	60
114. 5,7,3′,4′-Tetrahydroxy-3,6-dimethoxy-	357	367	10	61
115. 5,4′-Dihydroxy-3,6,7-trimethoxy-	342	~392	50	
(penduletin)		362	20	
116. Penduletin 4′-glucoside	331	349	18	
117. 5,7,3′-Trihydroxy-6,8,4′-trimethoxy-	345	379	34	4
(acerosin)				
118. 5,7,4′-trihydroxy-6,8,3′-	345	375	30	4
trimethoxy-(sudachitin)				
119. 5,7-Dihydroxy-6,8-dimethoxy-	323	340	17	2
120. 5-Hydroxy-6,7,8-trimethoxy-	316	334	18	2
121. 5,7-Dihydroxy-6,8,4′-trimethoxy-	332	361	29	3
(nevadensin)				
122. 5-Hydroxy-6,7,8-trimethoxy-3′,4′-	343	359	16	2
methylenedioxy-				
123. 5,4′-Dihydroxy-6,7,8-trimethoxy-	333	355	23	
(xanthomicrol)	330	352	22	62
124. Xanthomicrol 4′-methyl ether	328	350	22	
125. 5-Hydroxy-6,7,8,3′,4′-pentamethoxy-	341	362	21	62
126. 5,4′-Dihydroxy-6,7,8,3′-tetramethoxy-	341	360	19	62
127. 5,7,3′-Trihydroxy-6,8,4′-trimethoxy-3-	352	365	13	15
glucosidoxy-				
128. 5,7,4′-Trihydroxy-6,8,3′-trimethoxy-3-	354	425, *373*	19	5
glucosidoxy-				
129. 6-Hydroxyluteolin (?)	349	375	26	45

TABLE 2—*continued*

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
Miscellaneous flavones				
130. 5-Hydroxy-7-methoxy-2-glucosidoxy- (echiodin)	325	365	40	63
131. 5,2′-Dihydroxy-7-methoxy- (echiodinin)	340	380–385	40–45	64
	340	370	30	66
132. 5,3′-Dihydroxy-7,8,2′-trimethoxy- (wightin)	335	400	65	65
	338	403	65	66
133. 5-Hydroxy-3,6,3′,4′-tetramethoxy-8-methyl-	345	355	10	19
134. 5-Hydroxy-7-methoxy-(tectochrysin)	338	380	42	
	306	321	15	
	268	282	14	

TABLE 3. ETHANOLIC ALUMINUM CHLORIDE SPECTRA OF FLAVONES WITHOUT 3 AND 5 HYDROXYL GROUPS

Flavone	λ_{max} EtOH	λ_{max} $AlCl_3$	Shift	Reference
135. 3′,4′-Dihydroxy-	345	346	1	
136. 7,3′,4′-Trihydroxy-	342	343	1	
137. Luteolin 5-glucoside	345	345	0	46
138. 5,7-Di-*O*-methyl-8-*C*-glycosyl-luteolin (Parkinsonin B)	350	350	0	47
139. 5-*O*-Methyl-8-*C*-glucosyl-luteolin	352	360	8	47
140. 3,5-Di-*O*-methylquercetin	345	347	2	
141. 3,5,3′-Tri-*O*-methylquercetin	343	343	0	
142. 3,5,7-Tri-*O*-methylquercetin	~346	346	0	
143. 4′,7-Dihydroxyflavone	330	332	2	40
144. Bayin	331	332	1	40

approximately 60 nm in ethanolic aluminum chloride (Band I in the spectrum of the aluminum-flavonol complex has a single peak of high intensity (Band Ia) and usually an inflection of low intensity (Band Ib) at a lower wavelength); (3) with 5-hydroxyflavones, in which a 3-hydroxyl is absent or protected by glycosidation or alkylation, the bathochromic shift is smaller and the long wavelength band exhibits two peaks or inflections (the shift of the flavone Band I to the complex Band Ia is 20–45 nm); and (4) the magnitude of the above shifts is independent of the presence or absence of an *ortho* 3′,4′-dihydroxyl grouping, which does *not* complex with aluminum chloride in ethanol (1–2 drops of aqueous or alcoholic aluminum chloride added to a solution of the flavone in absolute ethanol in the cell).

Re-investigation of these conclusions was prompted particularly by the recent work of Blundstone[5] on the identification of quercetin glycosides in *Rheum rhaponticum*. He reported that quercetin 3-glycosides gave shifts whose magnitudes approached and, in one case, exceeded the shift claimed to be characteristic of 3-hydroxyflavones, e.g. quercetin 3-rutino-

[5] H. A. BLUNDSTONE, *Phytochem.* **6**, 1449 (1967).

side gave a shift of 78 nm. Thus, he was led to conclude that the reliability of the magnitude of shift obtained with aluminum chloride as a method of locating free 3-hydroxyl must be regarded with suspicion. As previously indicated [1] and in the numerous studies cited in Tables 1–3, aluminum chloride spectra have been correlated with structure on the basis of the apparent complexing ability of dilute solutions of the reagent with phenolic flavones in ethanol. Blundstone's measurements, however, were made in *methanol* and his comments are based on the erroneous assumption that the chelatogenic properties of aluminum chloride are necessarily identical in these different solvents. Other authors have also occasionally used methanolic aluminum chloride and exceptionally large shifts have *sometimes* been obtained, e.g. Hänsel *et al.*[6] reported a 70 nm shift for orientin, a 5-hydroxyflavone. In ethanolic aluminum chloride, however, orientin gives [7,8] the expected shift of only 30–35 nm.

Since methanolic aluminum chloride shifts have not been correlated with structure, the wide variation in the magnitude of these shifts has not hitherto been explained. Spectral comparisons now clearly indicate that in methanol, but not in ethanol, aluminum chloride complexes with 3′,4′-dihydroxyl groups as well as with 3- or 5-hydroxy-4-carbonyl groupings of flavones. As a result of this additional complex formation, larger (and consistent) shifts are obtained in methanol with those flavones which contain a free 3′,4′-dihydroxyl grouping. In the absence of this group the aluminum chloride spectra do not differ significantly in the two solvents. Measurement of aluminum chloride spectra of flavones in both ethanol and methanol solutions may, in fact, prove to be a useful adjunct to the boric acid–sodium acetate method [9] of detecting dihydroxyl groups.

RESULTS AND DISCUSSION

The spectra of five quercetin 3-glycosides and 3-methyl ethers were measured in ethanol and in methanol solutions. In accord with the previous claims [1] these undergo a median bathochromic shift of 44 nm (range 41–49 nm) in ethanol on addition of aluminum chloride, e.g. rutin (Fig. 1). In methanol solutions, however, they undergo a large and consistent bathochromic shift of 70–77 nm and the intensity of the band is considerably increased. In contrast to the behavior of these quercetin compounds, 3-alkylated kaempferol derivatives show identical aluminum chloride shifts (45–48 nm) in ethanol and in methanol (Fig. 2).

	λ_{max} EtOH	$AlCl_4$ shift	λ_{max} MeOH	$AlCl_3$ shift
Rutin	362	46	360	74
Quercitrin	352	46	350	77
Xanthorhamnin	364	41	359	70
3-*O*-Methylquercetin	360	43	358	74
3-7-Di-*O*-methylquercetin	364	44	358	76
3,4′-Di-*O*-methylkaempferol	352	48	353	48
Robinin	353	45	351	46

[6] R. HÄNSEL, C. H. LEUCKERT, H. RIMPLER and K. D. SCHAAF, *Phytochem.* **4**, 19 (1965).
[7] V. K. BHATIA, S. R. GUPTA and T. R. SESHADRI, *Phytochem.* **5**, 177 (1966).
[8] B. H. KOEPPEN, C. J. B. SMIT and D. G. ROUX, *Biochem. J.* **83**, 507 (1962).
[9] L. JURD, *Arch. Biochem.* **63**, 376 (1956).

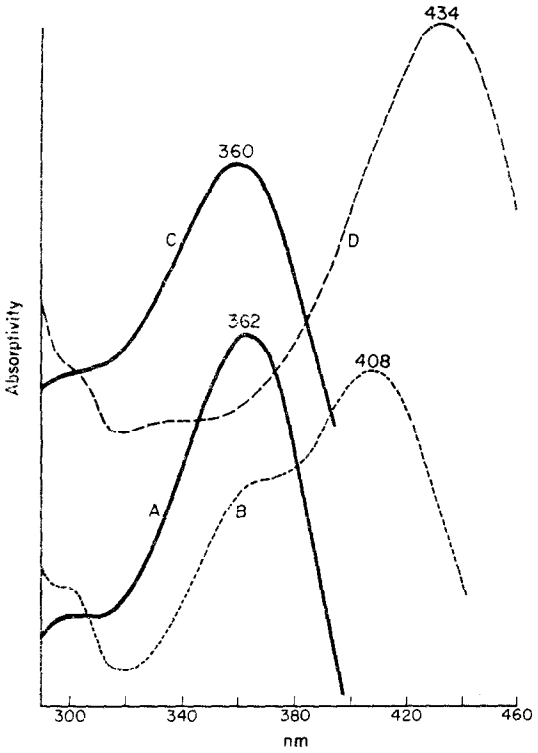


FIG. 1. SPECTRA OF RUTIN (83) ON (A) ETHANOL, (B) ETHANOLIC $AlCl_3$, (C) METHANOL, (D) METHANOLIC $AlCl_3$.

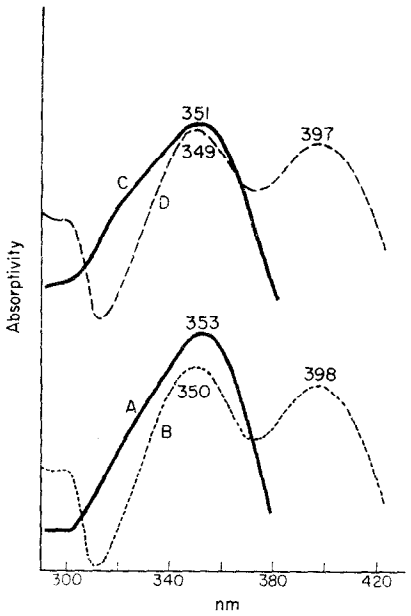


FIG. 2. SPECTRA OF ROBININ (74) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$. (C) METHANOL, (D) METHANOLIC $AlCl_3$.

These observations immediately suggest that the 3′,4′-dihydroxyl group in the quercetin derivatives accounts for the marked difference in their ethanolic and methanolic aluminum chloride spectra. Further comparisons support this conclusion:

	λ_{max} EtOH	$AlCl_3$ shift	λ_{max} MeOH	$AlCl_3$ shift
Luteolin (3′,4′,5,7-tetrahydroxyflavone)	351	39	350	71
4′-*O*-Methyl-luteolin	343	42	342	43
Apigenin (4′,5,7-trihydroxyflavone)	336	45	336	45
Xanthomicrol	333	22	332	23
4′-*O*-Methyl-xanthomicrol	328	22	328	23
Penduletin	342	20	341	22

Thus, apigenin and luteolin 4′-methyl ether, which lack a 3′,4′-dihydroxyl group, give identical aluminum chloride shifts in ethanol and methanol (42–46 nm). Luteolin, however, with a 3′,4′-dihydroxyl group, undergoes a shift of 39 nm with decreased intensity in ethanol and a shift of 71 nm with increased intensity in methanol. Like apigenin, more highly oxygenated 5-hydroxyflavones without a 3′,4′-dihydroxyl group, viz. xanthomicrol (4′,5′-dihydroxy-6,7,8-trimethoxyflavone), 4′-*O*-methyl-xanthomicrol, and penduletin (4′,5-dihydroxy-3,6,7-trimethoxyflavone) did not show significant differences in their ethanolic and methanolic aluminum chloride spectra.

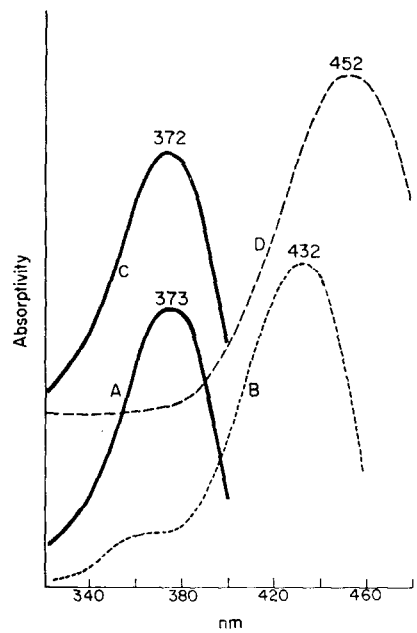


FIG. 3. SPECTRA OF RHAMNETIN (9) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$, (C) METHANOL, (D) METHANOLIC $AlCl_3$.

The spectra of ten 3-hydroxyflavones were also measured in these solvents. With ethanolic aluminum chloride all of the flavonols gave a consistent bathochromic shift of about 60 nm (range 58–62 nm). The 3-hydroxyflavones without a 3′,4′-dihydroxyl group gave the same shift (60 nm) in methanol. With those flavones containing a 3′,4′-dihydroxyl group, however, the shift in methanol increased markedly to 75–80 nm (Fig. 3).

	λ_{max} EtOH	$AlCl_3$ shift	λ_{max} MeOH	$AlCl_3$ shift
3,4′,7-Trihydroxyflavone	357	61	355	60
Kaempferol	368	60	364	59
3′-*O*-Methylquercetin	372	59	373	58
4′-*O*-Methylquercetin	371	60	371	59
4′,7-Di-*O*-methylquercetin	370	58	368	58
3,3′,4′,7-Tetrahydroxyflavone	364	61	361	79
Quercetin	373	58	373	79
5-*O*-Methylquercetin	365	62	365	80
7-*O*-Methylquercetin	373	59	372	80
Myricetin[10]	378	60	377	75

Addition of aluminum chloride to ethanolic solutions of 3′,4′-dihydroxyflavones, lacking hydroxyl groups in the 3- and 5-positions, did not significantly change their spectra. In methanol, however, these flavones underwent bathochromic shifts of 20–40 nm.

	λ_{max} EtOH	$AlCl_3$ shift	λ_{max} MeOH	$AlCl_3$ shift
3′,4′-Dihydroxyflavone	345	1	342	39
7,3′,4′-Trihydroxyflavone	342	1	341	37
3,5-Di-*O*-methylquercetin	345	2	345	21

These comparisons clearly indicate that larger aluminum chloride shifts may be expected in methanol (relative to ethanol) only with those flavones which contain *ortho*-dihydroxyl groups.

Structural Specificity of Ethanolic Aluminum Chloride Spectral Shifts

Ethanolic aluminum chloride spectra of an extensive variety of flavones are collected in Tables 1–3. This compilation is derived primarily from the recent data of many different authors and serves as a useful basis for evaluation of the specificity of the reagent for detecting 3- and 5-hydroxyl groups in flavones with a variety of hydroxylation patterns. This specificity of aluminum chloride has been questioned,[11] it being assumed[12] that complex formation with 3′,4′-dihydroxyl groups is responsible for the 35–45 nm shifts observed with quercetin and luteolin glycosides.

[10] J. KAGAN (*Phytochem.* **6**, 317 (1967)) reported myricetin undergoes a shift from 378 to 432 nm, i.e. 54 nm, in methanolic aluminum chloride.

[11] J. B. HARBORNE, in *Methods in Polyphenol Chemistry* (edited by J. B. PRIDHAM), p. 13, Macmillan, New York (1964).

[12] J. B. HARBORNE, *Chem. & Ind.* 1142 (1954).

3-Hydroxyflavones

Ethanolic aluminum chloride shifts reported for 3-hydroxyflavones are collected in Table 1. Twenty-five resokaempferol, fisetin, kaempferol, quercetin and myricetin derivatives give shifts of 55–63 nm, the average of the thirty-seven shifts reported being 59·7 nm.[13] The shifts obtained by Horowitz and Gentili[14] for quercetin (8) and isorhamnetin,[16] 70 and 65 nm respectively, are exceptionally large compared with the 57–60 nm shifts obtained on the same compounds by five other authors. As mentioned below, Horowitz and Gentili also obtained similarly large shifts with limocitrin and limocitrol derivatives. In their measurements these authors used ethanol saturated with aluminum chloride and the larger shifts apparently arise from the greater concentration of aluminum in these solutions.

The seven gossypetin derivates in Table 1 give shifts of approximately 60 nm (range 55–64 nm), with the exception of 5-*O*-methyllimocitrin (32), reported[15] Δ_λ 75 nm. Measured in this laboratory, limocitrin itself (3,5,7,4′-tetrahydroxy-3′,8-dimethoxyflavone) gave a shift of 60 nm.

Quercetagetin (34), quercetagetin 7-glucoside (35), and limocitrol (38) give shifts of 59–64 nm. The 6-*O*-methylquercetagetin derivatives, patuletin (36) and spinacetin (37), and isolimocitrol (39) gave larger shifts of 66–70 nm.

The data in Table 1 provide substantial evidence for the generalization that 3-hydroxyflavones undergo a characteristic bathochromic shift of approximately 60 nm in ethanolic aluminum chloride and that the magnitude of this shift is independent of the presence or absence of 5-hydroxy- and 3′,4′-dihydroxy groupings. Noted exceptions to this generalization may be 6-*O*-methyl-5,6,7-trihydroxy- and 5,6,7,8-tetrahydroxyflavone derivatives. On the basis of data on a limited number of representatives these compounds may give larger shifts of 66–70 nm.

5-Hydroxyflavones

In Table 2 the reported ethanolic aluminum chloride spectral shifts of ninety 5-hydroxyflavones are grouped according to structural types. In these flavones a 3-hydroxyl group is either absent or it is protected by glycosidation or etherification.

Apigenin, Luteolin and Tricetin Derivatives

Twenty-seven apigenin, luteolin and tricetin derivatives give an average bathochromic shift of 40 nm. The apigenin derivatives tend to show slightly higher shifts (42–48 nm) than luteolin and tricetin derivatives (33–42 nm). The spectral curves are similar in shape, although variations in the relative intensities of the aluminum complex Bands Ia and Ib may occur.

Two compounds in this group give exceptionally large shifts, viz. 4′,7-di-*O*-methylvitexin (47), 55 nm, and 6,8-dimethyl-7-*O*-methylapigenin (48), 68 nm. Although the magnitude of the 4′,7-di-*O*-methylvitexin shift approaches that of a 3-hydroxyflavone, its spectral curve (Fig. 4) is that of a typical 5-hydroxyflavone and distinctly different from those of 3-hydroxyflavones.

[13] Variations of a few nm in reported absorption maxima are not regarded as significant because of the use of different instruments and frequently slightly different conditions, e.g. anhydrous, aqueous or alcoholic aluminum chloride of different concentrations added to solutions of the flavone in absolute or 95% ethanol, in their measurements.

[14] R. M. Horowitz and G. Gentili, *J. Org. Chem.* **25**, 2183 (1960).

[15] B. Gentili and R. M. Horowitz, *Tetrahedron* **20**, 2313 (1964).

[16] S. J. Morris and R. H. Thomson, *Tetrahedron Letters* **2**, 101 (1963).

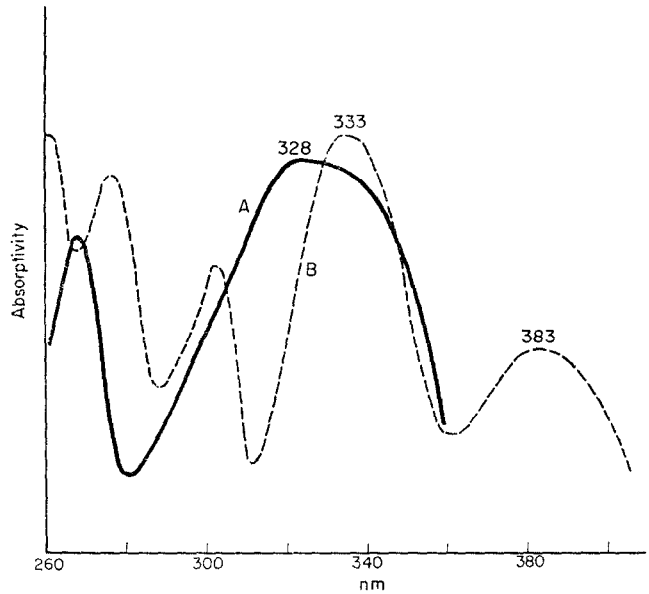


FIG. 4. SPECTRA OF 4′,7-DI-*O*-METHYLVITEXIN (47) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$.

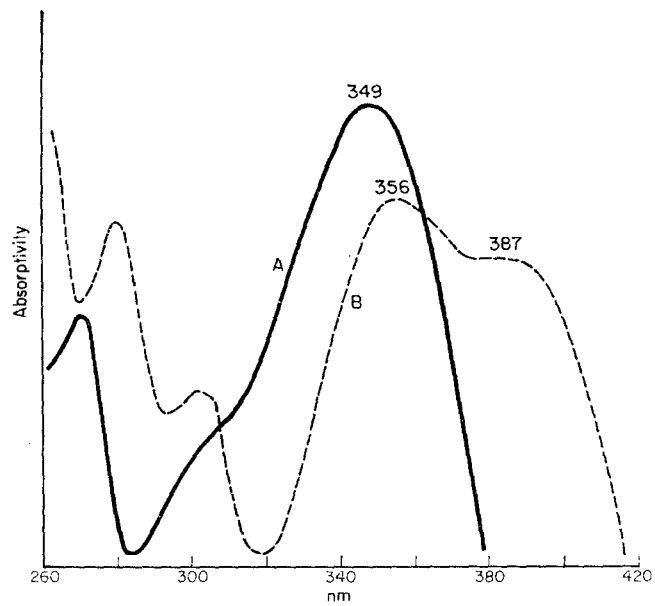


FIG. 5. SPECTRA OF TRICIN (65) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$.

Tricin (65) does not give an unusual aluminum chloride spectrum, although it was reported[16] to give a shift of only 5 nm. This value evidently refers to the shift to Band Ib of the aluminum complex. In agreement with Harborne and Hall[17] the tricin–aluminum chloride spectrum (Fig. 5) has a long wavelength band as a pronounced inflection at about

[17] J. B. HARBORNE and E. HALL, *Phytochem.* 3, 421 (1964).

380 nm. A similar explanation accounts for the 7 nm shift reported[18] for combretol (3,7,3′,4′,5′-penta-*O*-methyl myricetin) (100).

It is noteworthy that apigenin and luteolin glucuronides (46, 61, 62, 63) tend to give smaller shifts (12–29 nm) than the more usual glycosides and methyl ethers.

Kaempferol, Quercetin and Myricetin Derivatives

Thirty-eight of these derivatives with a free 5-hydroxyl and a glycosidated or alkylated 3-hydroxyl group give an average bathochromic shift of 42 nm. In this group of flavones there are no exceptional deviations, although, as in the case of apigenin and luteolin glucuronides, the glucuronide derivatives (79, 80, 92) show smaller shifts (26–34 nm) than glycosides and methyl ethers. The relative intensities of the aluminum complex Bands Ia and Ib vary.

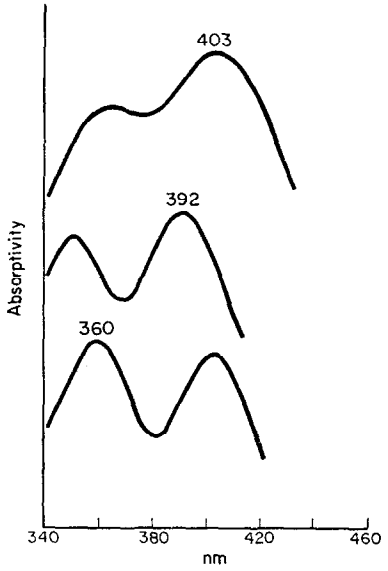


FIG. 6. SPECTRA IN ETHANOLIC ALUMINUM CHLORIDE OF (A) 3,3′-DI-*O*-METHYLQUERCETIN, (B) 3,7,4′-TRI-*O*-METHYLQUERCETIN, (C) 3-*O*-METHYLQUERCETIN.

As indicated by the spectra of 3-*O*-methylquercetin, 3,3′-di-*O*-methylquercetin and 3,4′,7-tri-*O*-methylquercetin (Fig. 6), as well as rutin (Fig. 1) and robinin (Fig. 2), this variation in relative intensities does not appear to be correlated with the presence of a 3′,4′-dihydroxyl group.

3-*Alkoxy*-5,7,8-*trihydroxyflavone Derivatives*

Three reported ethanolic aluminum chloride spectra of this type of 5-hydroxyflavone show shifts (52–60 nm) approaching those of 3-hydroxyflavones. Detailed spectra curves of these three compounds have not been published. However, limocitrin 3-glucoside (107), 5,7,4′-trihydroxy-8,3′-dimethoxy-3-glucosidoxyflavone, gave an aluminum chloride spectrum (shift 48; reported[15] shift, 57 nm) of the type commonly shown by 5-hydroxyflavones (Fig. 7; cf. Figs. 4,5).

[18] S. MONGKOLSUK, F. M. DEAN and L. E. HOUGHTON, *J. Chem. Soc.* 125 (1966).

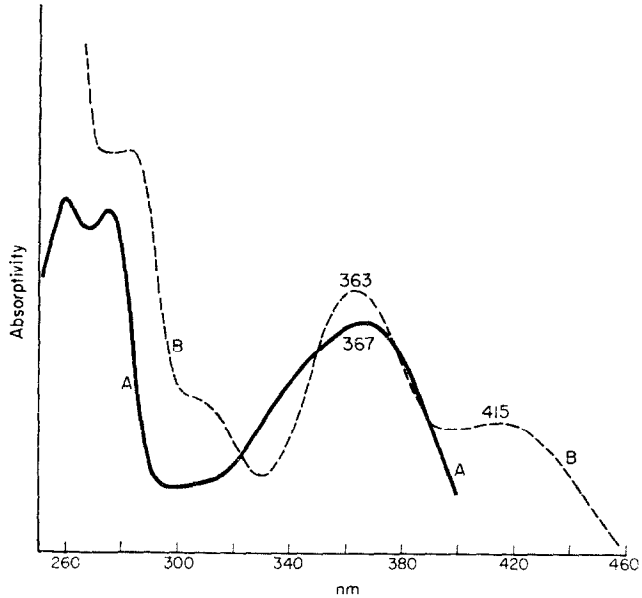


FIG. 7. SPECTRA OF LIMOCRITIN 3-β-D-GLUCOSIDE (107) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$.

5,6,7-Trihydroxy- and 5,6,7,8-Tetrahydroxyflavone Derivatives

Twenty flavones with a 5,6,7-oxygenation pattern give consistently smaller shifts (average shift, 21 nm) than shown by any other group of 5-hydroxyflavones, except glucuronides. Furthermore, in accord with the observation of Farkas and his co-workers [3] that the aluminum

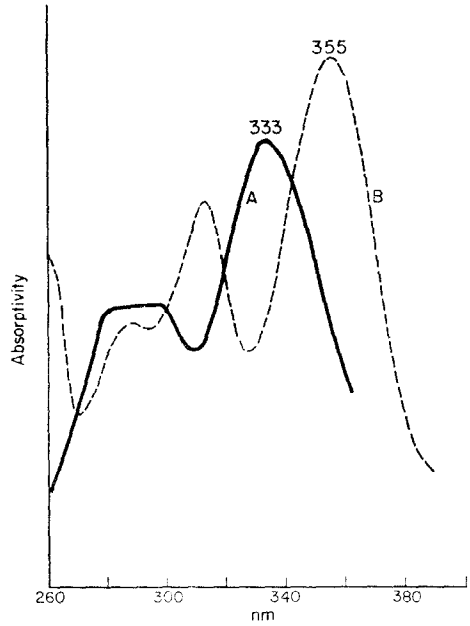


FIG. 8. SPECTRA OF XANTHOMICROL (123) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$.

chloride spectrum of nevadensin (121) has only a single peak at long wavelengths, the spectrum of the metal complex of xanthomicrol (123) (Fig. 8), 4′-*O*-methyl-xanthomicrol (124), and of the citrus flavones (125) and (126) also shows a single, well-defined peak in this region. This property, therefore, appears to be uniquely characteristic of flavones of this series. The 3-methoxyflavone, penduletin (115), 5,4′-dihydroxy-3,6,7-trimethoxyflavone, also shows (Fig. 9) a well-defined, single peak at 362 nm in ethanolic aluminum chloride. In this case, however, a slight inflection does occur at longer wavelength (about 400 nm).

The decrease in the magnitude of the aluminum chloride shift when a 5-hydroxyflavone contains a 6-hydroxy or 6-methoxy group is further indicated by the spectrum of 5-hydroxy-3,6,3′,4′-tetramethoxy-8-methyl flavone (133). This undergoes[19] a bathochromic shift of only 10 nm.

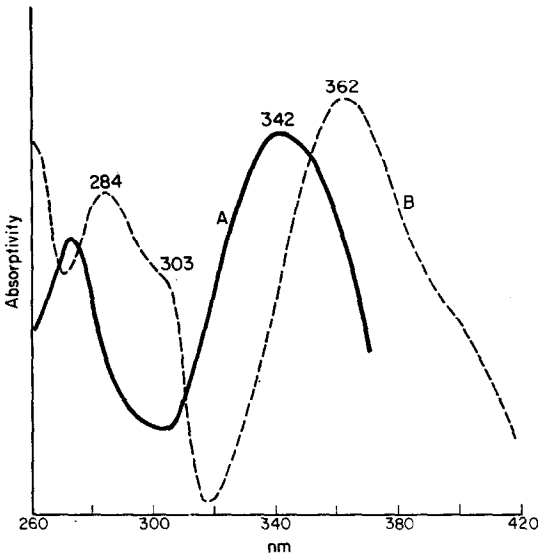


FIG. 9. SPECTRA OF PENDULETIN (115) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$.

Miscellaneous 5-Hydroxyflavones

In Table 2 the aluminum chloride spectra of five miscellaneous flavones, including the unusual 2′-hydroxyflavones, echoidin (130) and wightin (132), are recorded. With the exception of wightin (Δ_λ 60–70 nm) shifts vary from 40–45 nm. The spectrum of tectochrysin (134) (7-methoxy-5-hydroxyflavone) is a little unusual because of the absence of B ring substituents and is reproduced in Fig. 10.

In summary, the data in Table 2 supports the following generalizations: (1) In the absence of a free 3-hydroxyl, 5-hydroxyflavones derived from apigenin, luteolin, tricetin, kaempferol, quercetin and myricetin give shifts of about 34–48 nm with ethanolic aluminum chloride, the magnitude of the shift being independent of the presence or absence of a 3′,4′-dihydroxyl group. The long wavelength band of the aluminum-flavone complex exhibits two peaks or inflections of varying relative intensities. (2) Glucuronides of the above compounds give smaller shifts than glycosides and methyl derivatives. (3) Highly oxygenated 5-hydroxyflavones with an adjacent 6-hydroxy or 6-methoxyl group exhibit characteristically smaller

[19] D. ADINARAYANA and T. R. SESHADRI, *Tetrahedron* **21**, 3727 (1965).

shifts (average 21 nm). On the basis of a limited number of examples these compounds may also be unique in that the aluminum chloride spectra have a single peak in the long wavelength region. (4) 5,7,8-Trihydroxyflavone derivatives appear to be exceptional in giving unusually large shifts of 52–60 nm. Compounds of this type should be distinguished from 3-hydroxyflavones by the shape of the spectral curves of their complexes.

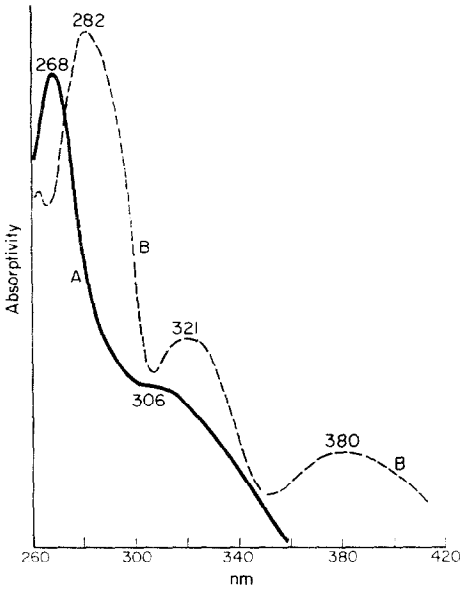


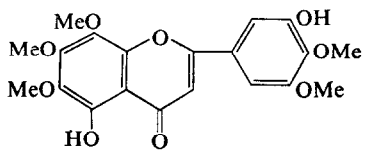
FIG. 10. SPECTRA OF TECTOCHRYSIN (134) IN (A) ETHANOL, (B) ETHANOLIC $AlCl_3$.

Flavones without 3- or 5-hydroxyl groups. In Table 3 the ethanolic aluminum chloride shifts are tabulated for ten flavones which lack free hydroxyl groups in the 3- and 5-positions. Seven of these flavones have a free 3′,4′-dihydroxyl group. Significant shifts were not given by any of these compounds. On the basis of these observations and the extensive data in Table 2, which showed that 5-hydroxyflavones, with and without 3′,4′-dihydroxyl groups, give shifts of the same order of magnitude, there seems to be no evidence to support the assumption that complex formation with this dihydroxyl grouping occurs in ethanolic aluminum chloride spectral measurements as usually performed.[20]

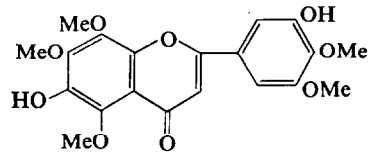
	$AlCl_3$ shift		$AlCl_3$ shift
Apigenin	43	Kaempferol 3-glucoside	37
Luteolin	40	Quercetin 3-glucoside	39
Luteolin 4′-methyl ether	40	3,7-Di-*O*-methylquercetin	44
Luteolin 3′-methyl ether	39	3,7,4′-Tri-*O*-methylquercetin	43

[20] Dr. R. M. HOROWITZ, private communication, has suggested that complex formation with 3′,4′-dihydroxyl may occur in ethanol saturated with aluminum chloride.

In tabulating these spectra, it was noted that a limited number of naturally occurring flavones have been assigned structures which are difficult to reconcile with their reported spectral shifts, viz. (1) digicitrin, a highly methoxylated flavone from *Digitalis purpurea*, has been assigned[21] the 5-hydroxyflavone structure (I). In contrast to the 20 nm shift given by flavones of similar structural type (Table 2, 117–128), the spectrum of digicitrin (λ_{max} 337, 282 nm) is unchanged[21] by aluminum chloride. If a lack of shift is confirmed, it is possible that the A ring hydroxyl of digicitrin should be reassigned, e.g. to the 6-position to give structure II. This latter structure is similar to that proposed for calycopterin, a constituent of *D. thapsii*,[22] (2) a compound from grass, identified[17] as tricin 5-monoglucoside (4′,7-dihydroxy-3′,5′-dimethoxy-5-glucosidoxy-flavone), gave a shift of about 49 nm with aluminum chloride.*



(I)



(II)

EXPERIMENTAL

Spectra of the flavones were determined in absolute ethanol and in absolute methanol on a Cary 15 recording spectrophotometer. One drop of 10% aqueous aluminum chloride solution was added to the cell and after 5 min the spectra were redetermined.

Model experiments with rutin indicated that formation of a complex under these conditions is complete within 5 min.

Acknowledgements—The author is indebted to Dr. G. Stout and Dr. R. M. Horowitz for specimens of xanthomicrol and limocitrin derivatives.

* *Footnote added in proof*—(*a*) In accord with similar 5-hydroxy-6-methoxyflavones (Table 2), scaposin (3′,5,7-trihydroxy-4′,5′,6,8-tetramethoxyflavone), λ_{max}^{EtOH} 337 nm, kindly provided by Dr. Mabry (M. B. THOMAS and T. J. MABRY, *Tetrahedron* **24**, 3675 (1968)), gave a shift of 20 nm with ethanolic aluminum chloride and Band I of the complex showed a single peak; (*b*) acacetin and 4′,7-di-*O*-methylvitexin give exceptionally large (52–55 nm, Table 2) shifts with aluminum chloride. In agreement with this cytoside (4′-*O*-methylvitexin), λ_{max}^{EtOH} 327, shifts 53 nm with ethanolic aluminum chloride (J. CHOPIN, M. L. BOUILLANT and A. DURIX, *Compt. Rend.* **260**, 4850 (1965)); (*c*) following receipt of this manuscript Markham and Mabry (*Phytochem.* **7**, 1197 (1968)) have described a procedure for detecting *ortho-dihydroxyl* groups with methanolic aluminum chloride.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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