Fluorometric Study on the Metal Chelates of Flavone Derivatives. I. Correlation between Fluorescence Intensity and Structure of Beryllium Chelates[1]

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The correlation between the structure and fluorescence intensity of flavone derivatives and their beryllium chelates was investigated. The ligands of type I, II in Chart 1 and their beryllium chelates did not produce fluorescence, while the ligands of type III, IV fluoresced by themselves and formed fluorescent chelates with beryllium ion. The ligands of type V which were non-fluorescent ligands formed fluorescent beryllium chelates.

One of flavone derivatives, morin, has been utilized as an excellent fluorometric reagent for the microdetermination of beryllium and other metal ions. There has not been an investigation dealing with the correlation between the fluorescence intensity and the structure of metal chelate of flavone derivatives. In the present work, a systematic study was made on the beryllium chelates of 27 flavone derivatives and it was found that there was a relationship between the structure and fluorescence intensity.

Results and Discussion

Table I lists the 27 flavone derivatives tested in the present study. The compounds marked with an asterisk were commercial products and the others were synthesized by the methods described in the experimental section. These ligands were classified into five types from their structure as shown in Chart 1. The condition for the measurement of fluorescence is described in the experimental section and the data obtained are shown in Table I as the relative value. The formation of beryllium chelates was indicated from changes in electronic absorption, excitation or emission spectra produced by the addition of beryllium ion. In the case of 3-hydroxyflavone, 3,3',4'-trihydroxyflavone, and 3,3',4',7-tetrahydroxyflavone, no differences were found among them, which seems to suggest no formation of chelates. From these data in Table I, the following facts are noted. The ligands having a hydroxyl group in 5-position (type I in Chart 1) and their beryllium chelates do not produce fluorescence, while the ligands having a hydroxyl group in 3-position (type III) fluoresce by themselves and form fluorescent chelates with beryllium. The ligands having two hydroxyl groups in both 3- and 5-position (type II) do not produce fluorescence with beryllium as well as by themselves, and when one of two hydroxyl groups, that in 5-position, is changed to a methoxyl group (type IV), they become fluorescent and form fluorescent chelates with beryllium. Introduction of a hydroxyl group into 7-position of 3,5-dihydroxy derivatives (type V) produces an interesting phenomenon in that non-fluorescent ligand forms a fluorescent chelate with beryllium.

From the experimental results observed above, an interesting correlation was found between the fluorescence emission of flavone derivatives or their beryllium chelates and their structural characteristics. Various substituent group have complicated effect on the

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1) This work was reported at the 89th Annual Meeting of the Pharmaceutical Society of Japan, April 6, 1969.
2) Location: *Mitahora, Gifu.*

TABLE I. Relative Fluorescence Intensity of Chromones, Flavones,
and Their Beryllium(II) Chelates

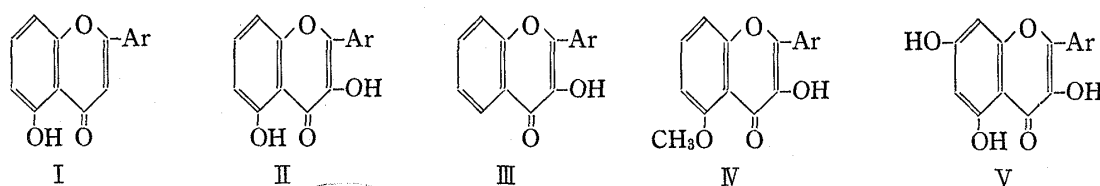| Compound	mp (°C)	Fluorescence intensity	
		Ligand	Be(II) chelate
5-OH-Chromone	126—127	0	0
5-OH-2-Me-Chromone	88— 89	0	0
5-OH-Flavone	158—159	0	0
2',5-OH-Flavone	272—273	0	0
5-OH-2'-OMe-Flavone	136—137	0	0
3',4',5-OH-Flavone	above 300	0	1
5,7-OH-Flavone	292—293	0	0
4',5,7-OH-Flavone[a]	345—350	0	0
3-OH-Flavone[a]	169—170	27	31
2',3-OH-Flavone	204—206	65	17
3-OH-2'-OMe-Flavone	213—214	5	12
3,4'-OH-Flavone	282—284	12	86
3-OH-4'-OMe-Flavone	236—237	30	86
3,3',4'-OH-Flavone	293—296	10	12
3-OH-3',4'-OMe-Flavone	187—189	65	95
3,7-OH-Flavone	264—265	62	136
3-OH-7-OMe-Flavone	178—179	15	94
2',3,7-OH-Flavone	158—159	205	8
3-OH-2',7-OMe-Flavone	204—206	7	18
3,4',7-OH-Flavone	271—273	36	137
3-OH-4',7-OMe-Flavone	193—194	3	150
3,3',4',7-OH-Flavone[a]	above 300	45	20
3,5-OH-Flavone	146—147	0	0
3-OH-5-OMe-Flavone	169—171	3	184
3,4',5-OH-Flavone	235—236	0	0
3-OH-4',5-OMe-Flavone	171—172	12	259
2',3,4',5,7-OH-Flavone[a]	285—290 (decomp.)	11	500
3,3',4',5,7-OH-Flavone[a]	314 (decomp.)	1	37
Quercetinsulfonic acid[a]	above 300	1	31

a) commercial product

Chart 1. Six Types of Flavone Derivatives

fluorescence intensity, but particularly, 3-hydroxyl group is considered to play a predominant part on the fluorescence emission. The fluorescence emission depends largely on the chelation formed between observed in case of chelates with other metal ions such as aluminum, magnesium, thorium, and yttrium, as well as beryllium. Simpson[3] reported that 4-carbonyl group formed a stronger hydrogen bond with 5-hydroxyl group than with 3-hydroxyl group, and we also obtained the same conclusion from the infrared spectra (Fig. 1). 3-Hydroxyflavone (Fig. 1b) shows a sharp vibration of ν_{OH} in the region of 3000—3500 cm^{-1} which suggests the presence of a free hydroxyl group, while 5-hydroxyflavone gives a broad absorption in the same region (Fig. 1a), which shows formation of a hydrogen bond. Therefore,
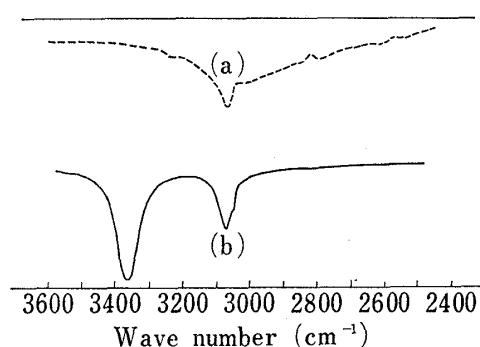
3) T.H. Simpson and L. Garden, J. Chem. Soc., 1952, 4638.

Fig. 1. Infrared Spectra of 5-Hydroxy-
flavone (a) and 3-Hydroxyflavone (b)
in Carbon Tetrachloride

the effect of 3-hydroxyl group is explained by assum-
ing that in the type II compounds containing two
hydroxyl groups in 3- and 5-position, intramolecular
hydrogen bond and beryllium chelate are formed
with hydroxyl group in 5-position, and 3-hydroxyl
group does not contributed to fluorescence emission,
while in the type V compounds containing another
hydroxyl group in 7-position, carbonyl group of
which results in non-fluorescence, and on formation
of beryllium chelate, 3-hydroxyl group takes part
in the chelation by influence of 7-hydroxyl group
which results in fluorescence. A series of changes
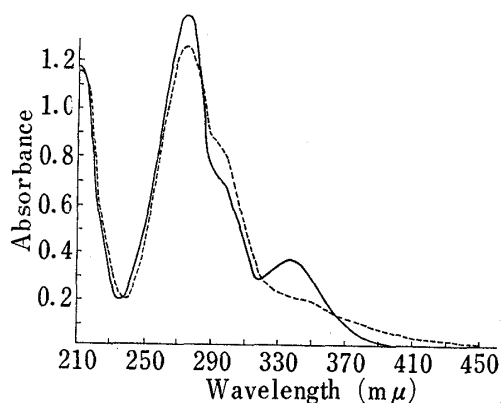observed between electronic absorption spectra of

Fig. 2. Absorption Spectra of 5-Hydroxy-
flavone and Its Beryllium(II) Chelate in
90% Ethanol

——————: 5-hydroxyflavone
------------: beryllium(II) chelate
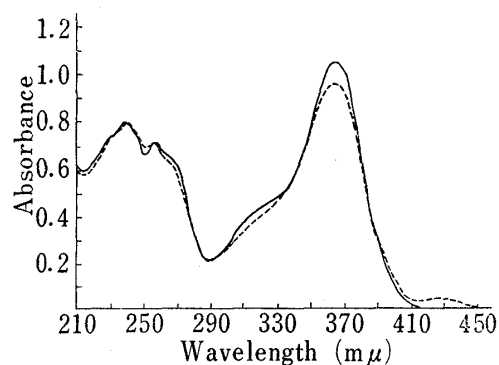

Fig. 3. Absorption Spectra of |3-Hydroxy-
5-methoxyflavone and Its Beryllium (II)
Chelate in 90% Ethanol

——————: 3-hydroxy-5-methoxyflavone
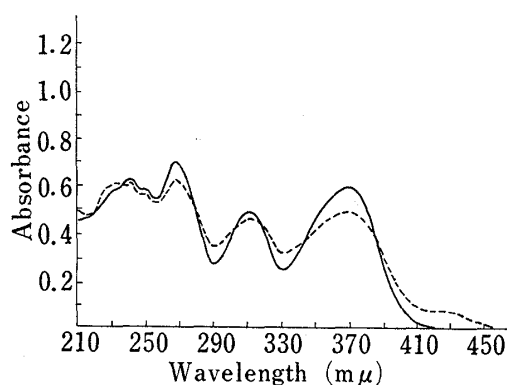------------: beryllium (II) chelate

Fig. 4. Absorption Spectra of 3,5-Di-
hydroxyflavone and Its Beryllium(II)
Chelate in 90% Ethanol

——————: 3,5-dihydroxyflavone
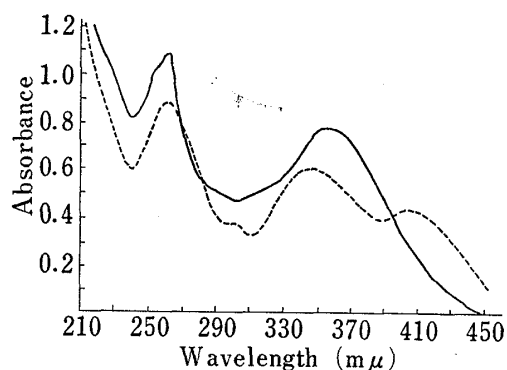------------: beryllium (II) chelate

Fig. 5. Absorption Spectra of Morin
and Its Beryllium(II) Chelate in 90%
Ethanol

——————: morin
------------: beryllium (II) chelate

free ligands and those of the corresponding beryllium chelates appear to support this specu-
lation. In Fig. 2,3, and 4 corresponding to the type I, IV, and II compounds, absorption
spectra of free ligands are very similar to those of their beryllium chelates, while in type V,
a significant difference is observed between the two kinds of spectra (Fig. 5), which shows a

change of the electronic arrangement in the molecule. This change leads to so large a difference in the absorption spectrum of type V compounds which is not observed in other types of flavone derivatives, and suggests a change in the position of chelation.

TABLE II. Absorption Spectra of Flavone Derivatives
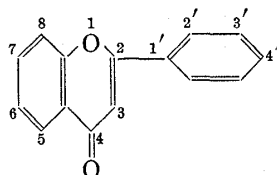
Compound	Position of OH						λ_{max} (mμ)	ε
	7	5	3	2'	3'	4'		
1		OH					338	7100
2			OH				347	18200
3		OH		OH			344	14200
4			OH	OH			336	12400
5	OH	OH				OH	339	22200
6	OH		OH			OH	356	22200
7	OH	OH					315	11700
8	OH		OH				343	18100
9		OH			OH	OH	355	20000
10			OH		OH	OH	372	22900
11			OH			OH	360	26000
12	OH		OH	OH			315	15000
13		OH	OH				369	12000

Effect of substituents on characteristics of absorption is listed in Table II. Compared with 5-hydroxy derivatives, 3-hydroxy derivatives show a bathochromic shift of absorption band in the longest wavelength and their intensities are stronger than those of the corresponding 5-hydroxy derivatives. These differences are reasonable from the general rule that an introduction of α-hydroxyl group to α,β-unsaturated ketone causes a remarkable bathochromic shift. Only one exception observed between 2',5-dihydroxyflavone and 2',3-dihydroxyflavone seems to be derived from steric hindrance of 2'-hydroxyl group. 4'-Hydroxyl group causes a bathochromic shift[4] (cf. No. 5—7, 6—8, 2—11), but 7-hydroxyl group conversely shows a hypsochromic shift, especially in the case of 5-hydroxy derivatives (cf. No. 1—7, 2—8, 4—12, 6—11).

Effect of various substituents on fluorescence intensity of 3-hydroxyflavone or its beryllium chelate is complicated. Introduction of 7-hydroxyl, 7-methoxyl, 4'-hydroxyl or 4'-methoxyl increased fluorescence intensity. However, introduction of 7-hydroxyl which results in increased fluorescence intensity causes a hypsochromic effect in absorption spectrum. Additional resonance due to hydroxyl group in 5-position of 3,5-dihydroxyflavone increases conjugation to a large extent, but produces no fluorescence.

From these facts observed as above, it seems quite probable that fluorescence emission is caused by change of the electronic arrangement in the ring formed through 3-hydroxyl and 4-carbonyl, but not by the additional resonance of the whole molecule. The reason why 3-hydroxyl not 5-hydroxyl is particularly effective for the fluorescence emission of beryllium chelate of flavone derivatives is an interesting question. We are carrying out further studies on the relation of fluorescence intensity to the wave number for $\nu_{c=o}$ of 4-cabonyl group.

4) S.K.K. Jatlar and B.N. Mattoo, J. Indian Chem. Soc., 33, 623, 641 (1956).

Experimental

Apparatus——Simadzu GSF-16 spectrofluorometer was used for determination of the fluorescence intensities of flavone derivatives and their metal chelates. Absorption spectra were measured with a Simadzu MPS-50L spectrophotometer.

Reagents——A beryllium nitrate stock solution was prepared by dissolving the salt (reagent grade) in distilled water. This solution containing 71 mg of $Be(NO_3)_2$ per ml was standardized by volumetry. A working solution was prepared by diluting the stock solution with distilled water. A stock solution of flavone derivatives of 0.5×10^{-3}M was prepared by dissolving the solid material in ethanol. Twenty-three of the flavone derivatives listed in Table I were prepared by modification of published methods,[5-10] and the others were commercial reagents.

5-Hydroxychromone[5]——2,6-Dihydroxyacetophenone (2 g) was treated with sodium powder (1.6 g) in ethyl formate (30 ml) at 0—5° under N_2 for 3 days. The reaction mixture was made acid and then steam distillated. The product was filtered, washed with water, dried and recrystallized from 30% ethanol. mp 125.5—127.5°. $FeCl_3$-reaction dark purple.

5-Hydroxy-2-methylchromone[6]——2-Hydroxy-6-methoxy-ω-acetylacetophenone (1 g) was converted to 5-hydroxy-2-methylchromone by gentle boiling with hydroiodic acid (10 ml). The reaction mixture was diluted with water, the product filtered, washed with water, dried and recrystallized from ethanol. mp 111—112°. $FeCl_3$-reaction bluish purple. *Anal.* Calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.58. Found: C, 68.07; H, 4.69.

5-Hydroxyflavone[7]——2,6-Dimethoxy-ω-benzoylacetophenone (1 g) was converted to 5-hydroxyflavone by gentle boiling with hydroiodic acid (10 ml) during 30 min. The reaction mixture was diluted with water, the product filtered, washed with water, dried and recrystallized from ethanol. mp 158—159°. $FeCl_3$-reaction green. *Anal.* Calcd. for $C_{15}H_{10}O_3$: C, 75.69; H, 4.24. Found: C, 75.45; H, 4.40.

2′,5-Dihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 2,6-dimethoxy-ω-(2-methoxybenzoyl)acetophenone. The product was recrystallized from ethanol. mp 272—273°. $FeCl_3$-reaction green. *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.93; H, 3.97. Found: C, 70.98; H, 4.09.

5-Hydroxy-2′-methoxyflavone[7]——2′,5-Dihydroxyflavone (0.4 g) was converted to 5-hydroxy-2′-methoxyflavone by gentle boiling for 6 hr with anhydrous potassium carbonate (0.2 g) and methyl iodide (0.3 g) in acetone solution. The reaction mixture was diluted with water. The product was filtered, washed with water, dried and recrystallized from ethanol. mp 136—137°. $FeCl_3$-reaction green. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.70; H, 4.51. Found: C, 71.53; H, 4.58.

3′,4′,5-Trihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 2,6-dimethoxy-ω-(3,4-dimethoxybenzoyl)acetophenone. The product was recrystallized from ethanol. mp above 300°. $FeCl_3$-reaction dark brown. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.72; H, 3.73. Found: C, 66.37; H, 3.97.

5,7-Dihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 2-hydroxy-4,6-dimethoxy-ω-benzoylacetophenone. The product was recrystallized from ethanol. mp 292—293°. $FeCl_3$-reaction purple. *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.93; H, 3.97. Found: C, 70.81; H, 4.15.

3-Hydroxy-2′-methoxyflavone[9]——2′-Hydroxy-2-methoxychalcone[11] (2 g) was dissolved in ethanol (60 ml) and 10% aqueous sodium hydroxide solution (30 ml), gently boiled and then treated with 30% H_2O_2 (6 ml). It was allowed to stand for 24 hr. The reaction mixture was then diluted with water and made acid. The product was filtered, washed with water, dried and recrystallized from ethanol. mp 213—214°. $FeCl_3$-reaction bluish purple. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.70; H, 4.51. Found: C, 71.48; H, 4.59.

2′,3-Dihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-2′-methoxyflavone. The product was recrystallized from ethanol. mp 204—206°. $FeCl_3$-reaction brown. *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.93; H, 3.97. Found: C, 70.86; H, 4.25.

3-Hydroxy-4′-methoxyflavone——This was made in the same way as 3-hydroxy-2′-methoxyflavone but using 2′-hydroxy-4-methoxychalcone.[12] The product was recrystallized from ethanol. mp 236—237°. $FeCl_3$-reaction purple. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.70; H, 4.51. Found: C, 71.53; H, 4.60.

3,4′-Dihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-4′-methoxyflavone. The product was recrystallized from ethanol. mp 282—284°. $FeCl_3$-reaction purple. *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.93; H, 3.97. Found: C, 71.15; H, 4.10.

5) A. Murata, T. Sudzuki and T. Ito, *Bunseki Kagaku*, **14**, 630 (1965).
6) C.R. Rao and V. Venkateswarlu, *Rec. Trav. Chim.*, **75**, 1324 (1956).
7) J. Tambor, *Chem. Ber.*, **41**, 787 (1908).
8) F.M. Dean and V. Podimung, *J. Chem. Soc.*, **1965**, 3978.
9) A. Oliverio and A. Schiavello, *Gazz. Chim. Ital.*, **80**, 788 (1950).
10) T.R. Seshadri and V. Venkateswarlu, *Pro. Indian Acad. Sci.*, **26A**, 186 (1947).
11) J. Tambor and H. Gubler, *Helv. Chim. Acta*, **2**, 101 (1919).
12) H. Pyan and G. C. Callaghan, *Proc. Roy. Irish Acad.*, **39B**, 124 (1929).

3-Hydroxy-3′,4′-dimethoxyflavone——This was made in the same way as 3-hydroxy-2′-methoxyflavone but using 2′-hydroxy-3,4-dimethoxychalcone.[13] The product was recrystallized from ethanol. mp 187—189°. FeCl₃-reaction purple. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.51; H, 4.74. Found: C, 68.81; H, 4.79.

3,3′,4′-Trihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-3′,4′-dimethoxyflavone. The product was recrystallized from ethanol. mp 293—296°. FeCl₃-reaction brown. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.72; H, 3.73. Found: C, 66.55; H, 3.61.

3-Hydroxy-7-methoxyflavone[8]——2′-Hydroxy-4′-methoxychalcone[14] (2 g) was dissolved in methanol (20 ml) and 20% aqueous sodium hydroxide solution (10 ml) and then treated with 30% H_2O_2 (4 ml) at 0—5°. It was allowed to stand for 8 hr at 0—5°. The reaction mixture was diluted with water and made acid. The product was filtered, washed with water, dried and recrystallized from ethanol. mp 178—179°. FeCl₃-reaction purple. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.70; H, 4.51. Found: C, 71.70; H, 4.51.

3,7-Dihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-7-methoxyflavone. The product was recrystallized from ethanol. mp 264—265°. FeCl₃-reaction purple. *Anal.* Calcd. for $C_{15}H_{10}O_4$: C, 70.93; H, 3.97. Found: C, 70.82; H, 3.97.

3-Hydroxy-2′,7-dimethoxyflavone——This was in the same way as 3-hydroxy-2′-methoxyflavone but 2′-hydroxy-2,4′-dimethoxychalcone.[15] The product was recrystallized from ethanol. mp 204—206°. FeCl₃-reaction green. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.51; H, 4.74. Found: C, 68.44; H, 4.66.

2′,3,7-Trihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-2′,7-dimethoxyflavone. The product was recrystallized from ethanol. mp 158—159°. FeCl₃-reaction green. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.72; H, 3.73. Found: C, 66.51; H, 3.61.

3-Hydroxy-4′,7-dimethoxyflavone——This was made in the same way as 3-hydroxy-2′-methoxyflavone but using 2′-hydroxy-4,4′-dimethoxychalcone.[16] The product was recrystallized from ethanol. mp 193—194°. FeCl₃-reaction purple. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.51; H, 4.74. Found: C, 68.33; H, 5.03.

3,4′,7-Trihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-4′,7-dimethoxyflavone. The product was recrystallized from ethanol. mp 293—296°. FeCl₃-reaction brown. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.72; H, 3.73. Found: C, 66.55; H, 3.61.

3-Hydroxy-5-methoxyflavone[10]——5-Methoxyflavanone (3 g) was dissolved in ethanol (90 ml), gently boiled and then treated with amyl nitrite (18 ml) and concentrated hydrochloric acid (30 ml) in small quantities during the couse of 15 min. The solution was allowed to stand for 2 hr and then diluted with water to 1200 ml and ether extracted. From the ether extract the flavonol was taken up in diluted sodium hydroxide solution. The alkaline solution was made acid, the product filtered, washed with water, dried and recrystallized from ethanol. mp 170—171°. FeCl₃-reaction purple. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 71.70; H, 4.51. Found: C, 71.44; H, 4.68.

3,5-Dihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-5-methoxyflavone. The product was recrystallized from ethanol. mp 146—147°. FeCl₃-reaction dark green. *Anal.* Calcd. for $C_{16}H_{12}O_4$: C, 70.93; ,H 3.93. Found: C, 71.22; H, 3.96.

3-Hydroxy-4′,5-dimethoxyflavone——This was made in the same way as 3-hydroxy-2′-methoxyflavone but using 2′-hydroxy-4,6′-dimethoxychalcone.[10] The product was recrystallized from ethanol. mp 171—172°. FeCl₃-reaction purple. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.51; H, 4.74. Found: C, 68.31; H, 3.81.

3,4′,5-Trihydroxyflavone——This was made in the same way as 5-hydroxyflavone but using 3-hydroxy-4′,5-dimethoxyflavone. The product was recrystallized from ethanol. mp 235—236°. FeCl₃-reaction green. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.72; H, 3.73. Found: C, 66.57; H, 3.81.

Measurement of Fluorescence Intensity and Absorption Spectra——A beryllium-to-ligand ratio of 20:1 was chosen for all experiments. The formed chelates remained stable at least 20 min. One ml of 0.5×10^{-3}M solution of ligand in ethanol and 1×10^{-2}M solution of $Be(NO_3)_2$ in distilled water were added to a 10 ml volumetric flask and the solution was diluted to 10 ml with ethanol. After 10 min, the fluorescence intensity was determined at the optimum excitation and emission wavelength which gave maximal fluorescence, and then the absorption spectrum was measured on the same solution.

13) S. Hattori, *Bull. Chem. Soc. Japan*, **2**, 171 (1927).

14) G. Bargellini and L. Monti, *Gazz. Chim. Ital.*, **44**, 25 (1914).

15) H.P. Vandrewalla and G.V. Jadhav, *Pro. Indian Acad. Sci.*, **28A**, 125 (1948).

16) S. Fujise and Y. Tachida, *Nippon Kagaku Zasshi*, **63**, 932 (1942).