Structure, stability and spectral properties of complexes of flavones with metal ions of group II

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Complexes of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one and its alkyl derivatives (flavones) with Me^{2+} (Me = Be, Mg, Ca, Zn and Cd) were synthesized and their spectral properties investigated by ^1H NMR, electronic absorption and fluorescence methods. ^1H NMR data enabled the constitutions of these complexes to be found, the spectroscopic techniques the determination of their stability constants. Complementary calculations supplied possible structures of the complexes and helped to explain the nature of the absorption and emission transitions. It was found that practically non-fluorescing flavones begin to emit radiation when they are bound in complexes. This information provides opportunities for applying flavone-metal ion complexes as photochromophores.

Получены комплексы 2-(3,4-дигидроксифенил)-3,5,7-тригидрокси-4H-хромен-4-она (кверцетина) и его алкилпроизводных (далее флавоны) с ионами Me^{2+} (Me = Be, Mg, Ca, Zn и Cd). Проведено исследование комплексов с использованием ^1H ЯМР, электронной абсорбионной и флуоресцентной спектроскопии. При помощи ^1H ЯМР спектроскопии установлено строение комплексов, в частности, расположение центров комплексообразования в молекулах флавонов. Спектральные исследования позволили рассчитать константы нестойкости полученных комплексов. Проведенные полуэмпирические расчеты возможной структуры комплексов дали результаты, совпадающие с экспериментальными данными, а также позволили объяснить природу электронных переходов в спектрах поглощения и испускания. Было показано, что нефлуоресциирующие полигидроксифлавоны и полиметоксифлавоны при образовании комплексов начинают флуоресцировать, причем интенсивность флуоресценции зависит от количественного соотношения метокси- и гидроксигрупп в молекуле. Данная информация может быть использована для создания фотохромофоров на основе соединений класса флавоноидов.

The fluorescence of hydroxyflavone-metal complexes has been utilized during the past 135 years (from 1867) in the qualitative and quantitative analysis of metal ions (of groups II–IV), in phytochemistry (for the identification of flavonoids in plant material) and in pharmaceutical chemistry (for drug analysis) [1, 2]. Interest in flavone-metal complexes has increased as new areas of applications have been found for these compounds. Among other things, flavone complexes are powerful antioxidants

[3, 4] and exhibit anti-inflammatory and antibacterial activity in animals and humans [5, 6].

It has recently been found that flavone complexes with group II metal ions additionally possess photochromic and electroluminescent properties. In view of this, it has been suggested that flavone-metal complexes be used as light sources in displays [7]. These complexes may therefore be regarded as new functional materials.

Flavones can contain from one to ten hydroxy groups (Fig. 1); hence, metal ion complexes of various compositions are possible, with different stabilities and spectral properties [8]. In this paper we present the results of experimental investigations and theoretical studies on group II metal ion (Be^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}) complexes with pentahydroxyflavone (quercetine) and its alkyl derivatives (Fig. 1). A further aim of the investigations was to find relations between the stabilities, properties and constitutions of these complexes.

The objects of the investigations were metal ion complexes with quercetine (**I**) and its methoxy- and methylenedioxy derivatives (referred to as flavones). The formulas and names of the flavones investigated are given in Fig. 1. Quercetine was purchased from Aldrich, and its methoxy- and methylenedioxy derivatives were synthesized according to refs. 9 and 10. Their purity was controlled by thin-layer chromatography.

Metal salts (magnesium and calcium perchlorates, and beryllium, zinc and cadmium chlorides — all from Aldrich) were dried under vacuum at 120°C. Methanol, used as solvent, was distilled after being dried over magnesium powder [11].

The concentration stability constants (a 1:1 metal:flavone stoichiometry of the complexes was assumed) were determined by spectrophotometric or spectrofluorometric titration, performed by measuring the absorption or fluorescence spectra following the addition of methanolic solutions of metal salts to solutions of flavones in the same solvent. The excitation wavelengths always corresponded to the isosbestic points, which were located on the basis of analysis of absorption spectra. Stability constants were determined using the Spectral Data Lab program [12] which includes the Stark-Barbanel method [13].

Absorption and fluorescence spectra were recorded on a Hitachi U4010 spectrophotometer and a Hitachi F4010 spectrofluorimeter,
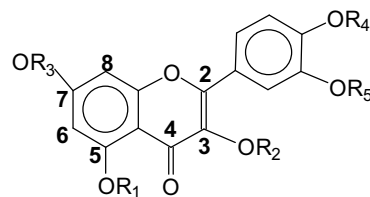

Fig. 1. Constitution of flavones occurring as ligands in the metal ion of group II complexes.

flavone	R_1	R_2	R_3	R_4	R_5
I	H	H	H	H	H
II	H	H	H	CH$_2$	
III	H	H	CH$_3$	CH$_3$	CH$_3$
IV	H	CH$_3$	CH$_3$	CH$_3$	CH$_3$
V	CH$_3$	CH$_3$	CH$_3$	CH$_3$	CH$_3$

I, 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one

II, 2-(1,3-Benzodioxol-5-yl)-3,5,7-trihydroxy-4*H*-chromen-4-one

III, 2-(3,4-Dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-4*H*-chromen-4-one

IV, 2-(3,4-Dimethoxyphenyl)-5-hydroxy-3,7-dimethoxy-4*H*-chromen-4-one

V,2-(3,4-Dimethoxyphenyl)-3,5,7-trimethoxy-4*H*-chromen-4-one

respectively. ^1H NMR spectra were measured on a Varian 300 spectrometer using DMSO-d_6 as solvent.

Unconstrained geometry optimisations of possible solvated complexes of Mg^{2+} with **I** in the ground state (S_0) and excited singlet (S_1) electronic states were carried out at the semiempirical PM3 (S_0 state) and PM3/CI (S_1 state) levels of theory [14] with standard procedures being employed together with the BFGS method [15−18] implemented in the MOPAC 93 program package [19]. The wavelengths of the electronic absorption ($S_0 \rightarrow S_1$) and fluorescence ($S_1 \rightarrow S_0$) transitions in the above-mentioned complexes were predicted at the PM3/CI level [14]. In the latter case, 400 ground and single excited state configurations were included in the calculations. Reasonable data were obtained if 2−3 molecules of the solvent (methanol) were included in the calculations. Heats of formation and Mulliken partial charges [20] were extracted directly from data files following the geometry optimisations, and the latter were used for computing the electron density changes accompanying $S_0 \rightarrow S_1$ transitions.

There are two complexes of Mg^{2+} with **I** in which the metal ion coordinates through the carbonyl oxygen atom and the hydroxy
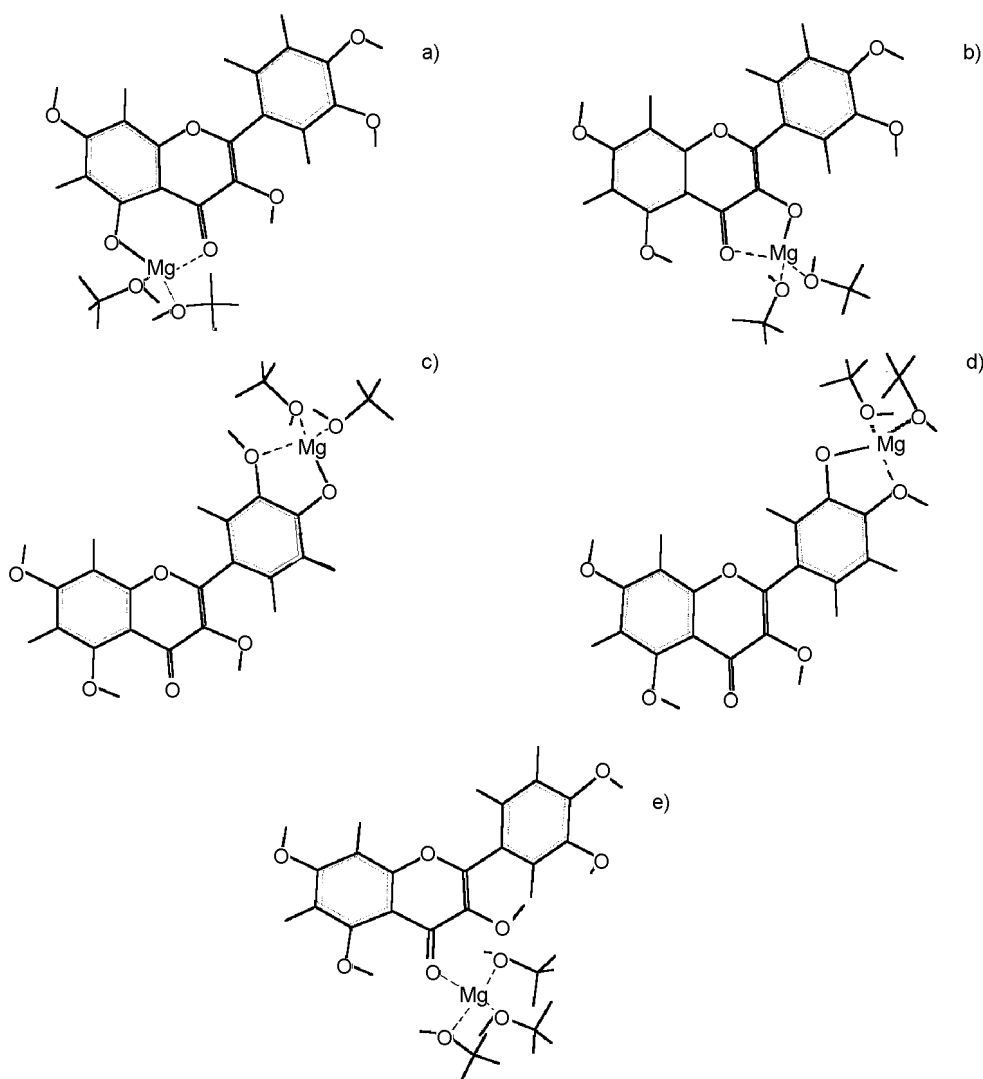
Fig. 2. PM3 optimized geometries of possible I-Mg^{2+} complexes in the ground electronic state

group in either position 5 (type **A**) or 3 (type **B**) (Fig. 2). Similarly, two complexes (types **C** and **D**) are possible when the metal ion coordinates through the oxygen atoms of the hydroxy groups attached to the phenyl ring in positions 4 and 3. Finally, a complex can also occur if the metal ion co-

ordinates through the carbonyl oxygen atom only (type **E**). Calculations showed that type **A**, **B**, **C** and **D** complexes are stable when, during complexation, one proton is removed from one hydroxy group in positions 5 or 3 (types **A** and **B**), or one of the hydroxy groups in positions 4 and 3 (types **C** and **D**). This means that type **A**, **B**, **C** and **D** complexes exhibit a formal charge of +1 and the type **E** complex a charge of +2. The enthalpies of formation of **A** and **B** are considerably lower than those of **C** and **D**; the former are therefore most probably predominant in solutions (Table 1).

As far as complexes of quercetine **(I)** with other metals are concerned, we had demonstrated earlier that their composition and structure are substantially affected by the radius and electronic configuration of the particular ions [21]. In the case of 1:1

Table 1. Enthalpies of formation ($\Delta_{f,298}H^0$, in kJ/mol) of I-Mg^{2+} complexes in the ground (S_0) and excited (S_1) electronic states

Entity (Fig. 2)	$\Delta_{f,298}H^0$ (S_0)	$\Delta_{f,298}H^0$ (S_1)
A	−1112.6	−849.6
B	−1173.8	−903.4
C	−1108.3	−936.8
D	−1103.8	−899.6
E	−444.1	−238.9

Table 2. ^1H NMR signals originating from protons of selected hydroxy groups in non-complexed and complexed flavones

Flavone	Position of the OH group	Non-complexed	Complexed				
			Be^{2+}	Mg^{2+}	Ca^{2+}	Zn^{2+}	Cd^{2+}
I	3	9.26	8.34				
	5	12.48		11.99	12.42	11.76	12.19
II	3	9.31	8.22				
	5	12.43		11.82	12.09	11.75	11.93
III	3	9.35	8.12				
	5	12.57		12.01	12.24	11.82	12.01
IV	5	12.59			12.59		12.59

metal:flavone complexes, types **A** and **B** are thermodynamically always the most stable. Some metal ions can, however, form complexes with a 1:2 stoichiometry in which two flavone molecules most probably coordinate through carbonyl oxygen atoms.

Which types of complexes flavones form with metal ions is shown in Table 2. The data in this Table indicate the coordination sites by metal ions. Thus: Be^{2+} always forms a type **A** complex, whereas the other ions — Mg^{2+}, Ca^{2+}, Zn^{2+} and Cd^{2+} — form type **B** complexes with **I**, **II** and **III**. The hydrogen atoms of both 3-OH and 5-OH groups always exhibit the highest chemical shifts for non-complexed flavones. The chemical shifts of these hydrogen atoms fall in value when flavones become ligands. The ^1H chemical shift of the hydrogen atom in the 5-OH group decreases qualitatively in the order: non-complexed $> Ca^{2+} > Cd^{2+} > Mg^{2+} > Zn^{2+}$, which correlates with the order of ionic radii (in Å): $Ca^{2+}(1.05) > Cd^{2+}(0.99) > Mg^{2+}(0.78) > Zn^{2+}(0.57)$ [22]. This implies that the larger ions bring about a reduction in the attenuation of hydrogen atom nuclei and, consequently, increase of chemical shifts.

Methylation of the hydroxy group in position 3 prevents **IV** from forming type **B** complexes. A type **A** complex is, however, formed by **IV** with Be^{2+} (radius = 0.31 Å [22]) and most probably with Mg^{2+} and Zn^{2+}, whose radii fit the dimensions of the cavity created by the carbonyl and 5-OH groups. This cavity is probably too small to contain the larger Cd^{2+} or Ca^{2+} ions. In the case of the latter two ions, however, the ^1H signal of the 5-OH group does appear and is almost identical to one of the non-complexed **IV**. We do not think, therefore, that Cd^{2+} and Ca^{2+} coordinate **IV**. Absorption

and fluorescence investigations showed that a complex is formed between **V** and Mg^{2+}, which can only be of type **E**. It is worth mentioning that type **E** complexes were identified when metal ions coordinated 3-methoxy-2-phenyl-4H-chromen-4-one (3-methoxyflavone) [23].

The long-wavelength absorption of non-complexed flavones (**I**, **II**, **III**, **IV** and **V**) falls within the long-wavelength range of the ultraviolet region (between 340–375 nm) (Table 3). Involvement of flavones in complexes with metal ions shifts this absorption to the visible region, i.e. between 395 and 430 nm. It should, however, be noted that the absorption of the **V**-Mg^{2+} complex appears at a shorter wavelength than any of the other complexes. The long-wavelength shift of absorption of complexed as opposed to non-complexed flavones most probably results from the fact that in all complexes, except those of **V** with Mg^{2+}, the ligands, on losing a proton, are more strongly bound to metals.

Non-complexed flavones fluoresce weakly (**V**), very weakly (**I** and **II**) or not at all (**III** and **IV**) (Table 3). Complexed flavones fluoresce much more strongly — fluorescence quantum yields attain values as high as several per cent. Fluorescence is the strongest in the case of complexes of **I** and **II** with metal ions. The **V**-Mg^{2+} complex also fluoresces relatively strongly. Fluorescence decreases as the degree of methylation of the hydroxy groups increases. Complexes of **III** and **IV** with metal ions exhibit the weakest fluorescence. Moreover, in the latter case the Stokes shift, is much stronger than in the case of the respective complexes of **I** and **II** with metal ions. This could be due to steric interference by the methoxy group in position 3, as well as intensification of

Table 3. Experimentally obtained spectral characteristics of non-complexed flavones and their complexes with metal ions[*]

Flavone	ν_{abs}, cm^{-1}	ν_{fl}, cm^{-1}	$\Delta\nu_{St}$, cm^{-1}	φ, %
Non-complexed				
I	26880	18460	8420	weak
II	28580	20440	8140	weak
III	28170	no fluorescense		
IV	28410	no fluorescense		
V	29410	21460	7950	5.5
Complexed with Be^{2+}				
I	23920	18490	5430	9.3
II	24650	19040	5610	4.6
III	24330	16810	7520	1.0
IV	25000	17830	7170	1.1
V	29410	no fluorescense		
Complexed with Mg^{2+}				
I	24390	19800	4590	4.5
II	24685	18870	5815	3.8
III	24210	16580	7630	0.3
IV	25190	17610	7580	0.3
V	26250	21140	5110	5.4
Complexed with Ca^{2+}				
I	23810	17200	6610	1.7
II	24000	19920	4080	1.4
III	24690	16420	8270	0.4
IV	28410	no fluorescense		
V	29410	no fluorescense		
Complexed with Zn^{2+}				
I	23360	19920	3440	8.2
II	24750	19460	5290	5.9
III	24270	16670	7600	0.3
IV	25910	17210	8700	0.4
V	29410	no fluorescense		
Complexed with Cd^{2+}				
I	25380	20000	5380	3.6
II	24020	19940	4080	3.9
III	24690	16080	8610	0.5
IV	28410	no fluorescense		
V	29410	no fluorescense		

[*] ν_{abs} and ν_{fl} indicate the positions of long-wavelength maxima in absorption and fluorescence spectra (cm^{-1}), $\Delta\nu_{St}$ — Stokes shifts and φ — percentage fluorescence quantum yields

the interfragmental (chromenephenyl) charge transfer upon excitation. The latter possibility was taken into account when we compared the changes in the sum of Mulliken partial charges corresponding to the chromene and phenyl fragments relevant to the $S_0 \rightarrow S_1$ transition (which accounts for the interfragmental charge transfer accompanying excitation). In the case of the complexes of **I** and **II** with Be^{2+}, charge separation increases by 0.26 and 0.37, while for the complexes of **III** and **IV** it increases by as much as 0.60 and 0.82, respectively. One of the consequences of this effect is a decrease in the π-contribution to the bond between the chromene and phenyl moieties in the excited state. This can widen the torsion angle between the chromene moiety and the phenyl ring, and raise the number of spin-orbital interactions, thereby decreasing the fluorescence quantum yield [24]. The results of geometry optimisations of the Be^{2+} complexes of **I** and **IV** demonstrated that the angle between the chromene moiety and the phenyl ring changes upon excitation from 7.5 to 7.0° in the former case, but from 44.0 to 89.0° in the latter. Therefore, high Stokes shifts and low fluorescence quantum yields of complexes **III** and **IV** with metal ions may be the result of marked conformational changes accompanying electronic transitions.

Analysis of electronic absorption and fluorescence spectra (Table 3) generally confirms the conclusions drawn from examination of ^1H NMR spectra (Table 2). It would, however, be difficult to say anything about the constitution of complexes exclusively on the basis of the data in Table 2. To achieve a deeper insight into the nature of absorbing and emitting entities we optimised their structures, in the case of **I**-Mg^{2+} complexes solvated by methanol molecules (Fig. 2 provides possible structures of these in the ground electronic states), and predicted wave numbers of absorption and fluorescence transitions (Table 4). Comparison of the data in Tables 3 and 4 reveals quite good conformity between experimental and theoretical (for type **B** complexes) absorption transitions in **I**-Mg^{2+}, which furnishes additional proof that the **B** form predominates in the S_0 state. The theoretically predicted values of fluorescence transitions for type **B** and **E** complexes fit the experimental value equally well, which suggests that both forms may emit radiation. However,

Table 4. Theoretically predicted wave numbers of long-wavelength absorption and fluorescence transitions for non-complexed quercetine (**I**) and **I**-Mg^{2+} complexes

Entity		ν_{abs}, cm^{-1}	ν_{fl}, cm^{-1}	$\Delta\nu_{St}$, cm^{-1}
I		29520	24480	5040
I−Mg^{2+}	−**A**	25880	23280	2600
	−**B**	24760	21800	2960
	−**C**	18740	10400	8340
	−**D**	22150	13835	8315
	−**E**	22870	17890	4980

the conformity between experimental and theoretical Stokes shifts is best in type **E** complexes, not quite so good in **B** complexes. It is difficult to say how far these findings support the coexistence of the **B** and **E** forms of the **I**-Mg^{2+} complex in the S_1 state, especially as experimental and theoretical data indicate that type **B** complexes predominate in the S_0 state. No simple explanation can be offered now, although it may be that the discrepancies between the experimental and theoretical characteristics are due to constitutional changes in the solvation spheres that accompany the relaxation of excited molecules, and which could not be easily taken into account in the calculations.

The stability constants for the complexes investigated are listed in Table 5. They were all calculated on the basis of electronic absorption and fluorescence spectral data assuming 1:1 metal:flavone stoichiometry. There are some indications that not only 1:1 complexes but also 1:2 complexes can exist [25]. However, since it is possible to differentiate the absorption and fluorescence of 1:1 and 1:2 complexes, it is difficult to judge which ones predominate. Stability constants for the ground and excited states are comparable, which implies that the complexes investigated preserve their composition and chemical constitution upon electronic excitation, unlike other flavones, which may undergo dissociation under such conditions [25]. The increase in the number of methoxy groups in flavones generally results in some increase in the complexation ability. In the case of Mg^{2+} and Zn^{2+}, an increase in the degree of methylation causes a change at the complexation site and is accompanied by a change in the stability constant. Stability constants increase gradually for type **B** complexes of Mg^{2+}

Table 5. Base 10 logarithms of the stability constants of flavone complexes with metal ions in the ground and excited electronic states determined on the basis of electronic absorption (abs) and fluorescence (fl) spectral data

Flavone	Method	Complexes				
		Be^{2+}	Mg^{2+}	Ca^{2+}	Zn^{2+}	Cd^{2+}
I	abs	**A** 4.37±0.02	**B** 4.03±0.03	**B** 3.29±0.04	**B** 4.44±0.02	**B** 4.38±0.03
	fl	4.52±0.03	3.97±0.03		4.39±0.03	4.75±0.04
II	abs	**A** 4.52±0.08	**B** 4.27±0.05	**B** 3.80±0.05	**B** 4.75±0.01	**B** 4.43±0.03
	fl	4.52±0.03	4.21±0.04		4.66±0.04	4.40±0.05
III	abs	**A** 4.87±0.02	**B** 5.24±0.02	**B** 4.54±0.04	**B** 4.97±0.02	**B** 4.46±0.03
	fl	4.75±0.03	5.11±0.02	4.54±0.04	4.70±0.03	4.46±0.03
IV	abs	**A** 5.27±0.02	**A** 4.42±0.02		**A** 4.37±0.02	
	fl	5.28±0.03	4.42±0.03		4.37±0.03	
V	abs		**E** 3.50±0.04			
	fl		3.60±0.04			

with **I**, **II** and **III** and decrease for type **A** and **E** complexes with **IV** and **V**, respectively. It is clear from these data that type **E** complexes are relatively less stable than those of type **A** or **B**.

Stability constants are generally high, hence components are almost completely complexed in solution. One can also expect such complexes to be readily formed in the solid phase. Since, following photoexcitation, these compounds emit radiation much more efficiently than neat flavones, they can be considered potential chromophoric materials.

This paper provides basic information on the structure, stability and spectral properties of the complexes of pentahydroxyflavone (quercetine) and its alkyl derivatives (flavones) with selected ions of group **II**, namely Be^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+} and Cd^{2+}. The most stable complexes are those in which the carbonyl oxygen atom and the oxygen atom of a neighbouring hydroxy group are involved. The complexes formed exhibit comparable stability in the ground and excited electronic states. Absorption of complexes is shifted towards the long-wavelength region relative to that of non-complexed flavones. Almost non-fluorescing flavones begin to emit radiation when they are bound in complexes. This fluorescence weakens if the hydroxy groups on the lateral phenyl ring are methylated. The results of complementary theoretical studies confirmed the experimental findings and helped to explain them. The results of this study suggest that the application of flavone-metal ion complexes as photochromophores looks promising.

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Структура, стійкість та спектральні властивості комплексів флавонів з іонами металів II групи

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Одержано комплекси 2-(3,4-дігідроксифеніл)-3,5,7,-тригидрокси-4H-хромен-4-ону (кверцетину) і його алкілпохідних (далі флавони) з іонами Me^{2+} (Me = Be, Mg, Ca, Zn и Cd). Проведено дослідження комплексів з використанням ^1H ЯМР, електронної абсорбційної та флуоресцентної спектроскопії. За допомогою ^1H ЯМР спектроскопії встановлено будову комплексів і знаходження центрів комплексоутворення в молекулах флавонів. Спектральні дослідження дозволили отримати константи стійкості одержаних комплексів. Проведені полуемпіричні розрахунки можливої будови комплексів дали результати, що співпадають з експериментальними даними, а також дозволили пояснити природу електронних переходів у спектрах поглинання та випромінення. Показано, що невипромінюючі полігідроксифлавони та поліметоксифлавони при утворенні комплексів починають флуоресціювати, при чому інтенсивність флуоресценції залежить від кількісного співвідношення метокси- та гідроксигруп в молекулі. Одержана інформація може бути використана для синтезу фотохромів на основі сполук класу флавоноїдів.