

Structural and spectroscopic study of 5,7-dihydroxy-flavone and its complex with aluminum

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Abstract

The structure, stability and molar absorptivity of the complex formed between AlCl_3 and 5,7-dihydroxy-flavone in methanol were investigated using UV-Vis spectroscopy and the AM1 method. The molar ratio method and Job's method of continuous variation were applied to ascertain the stoichiometric composition of the complex in methanol at constant ionic strength. A 1:2 complex was indicated by both methods. The molar absorptivity and stability constant of the complex were determined using a simple and accurate procedure that requires solutions having the ligand and metal ion in the stoichiometric proportion. The high stability constant demonstrates that the complexation reaction is total. The structure of this complex, obtained by the quantum semi-empirical AM1 method, indicates that two classes of metal–ligand interactions are involved in the formation of the metal complex: (a) two simple covalent bonds between the aluminum atom and the oxygen atoms of *o*-hydroxyl groups of 5,7-dihydroxy-flavone; (b) two stronger Coulombic interactions between the aluminum atom and the carbonyl oxygen atoms of the ligand.

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1. Introduction

Flavonoids are natural products widely distributed in the vegetable kingdom and currently consumed in large amounts in the daily diet [1,2]. They are known to have a broad spectrum of biological and pharmaceutical activities and an elevated chemical reactivity [3–6]. Their complexing capacity, widely used for elucidating the structure of natural flavonoids, can also contribute to the bioactivity of these compounds, by acting as carriers and regulators of metal concentration. Some flavonoids possess a strong antioxidant effect by their radical-scavenging activity and metal ion chelation [7–9].

In order to determine the chelating capacity of metallic ions of simple flavonoids and benzophenones of biological interest, we recently studied the complexing reaction of 2-hydroxy-benzophenone and 2,4-dihydroxy-benzophenone

with AlCl_3 [10–12]. In this work, we determine the stoichiometry of the complex formed between AlCl_3 and 5,7-dihydroxy-flavone in methanol, its equilibrium constant and molar absorptivity. On the other hand, the structures of free and complexed molecules of the ligand are determined using the semiempirical AM1 method.

2. Experimental

2.1. Reagents

Fig. 1 shows the structure and chemical numbering system of 5,7-dihydroxy-flavone (**1**). Compound **1** from Sigma was purified by repeated crystallization from ethanol–water. The purity control was performed determining its melting point [13] ($\text{mp} = 285^\circ\text{C}$) and its TLC chromatographic properties [14]. Crystallized anhydrous aluminum chloride, crystallized sodium chloride, and methanol (MeOH) of spectroscopic grade from Fluka, were used without further purification.

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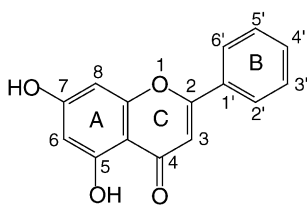


Fig. 1. Structure and chemical numbering system of 5,7-dihydroxy-flavone.

2.2. Procedures

All solutions were prepared by weighing with an accuracy of ± 0.0001 g. The experiments comprised: (a) the determination of the stoichiometric composition of complex formed by AlCl_3 and **1** in MeOH; (b) the determination of the molar absorptivity (ϵ_C) and stability constant (K_C) of this complex.

The molar ratio method, introduced by Yoe and Jones [15], was used for the spectrophotometric determination of the complex composition. For this method, solutions containing a constant concentration of **1** (1.124×10^{-4} M) and variable concentrations of AlCl_3 (from 1.068×10^{-5} to 2.926×10^{-4} M) were prepared in MeOH containing NaCl (2.80×10^{-2} M) for constant ionic strength. Under these operative conditions, about 1 h was necessary to reach the complexation equilibrium at 25.0 ± 0.1 °C. After this, the UV-Vis spectra were recorded on a Shimadzu UV 160 A double beam spectrophotometer with 1 cm thermostatically controlled cells, between 215 and 450 nm at the same temperature.

For the application of the continual variation method (Job's method) [16], the solutions were prepared by mix-

ing different volumes of equimolar stock solutions of **1** and AlCl_3 (5.10×10^{-3} M). In these solutions, the sum of the total concentration of ligand and metal was constant (2.48×10^{-4} M). Solutions containing only compound **1** in the same concentration as in the mixture were used for corrections of Job's plot.

In the determination of the ϵ_C and K_C of the complex, a spectrophotometric method designed by Ferretti et al. [17] was used. This procedure requires the preparation of solutions maintaining the molar ratio of ligand to metal ion, in the stoichiometric proportion. With this purpose, adequate aliquots of stock solutions of **1** and AlCl_3 were added to volumetric flasks containing approximately 4 g of MeOH to obtain a progressively increased concentration of the complex. The analytic concentrations of **1** and AlCl_3 in the reaction mixture were from 3.61×10^{-5} to 1.58×10^{-4} M and from 1.80×10^{-5} to 7.84×10^{-5} M, respectively. The ionic strength was adjusted to the value 2.80×10^{-2} with NaCl. After maintaining the reacting solutions for 1 h at 25 ± 0.1 °C, the respective equilibrium absorbances (A_e) were read at 382 nm, which is the maximum absorption wavelength of the complex in MeOH. Fig. 2 shows the UV-Vis spectra of **1**, both free and complexed with AlCl_3 in MeOH. At 382 nm the absorption of the free ligand is lower than in the complex.

3. Calculations

The molecular formula of the ligand chrysin or 5,7-dihydroxy-flavone is $\text{C}_{15}\text{H}_{10}\text{O}_4$, while that of the obtained

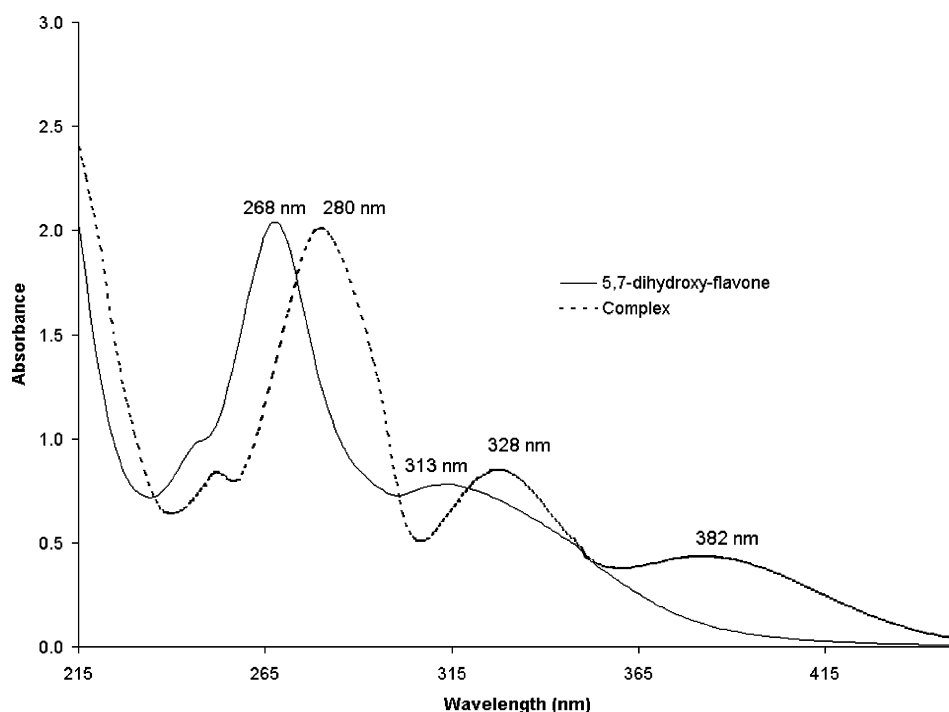


Fig. 2. UV-Vis spectra of 5,7-dihydroxy-flavone free and complexed with AlCl_3 in methanol.

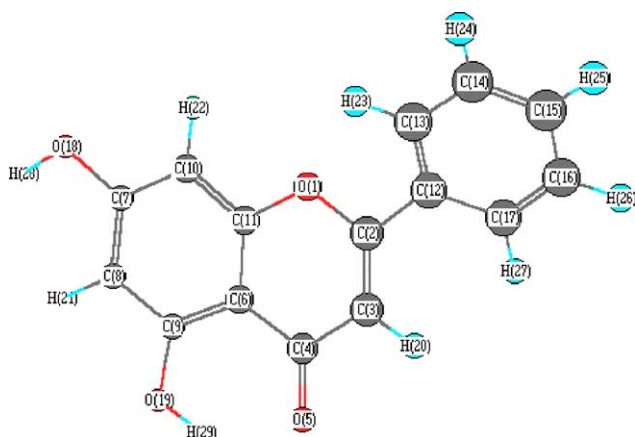


Fig. 3. Practical numbering system adopted in the calculations.

complex is $C_{30}H_{18}O_8-Al-Cl$. It is evident that this metallic complex possesses a high number of heavy atoms, which implies that the study of its structure by *ab initio* or DFT methods would require a high CPU time. Therefore, the structure of the complex was studied by the semiempirical MO AM1 method [18] included in the Gaussian 98 [19] program packages. It must be noted that the AM1 method does not allow analyzing the hexacoordinated aluminum atom in the obtained complexes; however, it is useful for description of the structural changes undergone by several compounds. This method has been previously used to explain the conformation, hydrogen bonding and UV solvatochromic shifts of benzophenones in primary alcohols [20]. Furthermore, the AM1 method has proved to be useful for satisfactorily describing the structural differences between free and chelated benzophenone molecules [10] and flavonoids [21,22] used as ligands of various metallic

ions. The calculations were performed on an Intel Pentium IV 1.4 GHz CPU with 512 MB of RAM. Fig. 3 shows the practical numbering system adopted for carrying out the calculations.

4. Results and discussion

The UV-Vis spectrum of **1** in MeOH (Fig. 2) is characterized by two major absorption bands with maxima at 313 nm (band I) and 268 nm (band II). Band I is considered to be associated with the absorption due to the B-ring cinnamoyl system, and band II with the absorption involving the A-ring system [23]. In particular, this compound, which is oxygenated in the A-ring but not in the B-ring, shows a spectrum with a pronounced band II and a weak band I. With $AlCl_3$, flavones containing hydroxyl groups at C-3 or C-5 form stable complexes between C-4 keto function and either the 3- or 5-hydroxyl group, producing a large bathochromic shift of band I. In Fig. 2, a bathochromic shift of 69 nm can be observed for band I, greater than that reported for 5(OH)flavone (60–61 nm) [23,24] and 3(OH)flavone (56–58 nm) [22,23].

4.1. Complex stoichiometry in methanol

The stoichiometry of the complex was determined applying the molar ratio method. The absorbance of band I of **1** decreases at 313 nm and a new band, which increases with the amount of added $AlCl_3$, appear at 382 nm. The data obtained from these experiments at 25.0 ± 0.1 °C are summarized in Fig. 4. In this figure, we plotted A_e against the $[AlCl_3]/[I]$ ratio. The straight lines built with the data of A_e obtained at 313 and 382 nm intersect at $[AlCl_3]/[I] = 0.5$, revealing the formation of an $Al(III)/I = 1:2$ complex. It

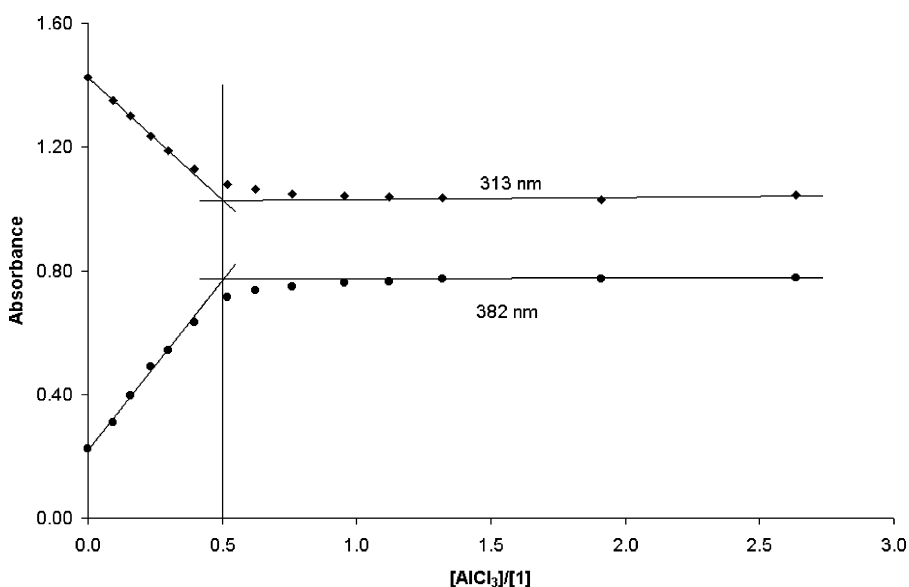


Fig. 4. Determination of the stoichiometry of the complex formed between $AlCl_3$ and 5,7-dihydroxy-flavone in methanol, by applying the molar ratio method.

can also be observed that the molecule of **1** is completely complexed for a ratio $[AlCl_3]/[I] = 1$.

Analyzing the complexation reactions between aluminum and flavonoids in MeOH, it can be stated that the chelating power of **1** is similar to that reported for 3(OH)flavone but greater than that determined for 5(OH)flavone [24] and 3',4'(OH)₂flavone [25]. Boudet et al. [22] have observed a complex $Al(III)/3(OH)flavone = 1:2$, and a ratio $[AlCl_3]/[ligand]$ of **1** is necessary to obtain a full complexation of 3(OH)flavone. Cornard et al. have studied the complexation of 5(OH)flavone [24] and 3',4'(OH)₂flavone [25] and have determined the formation of complexes of 1:1 stoichiometry. These molecules are completely complexed for a ratio $[AlCl_3]/[ligand]$ of 1.5 and 2, respectively.

Job's method (continual variation method) was applied to validate the stoichiometric composition of the chelate. The absorbance plots at 382 nm (Fig. 5) against the molar fraction of **1** (X_1) have a maximum at $X_1 = 0.64$ (close to 0.66), confirming that the stoichiometric ratio of $AlCl_3$ to **1** in the complex is 1:2.

4.2. Stability constant and molar absorptivity of the complex

Considering that the stoichiometry of complex is 1:2 and omitting the charges for the sake of simplicity, the complexation reaction can be written as,



where M represents $AlCl_3$; L represents ligand **1**, and C represents the metal complex. From now on, t is the time; M_0 and L_0 the molar concentrations of $AlCl_3$ and **1** for $t = 0$, respectively; and x the molar concentration of complex at

equilibrium ($t = \infty$). Considering that L and C, but not M, absorb some radiation at the selected wavelength (as frequently occurs), the total absorbance of the reacting solution in the equilibrium will be:

$$A_e = \varepsilon_L(L_0 - 2x)\ell + \varepsilon_C x \ell \quad (2)$$

where ε_L is the molar absorptivity of the ligand and ℓ the optical path length. Under the experimental conditions adopted in this work, $L_0 = 2M_0$, the stability constant of the complex is expressed as,

$$K_C = \frac{x}{(M_0 - x)(L_0 - 2x)^2} = \frac{2x}{(L_0 - 2x)^3} \quad (3)$$

Combining Eqs. (2) and (3) and solving the obtained cubic equation, the following expression is obtained [17]:

$$\frac{A_e}{\ell M_0} = \varepsilon_C + \frac{2\varepsilon_L - \varepsilon_C}{(4K)^{1/3}} \cdot \frac{1}{(M_0)^{2/3}} \quad (4)$$

Considering $\ell = 1$ cm, the experimental data obtained at ionic strength 2.80×10^{-2} and temperature 25.0 ± 0.1 °C were plotted according to Eq. (4) as shown in Fig. 6. The values obtained for ε_C and K_C from the intercept and slope of the straight line were:

$$\varepsilon_C (382 \text{ nm}) = 12,092 \text{ l mol}^{-1} \text{ cm}^{-1}$$

$$K_C (\text{MeOH}) = 4.55 \times 10^{11}$$

It is interesting to highlight that Eq. (4), besides permitting the determination of the ε_C and K_C values, would constitute an alternative for confirming the 1:2 stoichiometry. The high value of K_C shows that equilibrium is totally displaced towards the formation of the complex. The value of K_C for this complexation reaction in MeOH is comparable to

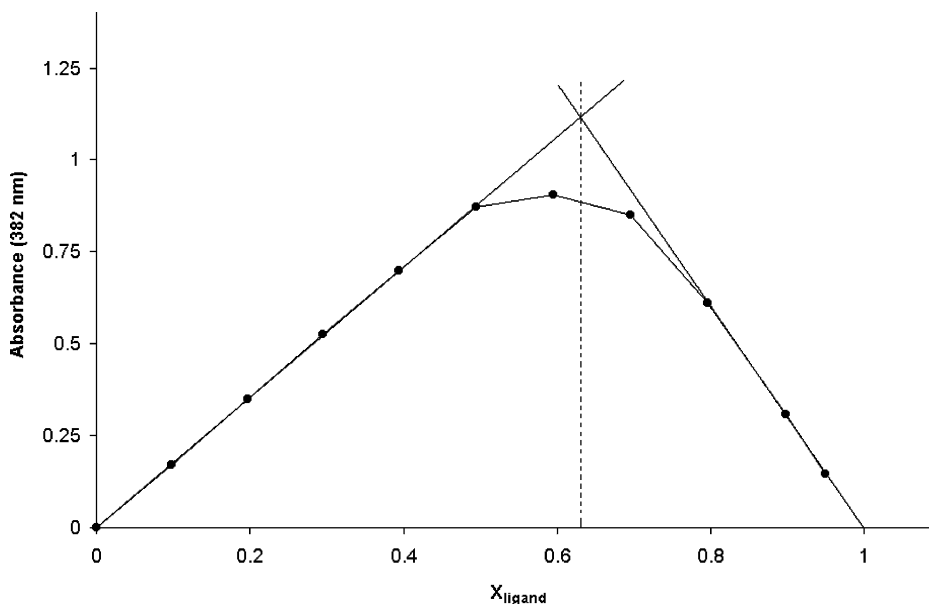


Fig. 5. Method of continual variations. Absorbance plot at 382 nm (λ_{max} of chelate) vs. molar fraction of ligand.

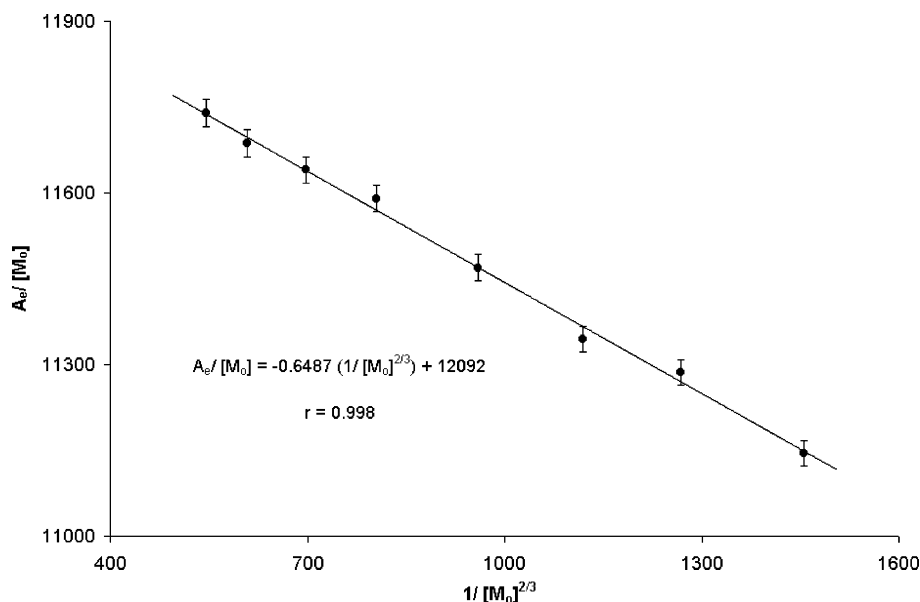


Fig. 6. Determination of stability constants and molar absorptivity of the complex formed between AlCl_3 and 5,7-dihydroxy-flavone in methanol. Error bars = 0.2%.

that reported for the chelation of aluminum by 3(OH)flavone (2×10^{12}) [25], also with 1:2 stoichiometry. Other flavonoids and benzophenones that complexate aluminum in a 1:1 stoichiometric relationship have lower equilibrium constant values (approximately 10^6) [10,12,25].

4.3. Structural properties of the complex

It is evident that the ligand suffers some structural changes when it reacts with AlCl_3 to form the metal complex (Figs. 3 and 7). Recently, Lau et al. [26] carried out a very exhaustive conformational study on **1** by means of ab initio methods at the RHF/6-31G(d) level of theory. These authors determined (in vacuum) that by rotation of the plane that involves the aromatic B-ring with respect to the plane containing the rest of the molecule around the single bond $\text{C}_2 - \text{C}_{1'}$ (Fig. 1), the dihedral angle D ($\text{C}_{17}\text{C}_{12}\text{C}_2\text{C}_3$) (Fig. 3) varies between 26.40° – 27.43° . Table 1 reports the optimized values of several structural properties calculated for both ligand and complex with the AM1 method. The data indicate that **1** has a non-planar structure.

Our calculations performed in MeOH at 298 K for ligand and complex show that the dihedral angles D ($\text{C}_{17}\text{C}_{12}\text{C}_2\text{C}_3$) are 27.5° and 26.7° , respectively. This implies that the folding of phenyl B-ring of both species with respect to the plane that comprises the rest of the molecule practically remains constant. This is to say, the above mentioned dihedral angle of the free molecule of **1** is not substantially modified when it is complexed. On the other hand, and as it should be expected, other molecular magnitudes are very different. As an example, the dipole moment (DM) of the complex is higher than **1**. This fact is closely linked to the changes in the bond lengths and net charges of the atoms involved in the forma-

Table 1

Calculated structural magnitudes with the AM1 method and using at the COSMO model for the ligand and metal complex methanol, at 298 K

Magnitude	Methanol	
	Ligand	Metal complex
DM	4.61	7.11
D ($\text{C}_{17}\text{C}_{12}\text{C}_2\text{C}_3$)	27.5	26.7
D ($\text{C}_{47}\text{C}_{42}\text{C}_{37}\text{C}_{32}$)	–	26.9
q (O_5)	–0.476	–0.401
q (O_{19})	–0.292	–0.410
q (O_{35})	–	–0.398
q (O_{49})	–	–0.408
q (Al_{21})	–	0.900
q (Cl_{20})	–	–0.385
r ($\text{Cl}_{20}-\text{Al}_{21}$)	–	2.219
r (C_4-O_5)	1.255	1.268
r (C_9-O_{19})	1.364	1.347
r ($\text{C}_{34}-\text{O}_{35}$)	–	1.268
r ($\text{C}_{39}-\text{O}_{49}$)	–	1.345
r ($\text{O}_{19}-\text{Al}_{21}$)	–	1.745
r ($\text{O}_{49}-\text{Al}_{21}$)	–	1.744
d ($\text{O}_5-\text{Al}_{21}$)	–	2.347
d ($\text{O}_{35}-\text{Al}_{21}$)	–	2.331
A ($\text{C}_6\text{C}_4\text{O}_5$)	123.2	124.2
A ($\text{C}_6\text{C}_9\text{O}_{19}$)	124.0	121.8
A ($\text{C}_{36}\text{C}_{34}\text{O}_{35}$)	–	124.4
A ($\text{C}_{36}\text{C}_{39}\text{O}_{49}$)	–	122.4
A ($\text{C}_9\text{O}_{19}\text{Al}_{21}$)	–	118.7
A ($\text{C}_{39}\text{O}_{49}\text{Al}_{21}$)	–	121.0

DM: dipolar moment (Debye); D : dihedral angle between the indicated atoms ($^\circ$) (Figs. 3 and 7); q : total atomic charge of the indicated atom (a.u.); r : bond length between the indicated atoms (\AA); d : Coulombic interaction distance between the indicated atoms (\AA); A : bond angle between the indicated atoms ($^\circ$).

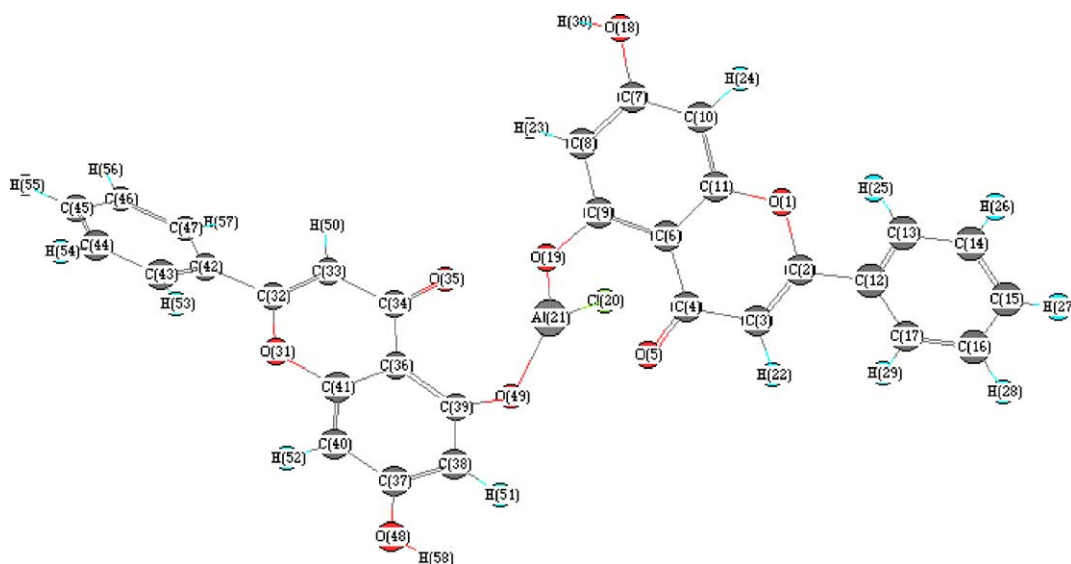


Fig. 7. Structure of the complex formed between AlCl_3 and 5,7-dihydroxy-flavone in methanol.

tion of the complex, namely: O_5 , O_{19} , O_{35} , O_{49} and Al_{21} . The union bond lengths and net charges of the chlorine and aluminum atoms in a normal AlCl_3 molecule are:

$$r(\text{Al}-\text{Cl}) = 1.874 \text{ \AA}; \quad q(\text{Al}) = 0.460 \text{ a.u.};$$

$$q(\text{Cl}) = -0.153 \text{ a.u.}$$

From Table 1, it can be seen that the three above magnitudes are markedly higher in the complex, as is also the case of the $q(\text{O}_{19})$, $q(\text{O}_{49})$ charges and the $r(\text{C}_4-\text{O}_5)$, $r(\text{C}_{34}-\text{O}_{35})$ bond lengths. It is obvious that the simultaneous increase of a bond's length and the charges of the implied atoms will markedly increase the polarization of the bond, and therefore the DM of the complex molecule. This explains why the DM of the complex is much higher than the DM of the free ligand.

It was proposed that two classes of metal–ligand interactions are involved in the formation of the metal complex: (a) two simple covalent bonds between the aluminum atom and the oxygen atoms of *o*-hydroxyl groups of **1** ($r(\text{O}_{19}-\text{Al}_{21})$ and $r(\text{O}_{49}-\text{Al}_{21})$); (b) two stronger Coulombic interactions between the central atom and the carbonyl oxygen atoms of the ligand ($d(\text{O}_5-\text{Al}_{21})$ and $d(\text{O}_{35}-\text{Al}_{21})$). From Table 1, it can be observed that although the $d(\text{O}_{35}-\text{Al}_{21})$ distance is lower than $d(\text{O}_5-\text{Al}_{21})$, the difference between these two magnitudes is very small (under 1%). Furthermore, it must be pointed out that the values for $r(\text{O}_{19}-\text{Al}_{21})$ and $r(\text{O}_{49}-\text{Al}_{21})$ are practically identical. It is also observed that the bond lengths *o*-C–OH ($r(\text{C}_9-\text{O}_{19})$, $r(\text{C}_{39}-\text{O}_{49})$) in the free molecules of **1** suffer a slight decrease of 0.017 Å when they are complexed. The opposite occurs in the case of the double bond C=O ($r(\text{C}_4-\text{O}_5)$, $r(\text{C}_{34}-\text{O}_{35})$). The carbonylic bond lengths in the complex are approximately 0.013 Å above the length bond C=O that characterizes the free ligand. Opposite behaviors are also observed in the total atomic

charges of the oxygen atoms of carbonyl ($q(\text{O}_5)$, $q(\text{O}_{35})$) and hydroxyl ($q(\text{O}_{19})$, $q(\text{O}_{49})$) groups. While the $q(\text{O}_5)$, $q(\text{O}_{35})$ of the complex are lower (in absolute value) than the $q(\text{O}_5)$ values of free molecules, the opposite occurs in the case of the $q(\text{O}_{19})$ charges. These observations permit to conclude that the formation of the complex implies an increase of length bond C=O together with a decrease of $q(\text{O}_5)$, and a decrease of length bond *o*-C–OH accompanied by an increase of $q(\text{O}_{19})$. Overall, the structural changes suffered by **1** upon forming the complex bear a certain similarity with those previously observed in the complexation reaction of AlCl_3 with *o*-hydroxy-benzophenones [10].

5. Conclusions

The UV-Vis spectra of **1** in MeOH exhibit two main absorption bands at 313 and 268 nm. This compound, with AlCl_3 in pure MeOH at 25 °C maintaining the ionic strength constant, reacts easily reaching the complexation equilibrium at about 1 h. Therefore, band I suffers bathochromic shifts of 69 nm. Applying the molar ratio method and Job's method, it was determined that the stoichiometric composition of the complex formed is 1:2. Furthermore, additional experiments allowed obtaining the value of the constant of stability. Considering the elevated value of the equilibrium constant of the investigated complexation reaction, it can also be inferred that 5,7-dihydroxy-flavone constitutes an analytical reagent of great interest due to its potential use in the quantitative determination of metallic ions. The structure of the complex was satisfactorily explained by means of the theoretical study performed using the AM1 method. The calculated length bonds, energy and reactivity indices permitted to conclude that the formation of the complex

implies an increase of bond C=O length together with a decrease of q (O_5), and a decrease of length bond o -C–OH accompanied by an increase of q (O_{19}). This is in agreement with the observed MD. Two classes of metal–ligand interactions are involved in the formation of the metal complex: (a) two simple covalent bonds between the aluminum atom and the oxygen atoms of o -hydroxyl groups of **1**; (b) two stronger Coulombic interactions between the aluminum atom and the carbonyl oxygen atoms of the ligand.

Acknowledgements

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