Novel Synthesis of 3,3′-Dihydroxyflavone and Apparent Formation Constants of Flavonoid−Ga(III) Complexes

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Flavonoids are a wide variety of polyphenolic compounds. They have many biological properties. They also exhibit a metallic ion complexation capacity, which is important since it has been found that biological properties these ligands present can be enhanced when complexation occurs. In this work four flavonoid−Ga(III) complexes were studied: 2′,4′,3-trihydroxychalcone−Ga(III), 2′,3-dihydroxychalcone−Ga(III), 3,7,3′-trihydroxyflavone−Ga(III), and 3,3′-dihydroxyflavone−Ga(III). All ligands were synthesized by our working group, contributing a novel, cheaper, and more simple way to obtain 3,3′-dihydroxyflavone. The stoichiometry of the complexes was determined using the Yoe−Jones method. Both chalcone−Ga(III) complexes presented 1:1 L:M stoichiometry; meanwhile flavone−Ga(III) complexes presented 2:1 L:M stoichiometry. The p*K* values were determined by means of a spectrophotometric method. Values obtained were: 2′,4′,3-trihydroxychalcone−Ga(III), pK = 4.35 ± 0.38; 2′,3-dihydroxychalcone−Ga(III), pK = 3.40 ± 0.17; 3,7,3′-trihydroxyflavone−Ga(III), pK = 7.74 ± 0.19; and 3,3′-dihydroxyflavone−Ga(III), pK = 8.46 ± 0.27.

Introduction

There is a wide variety of polyphenolic compounds named flavonoids. These are extensively distributed in plants, and they are considered to be dietary antioxidants. Flavones and chalcones are two different subclasses of this family. Flavones have been studied because of their biological properties such as radical scavenging[1] and anti-inflammatory[2] and anthelmintic[3] activities, while chalcones are important due to their anti HIV[4] and antitumoral activity[5] among others.

Chalcones can be found in nature and extracted from different plants but also can be synthesized through Claisen−Schmidt's reaction. This method involves the reaction between acetophenone and benzaldehyde, both appropriately substituted, and aqueous alkaline solution. An α,β-unsaturated ketone is formed under these conditions.[6]

Flavones can also be synthesized by cycling chalcones in an alkaline medium[7] or through oxidation using selenium or heating with palladium.[6] In this work a new method to synthesize these compounds is informed, which successfully achieved 3,3′-dihydroxyflavone starting from 2-hydroxyacetophenone and 3-hydroxybenzaldehyde. This means that the synthesis of the intermediate chalcone was not necessary, giving as a result a cheaper and simpler method.

Many flavonoids have a metallic ion complexation capacity.[8−10] The study of this capacity is important because it has been found that the biological properties of these ligands can be enhanced when complexation occurs.[11] In this work the gallium(III) ion was chosen because of its many applications in industry as a semiconductor. Many Ga(III) complexes are being studied due to their cytotoxic potential[12] and for tumor detection.[13] The molar ratio of each complex was evaluated by the Yoe−Jones method,[14] whereas formation of the complexes between these compounds and Ga(III) was determined spectrophotometrically using a graphic linear method previously developed by our work group.[15]

Experimental Section

Reagents. 2-Hydroxyacetophenone, 2,4-dihydroxyacetophenone, and 3-hydroxybenzaldehyde were purchased from Fluka. KOH was provided by Cicarelli and HCl by Tetrahedron. H_2O_2 was obtained from Parafarm. Ga(III) solutions were prepared using $GaCl_3$ provided by Alfa-Aesar. Methanol (Merck spectroscopic grade) was used for preparating all methanolic solutions.

Apparatus. An Agilent 8454 diodo-array spectrophotometer was used to record the ligands and their complex spectra as well as to identify the synthesized compounds.

Synthesis. 2′,3-Dihydroxychalcone was synthesized by condensing 2-hydroxyacetophenone and 3-hydroxybenzaldehyde in an alkaline medium. An ethanolic equimolar solution of both reactants was mixed with a 13 M KOH solution. The mixture was kept at 4 °C for three days. Later it was water-diluted and acidified with HCl:H_2O 1:1. The yellow product obtained was filtered and recrystallized from a water−ethanol mixture. Purification was carried out by chromatography, using a Sephadex LH 20 column and methanol as eluent.

2′,4′,3-Trihydroxychalcone was synthesized by condensing in an alkaline medium 3-hydroxybenzaldehyde and 2,4-dihydroxyacetophenone. An ethanolic equimolar solution of both reactants was mixed with a 12.5 M KOH solution. The mixture was kept at 4 °C for seven days. Later it was water-acidified using HCl:H_2O 1:1. The yellow product obtained was filtered and recrystallized from a water−ethanol mixture.

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Table 1. Identification Data of Synthesized Flavonoids

compound	Rf (TLC)	UV $\lambda_{\max}$ (MeOH) (ε)
2′,4′,3-trihydroxychalcone	0.18	356 (20947) 253 (8968)
3,7,3′-trihydroxyflavone	0.14	318.6 (16052) 244.8 (15447)
2′,3-dihydroxychalcone	0.26	311 (20144) 253 (10058)
3′,3-dihydroxyflavone	0.32	334 (20665) 304 (12808) 244 (24783)

3,7,3′-Trihydroxyflavone was synthesized, purified, and identified according to the method reported by Tanaka and Sujino[7] using a methanolic solution of 2′,4′,3-trihydroxychalcone, where 8 M NaOH and a 30 % hydrogen peroxide solution were added. The mixture was stirred at room temperature for 2 h. Then, it was neutralized with 6 M HCl solution. The yellow product obtained was filtered and recrystallized from a water–ethanol mixture.

All flavonoid syntheses were followed using thin layer chromatography (poliamide 11 F_{254}, mobile phase: methanol:acetic acid:water, 90:5:5), and the very same method was used to determine the purity of the compounds.

Novel 3,3′-Dihydroxyflavone Synthesis. 3,3′-Dihydroxyflavone was also obtained by condensation between 2-hydroxyacetophenone and 3-hydroxybenzaldehyde in an alkaline medium. An equimolar ethanolic solution of both reactants was prepared and mixed with a 13 M KOH solution and a 30 % hydrogen peroxide solution. The mixture was stirred for 7 h; then it was diluted and neutralized with $HCl:H_2O$ 1:1. The precipitated compound was filtered and recrystallized from a water–ethanol mixture. The synthesis yield was 33 %.

Synthesis was followed using thin layer chromatography (poliamide 11 F_{254}, mobile phase: methanol:acetic acid:water, 90:5:5), and the very same method was used to determine the purity of this compound.

Identification of Synthesized Compounds. To identify the synthesized flavonoids, an Agilent 8454 diodo-array spectrophotometer was used to compare the ultraviolet visible spectrum of the synthesized compounds with the spectra of previously identified 2′,4′,3-trihydroxychalcone, 2′,3-dihydroxychalcone, 3,7,3′-trihydroxyflavone, and 3,3′-dihydroxyflavone. Thin layer chromatography assays were also performed. Results can be observed in Table 1.[1] The structures of studied flavonoids are shown in Figure 1.

Stoichiometry Determination. To determine the stoichiometries of the studied complexes, the Yoe–Jones method was used. This is a spectrophotometric method that allows the determination of molar composition of metallic complexes. The method consists of preparing a set of solutions keeping constant the metallic ion concentration and varying the ligand concentration. The absorbance of these solutions was measured and used to plot a graphic of absorbance versus ligand/metal concentration ratio, L/M. The intersection points between straight lines of experimental data indicate the ligand/metal molar ratio.

Apparent Stability Constant Determination. A graphic linear method was used to find out the stability constant of the complexes. This easy and of extensive applicability method, previously developed by our work group,[15] can be applied to mono- and polynuclear complexes. It only requires the analytical concentration of reactants and absorbances of the equilibrium solutions. The general expression for L_nM complexes is:

$$[L]_j^n + n^2[L]_j^{n-1}[M]_j = \frac{([L]_r^n + n^2[L]_r^{n-1}[M]_r + K')A_r}{[L]_r^n + [M]_r}\frac{[L]_j^n[M]_j}{A_j} - \frac{1}{K} \tag{1}$$

where [L] is the ligand concentration, [M] metallic ion concentration, K is the apparent equilibrium constant, $K' = 1/K$, A is the reacting solution absorbance, and r and j indicate two equilibrium solutions.

The graphic representation of $[L]_j^n + n^2[L]_j^{n-1}$ $[M]_j$ versus $[L]_j^n[M]_j/A_j$ is a straight line that allows the estimation of the apparent formation constant from the slope and from the intercept. The average of these two values is the apparent formation constant value for every experiment performed. These experiments are carried out three times for every complex studied.

Some of the studied systems presented 1:1 L/M stoichiometry. The expression for LM complexes, considering eq 1 is:

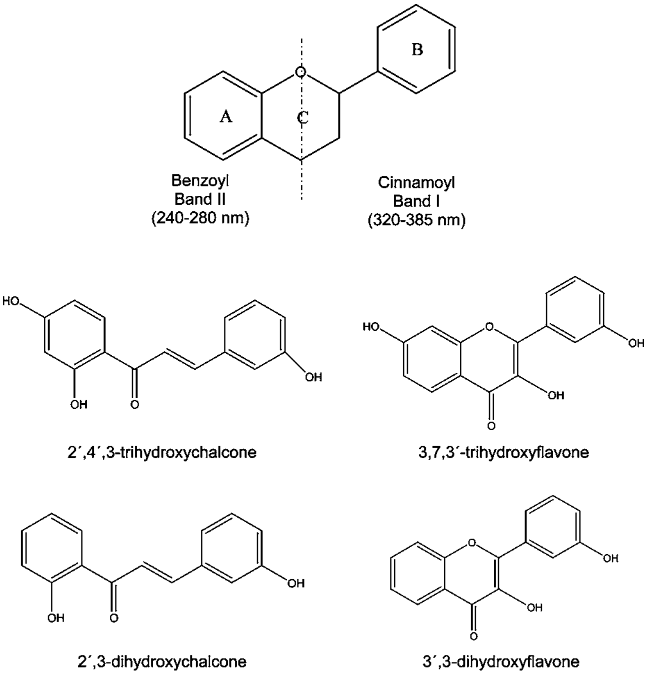
$$[L]_j + [M]_j = \frac{([L]_r + [M]_r + K')A_r}{[L]_r + [M]_r}\frac{[L]_j[M]_j}{A_j} - \frac{1}{K} \tag{2}$$

and to obtain the straight line for this kind of complex the graphic representation of $[L]_j + [M]_j$ versus $[L]_j[M]_j/A_j$ must be done (eq 2). In the case of a L_2M complex, according to eq 3, a graphic of $[L]_j^2 + 4\,[L]_j[M]_j$ versus $[L]_j^2[M]_j/A_j$ gives a straight line that allows estimation of the apparent formation constant from the slope and from the intercept.

$$[L]_j^2 + 4[L]_j[M]_j = \frac{([L]_r^2 + 4[L]_r[M]_r + K')A_r}{[L]_r^2 + [M]_r}\frac{[L]_j^2[M]_j}{A_j} - \frac{1}{K} \tag{3}$$

Results and Discussion

After the flavonoids were synthesized, purified, and identified, some tests were performed to ensure the formation of complexes. Tests were carried out preparing methanolic solution of



2′,4′,3-trihydroxychalcone

3,7,3′-trihydroxyflavone

2′,3-dihydroxychalcone

3′,3-dihydroxyflavone

Figure 1. Skeleton of flavonoids and studied flavonoid structures.

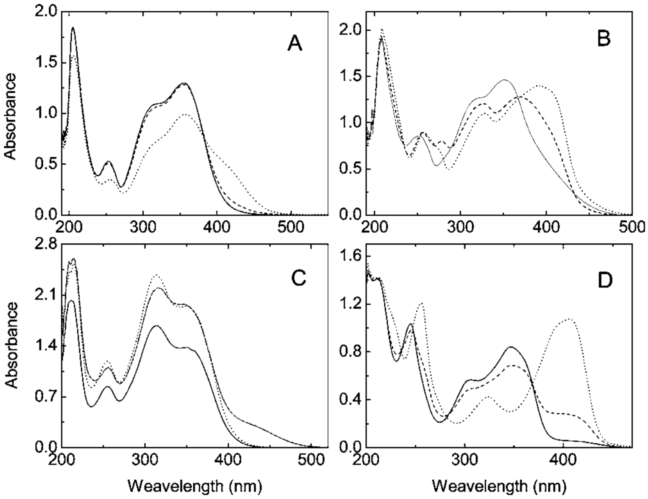


Figure 2. Spectral changes of the ligands upon the addition of the metallic ion soution **A** [2′,4′,3-trihydroxychalcone (solid curve), 2′,4′,3-trihydroxychalcone + Ga(III) L/M = 4 (dashed curve), 2′,4′,3-trihydroxychalcone + Ga(III) L/M = 0.25 (dotted curve)], **B** [3,7,3′-trihydroxyflavone (solid curve), 3,7,3′-trihydroxyflavone + Ga(III) L/M = 4.67 (dashed curve), 3,7,3′-trihydroxyflavone + Ga(III) L/M = 0.28 (dotted curve)], **C** [2′,3-dihydroxychalcone (solid curve), 2′,3-dihydroxychalcone + Ga(III) L/M = 4.13 (dashed curve), 2′,3-dihydroxychalcone + Ga(III) L/M = 0.25 (dotted curve)], and **D** [3,3′-dihydroxyflavone (solid curve), 3,3′-dihydroxyflavone + Ga(III) L/M = 3.55 (dashed curve), 3,3′-dihydroxyflavone + Ga(III) L/M = 0.21 (dotted curve)].

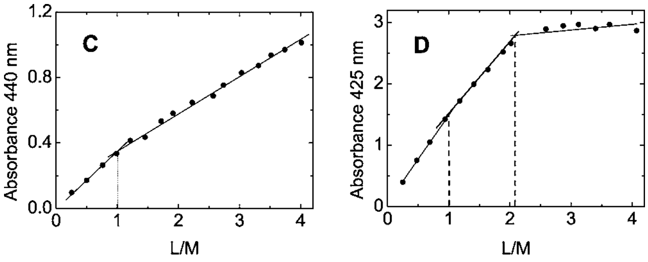


Figure 3. Stoichiometry determination. **C**: 2′,3-dihydroxychalcone−Ga(III), **D**: 3,3′-dihydroxyflavone−Ga(III).

every ligand and the metallic ion. Ligand solution spectra were recorded before and after adding Ga(III) solution. The formation of the complex was confirmed by comparing the spectra obtained, where a bathochromic shift was observed. The complex formation was evaluated using two values of ligand/metal concentration ratio (L/Me): 0.25 and 4. Spectra are shown in Figure 2.

Flavonoids display two major absorption bands in the ultraviolet−visible region. Band I corresponds to the absorption in the (320 to 385) nm range and is related to the cinnamoyl system. The absorption in the (240 to 280) nm range, Band II, corresponds to the benzoyl system (see Figure 2). Compared with flavonoids, absorption spectra Band II in complexes are shifted to higher wavelengths. This red shift is caused by the increased conjugative effect when the complex is formed to give a new ring with the metallic ion.

To select the appropriate conditions for metallic complex formation, two tests were carried out, using an excess of one of the reactants in each one. Results showed that 2′,4′,3-trihydroxychalcone only complexes Ga(III) when the ligand concentration is lower than the metallic ion concentration. The opposite effect is observed in the complexation between 2′,3-dihydroxychalcone and Ga(III). Both flavones can complex the metallic ion without regard of which reactant is present in excess.

Stoichiometries of complexes were determined by the Yoe−Jones method, previously described. Figure 3 shows the 2′,3-dihydroxychalcone−Ga(III) and 3,3′-dihydroxyflavone−Ga(III) graphic obtained. The observed stoichiometry for both chalcone−Ga(III) complexes was 1:1 L/M, while flavone−Ga(III) complexes presented stoichiometry 2:1 L/M.

The apparent equilibrium constants of the complexes were determined at 25 °C using the graphic linear method described before. The ligand/metal ratios used were: 2′,4′,3-trihydroxychalcone−Ga(III), 0.26; 3,7,3′-trihydroxyflavone−Ga(III), 0.22; 2′,3-dihydroxychalcone−Ga(III), 2; and 3,3′-dihydroxyflavone−Ga(III), 6. The experiments to determine the apparent equilibrium constant values were carried out three times for every complex. Figure 4 shows the experimental data obtained for one of the three experiments carried out for every studied complex. Data from the

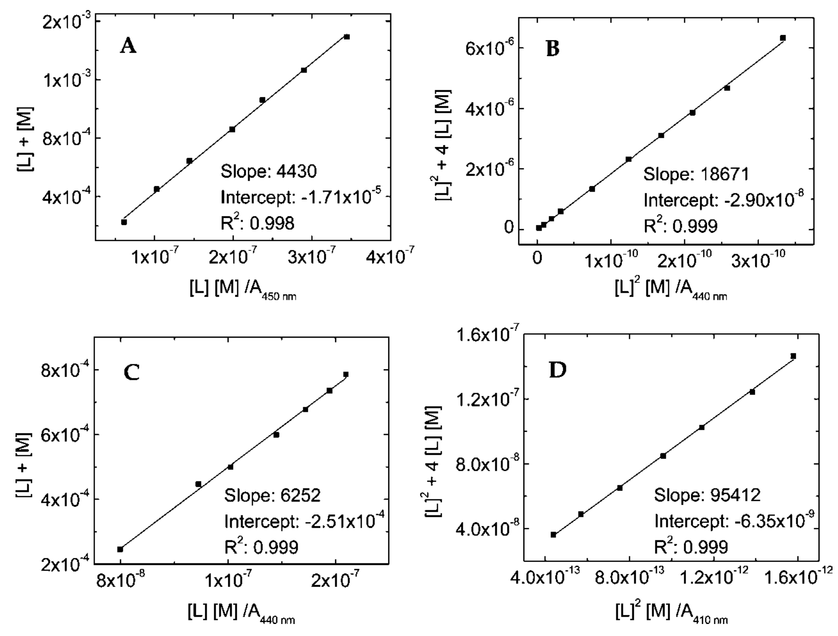


Figure 4. Graphical apparent equilibrium constant determination: experimental data obtained for one of the three experiments carried out for every studied complex. **A:** 2′,4′,3-trihydroxychalcone-Ga(III), **B:** 3,7,3′-trihydroxyflavone-Ga(III), **C:** 2′,3-dihydroxychalcone-Ga(III), **D:** 3,3′-dihydroxyflavone-Ga(III).

Table 2. Stoichiometries, Apparent Equilibrium Constant, and p*K* Values of Studied Systems at 25 °C

system	stoichiometry	*K*	p*K*
2′,4′,3-trihydroxychalcone−Ga(III)	1:1	$2.239 \cdot 10^{4}$	4.35 ± 0.38
3,7,3′-trihydroxyflavone−Ga(III)	2:1	$5.495 \cdot 10^{7}$	7.74 ± 0.19
2′,3-dihydroxychalcone−Ga(III)	1:1	$2.512 \cdot 10^{3}$	3.40 ± 0.17
3,3′-dihydroxyflavone−Ga(III)	2:1	$2.884 \cdot 10^{8}$	8.46 ± 0.27

12 experiments performed can be observed in the Supporting Information section. Stoichiometries, apparent equilibrium constants and p*K* values of every system are listed in Table 2.

Considering that both chalcones presented 1:1 L/M stoichiometry, the graphic representation of $[L]_j + [M]_j$ versus $[L]_j[M]_j/A_j$ for a set of known concentration solutions in equilibrium gave a straight line that allowed the estimation of the apparent formation constant from the slope and from the intercept. The average of these two values was the apparent formation equilibrium constant. The values obtained from the three experiments carried out for every chalcone were used to calculate the informed average apparent formation equilibrium constant, p*K* values, and experimental errors (see Table 2).

2′-Hydroxychalcones may complex metallic ions through a stable six-atom ring. This ring might involve an oxygen atom from the carbonyl group and an OH group in $C_{2'}$. The apparent equilibrium constant values for chalcone−Ga(III) systems show that the presence of an hydroxyl group in the 4′ position of the ligand increases the complex stability. This effect might occur due to the increased oxygen electronegativity of the carbonyl group. The stoichiometry and apparent equilibrium constant value for trihydroxylated chalcone shows that it could be considered as an analytical reagent for Ga(III).

3,3′-Dihydroxyflavone and 3,7,3′-trihydroxyflavone formed L_2M complexes. In these cases, according to eq 3, a graphic of $[L]_j^2 + 4\,[L]_j[M]_j$ versus $[L]_j^2\,[M]_j/A_j$ gave a straight line that allowed the estimation of the apparent formation constant from the slope and from the intercept. The average of these two values was the apparent formation equilibrium constant. Again, the values obtained from the three experiments carried out for every flavone were used to calculate the informed average apparent formation equilibrium constant, p*K* values, and experimental errors.

Flavone-metallic ion coordination could involve oxygen atoms from the OH in C_3 and carbonyl group, forming a stable five-atom ring. These results agree with those reported by De Souza and De Giovanni[16] who used pentahydroxylated flavones as ligands. According to the values in the table, the introduction of an additional hydroxyl group in position 7 could not contribute to stability of the complex.

Conclusions

In this work four flavonoids were synthesized. 2′,3-Dihydroxychalcone, 2′,4′,3-trihydroxychalcone, and 3,7,3′-trihydroxyflavone were successfully synthesized using a common method of synthesis. A novel synthesis method to achieve highly pure 3,3′-dihydroxyflavone was developed. This method obtains the compound in a simpler and more economic way, without the intermediate chalcone synthesis step.

The four studied flavonoids were able to complex the Ga(III) metallic ion. The stoichiometry and values of the apparent formation equilibrium constants of all complexes were determined. Chalcone−Ga(III) complexes are formed involving a six-membered ring and presented 1:1 stoichiometry. The apparent equilibrium constant values for chalcone−Ga(III) systems show that introducing an hydroxyl group in the 4′-position of the ligand increases the oxygen electronegativity of the carbonyl group and, thus, the complex stability. The apparent equilibrium constant value for trihydroxylated chalcone shows that it could be considered as an analytical reagent for Ga(III).

Flavone−Ga(III) complexes are formed involving a five-membered ring and presented 2:1 stoichiometry. According to the values in the table, the introduction of an additional hydroxyl group in position 7 could not contribute to stability of the complex.

Supporting Information Available:

Data from the three apparent constant determination carried out for every system studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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