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Spectroscopic and structural study of complexes of quercetin with Al(III)

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Abstract

Complex formation between aluminium and quercetin (Q) in methanol was studied by the combined use of spectroscopic measurements and quantum chemical calculations. Quercetin presents in its structure three possible chelating sites in competition. UV–visible spectroscopy has showed the successive formation of two complexes of stoichiometry Al(III):Q of 1:2 and 2:1, respectively. The first site involved in the complex formation process is the 3-hydroxychromone and the second one is the *ortho*-dihydroxyl group. Semiempirical treatment, using the AM1 hamiltonian, permitted calculation of the structural modifications engendered by the ligand through chelation of one then two aluminium ions. The electronic and vibrational spectra have been calculated with the same method in order to compare them to the experimental spectra and so confirm the involved chelating sites. The simulated electronic spectra obtained from the complex models are in good agreement with the experimental UV–visible absorption spectra. In the same way the vibrational spectra of the complexes validate the proposed complex formation mechanism. The pH influence on the complexes stoichiometry and on the preferentially occupied chelating sites has been also investigated.

Keywords: Quercetin; Al(III) complexation; Stoichiometry; UV–Vis spectroscopy; Raman spectroscopy; AM1 calculation

1. Introduction

Flavonoids, 2-phenyl-benzo-α pyrones, are polyphenolic compounds that occur ubiquitously in plant kingdom. A multitude of substitution patterns in the two benzene rings (A and B) of the basic structure occur in nature. Variations in their heterocyclic rings gives rise to flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavones. Over 4,000 different naturally occurring flavonoids have been described [1] and this list is still growing. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most common flavonols present in nature that has attracted the attention of many researchers because of its biological and pharmaceutical properties [2–4]. Quercetin is a strong antioxidant and a major dietary flavonoid. Although the antioxidant activity of the polyhydroxyflavones is primarily a function of their ability to act as free radical acceptors, the metal-complexing properties of these molecules may make some contribution to their total activity. So, the antioxidant action of quercetin appears to be a combination of reaction with free radicals and metal ion complexing. Some studies have revealed that quercetin functions as antioxidant mainly by chelating metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}) and by scavenging peroxyl radicals whereas their OH radical scavenging effect is much less important [5–8]. In contrast, Masataka et al. report that the presence of aluminium ions could attenuated the antioxidant action of flavonoids such as quercetin [9]. As metal chelators, the flavonoids play an important role in both the bioavailability and toxicity of a variety of metals. For example, the complexation of Al(III) by quercetin reduces the overload of aluminium in the diet, a metal which has been implicated in neurological and bone disorders [10,11].

Quercetin possesses three possible chelating sites in competition: the 3-hydroxychromone, the 5-hydroxychromone and the 3′4′-dihydroxyl groups (Fig. 1). Quercetin has been used before as colorimetric reagents for the spectrophotometric and also for fluorimetric determination of metal ions [12,13]. The complexes formed between quercetin and metal exhibit highly sensitive molecular fluorescence properties and are used in analytical methods for the detection of trace and ultra trace of metals. A great number of methods for studying the binding of Al(III) to the quercetin molecule, such as UV–Vis or fluorescence spectroscopies, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopies have been used. But these studies

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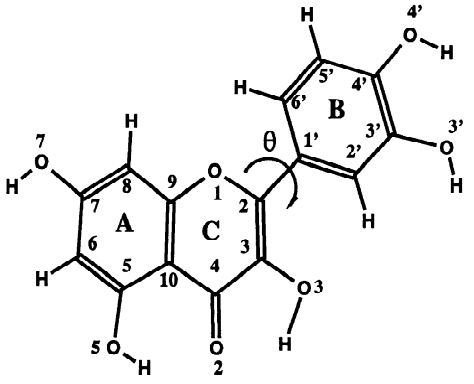

Fig. 1. Representation and atomic numbering adopted for quercetin.

do not reveal information on the coordination and the structure of the metal ion binding sites as well as conformational changes that occur in the ligand.

The present contribution is essentially an attempt to compare the chelating power between sites of different type present in a polyfunctional natural ligand: the quercetin molecule. The aim of this work is to determine: (i) the stoichiometric composition and the formation mechanism of different complexes obtained between quercetin and Al(III) in methanoic solution in different pH conditions, (ii) the preferential site implicated in each chelation process step and (iii) the structural modifications of the ligand engendered by the chelation. The joint utilisation of spectroscopic measurements (UV–Vis and Raman spectroscopies) and quantum chemical calculations seems to be convenient to reach the research goal.

It is obvious that a better knowledge of the chelation process between Al(III) and quercetin and of the structural modifications of the ligand in the complex could permit a better understanding of the changes observed in the antioxidant properties of quercetin in presence of aluminium.

2. Experimental

2.1. Reagents and methods

Quercetin (Q) was obtained from Extrasynthèse (France). Because of the very poor solubility of quercetin in water, spectroscopic-grade methanol was used. The stoichiometry of complexes was determined by the molar ratio method. A concentration of 4×10^{-5} M of quercetin in methanol was kept constant whereas $AlCl_3$ was varied from 4×10^{-7} to 4×10^{-3} M. For the study in acidic medium, both quercetin and $AlCl_3$ solutions was realised in a methanol–water (90:10) mixture, containing NaCl (10^{-1} M) for constant ionic strength. In these conditions, the aqueous solution consisted of 3.2×10^{-2} M HCl (apparent pH 2.5).

2.2. Instrumentation

Absorption spectra were run at room temperature in 1 cm quartz cells on a double-beam spectrophotometer (Cary Model 13E). The Fourier transform (FT) Raman spectra were recorded with 4 cm^{-1} resolution on a Bruker IFS 88W instrument equipped with an FRA 106 FT-Raman accessory. The excitation in the near-infrared range (1.06 μm) permits one to avoid the fluorescence emission of studied samples.

2.3. Calculations

The size of the system, and notably of the complexes, does not allow one to use ab initio or DFT calculations to optimise the molecular conformation or to calculate vibrational and electronic spectra. In order to arrive at meaningful results, it has been necessary first to test computational methods to establish their suitability for the study of flavonoid structures. Ours previous work [14,15] has shown, that among the semiempirical methods, the AM1 (Austin Model 1) method [16] is the most appropriate to determine the structure of the flavonoid compounds and to reproduce the experimental data, like electronic or vibrational spectra. In a previous article [17], we have studied the free quercetin molecule using the AM1 quantum mechanical semiempirical method and good agreement was observed between the calculated and experimental Raman and electronic spectra. Other studies concerning the molecular modelling of quercetin and of its radical species have also used the AM1 method with success [18]. The same semiempirical method implemented in the Hyperchem (version 5.0) program [19] was used, here, to carry out all the calculations. The geometries of the free and complexed molecules were fully optimised without restrictions with a gradient criterion set to 10^{-3}. The electronic absorption spectra were calculated by using a configuration interaction treatment. The contribution of singly excited configurations between the nine highest occupied molecular orbitals and the nine lowest unoccupied molecular orbitals have been considered.

3. Results and discussion

3.1. Stoichiometry and involving sites

In earlier studies [20–22], we reported the behaviour towards Al(III) complexation of flavonoids presenting only one chelating site: 3-hydroxyflavone (3HF), 5-hydroxyflavone (5HF) and 3′,4′-dihydroxyflavone (3′4′diOHF). Table 1 summarises the main characteristics (stoichiometries and stability constants) of complexes obtained in different medium: (i) pure methanol, (ii) alkaline medium (methanol containing sodium methanoate, 10^{-1} M) and (iii) acidic medium consisting of a mixture of methanol–

Table 1
Stoichiometry of complexes of Al(III) with 3′4′diOHF, 3HF and 5HF in methanol in different media and stability constants

		3′4′-Dihydroxyflavone	3-Hydroxyflavone	5-Hydroxyflavone
Pure methanol	Stoichiometry	1:1	1:2	1:1
	Stability constant	5×10^{6}	2×10^{12}	3×10^{6}
Methanol (alkaline medium)	Stoichiometry	1:3	1:2	1:1
Methanol (acidic medium)	Stoichiometry	–	2:1	1:1*

* Obtained for a large amount of Al(III).

water (90:10) with hydrochloric acid, 10^{-2} M. In pure methanol, the results clearly show that among the three possible complexing sites, the 3-hydroxychromone group presents the strongest chelating power, indeed two 3HF ligands bonded the Al(III) ion whereas a 1:1 stoichiometry is observed for the complex of both 5HF and 3′4′diOHF. These two last molecules have roughly the same chelating power towards Al(III), their complexes presenting stability constants of the same magnitude. In presence of MeO^{-}, the different sites can be classified according to their ability to complex Al(III): *ortho*-dihydroxyl>3-hydroxy-4-carbonyl>5-hydroxy-4-carbonyl. In acidic conditions, only 3HF easily forms a complex of stoichiometry Al_2(3HF), whereas the complex formation with Al(III) is difficult with 5HF and does not occur with 3′4′diOHF. We have also observed that the complex Al(3′4′diOHF) formed in pure methanol is dissociated by addition of acid to the medium.

Absorption spectra of quercetin in methanoic solution with different concentration of $AlCl_3$ are presented in Fig. 2. Quercetin exhibits a strong absorption band (band I) at 372 nm. Spectral changes accompanying addition of Al(III) to the solution show that the absorption peak at 372 nm decreases, while a band centred at 428 nm increases with the amount of metal added. For ratios $[AlCl_3]/[Q]$ less than 0.5, a first isobestic point is observed at 392 nm, indicating the presence of two species in equilibrium and therefore the formation of a first complex. For ratios greater than 0.5, a second isobestic point is observed at 434 nm, accompanied by the increase of a new band at 456 nm, characteristic of the formation of a second complex. Spectra in Fig. 2 clearly show that there are two chelating processes occurring consecutively and implicating two binding sites. As all spectra cross at least one of the two isobestic points, the simultaneous presence of free and two forms of the complex is not observed. This fact indicates that the formation of the second complex only begins when all quercetin molecules are already involved in the first complex. The molar ratio plots at 428 nm (λ_{max} of the first complex: complex I) and at 456 nm (λ_{max} of the second complex: complex II) show inflection at $[AlCl_3]/[Q]=0.5$ and 2, respectively, indicating a stoichiometry Al(III):Q of 1:2 for complex I and 2:1 for complex II (Fig. 3).

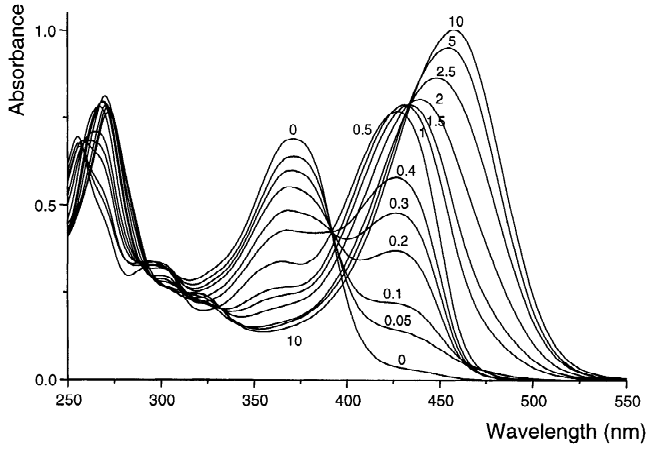

Fig. 2. Electronic absorption spectra of quercetin in methanol in absence and in presence of Al(III). The molar ratios $[AlCl_3]/[Q]$ are indicated on each spectrum.

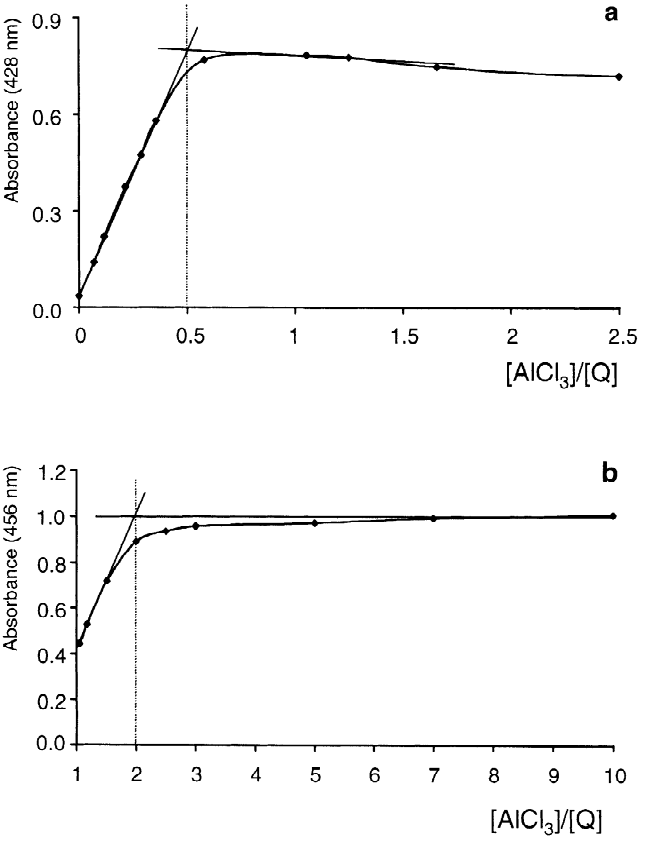

Fig. 3. Absorbance versus $[AlCl_3]/[Q]$ molar ratios plots at 428 nm (a) and 456 nm (b).

By electrospray mass spectrometry, Deng and Van Berkel [23] have observed a complex of stoichiometry 1:2 for the Al–quercetin system in methanol, and in small amounts by increasing the Al(III) concentration, another complex of stoichiometry 1:1 was observed. These two species being singly charged indicated the presence of $[AlL_2]^+$ and $[AlRL]^+$ with R=H^-, OH^- or MeO^- and L=deprotonated Q. In this condition, the authors concluded that Al(III) binding involves either the 5- or 3-hydroxyl group and the 4-keto group. However, they did not experimentally observed the 2:1 complex. This could be explained by the fact that the formation of the 2:1 complex necessitates a higher Al(III)/Q concentration ratio. The UV–visible spectra recorded in this study do not show the presence of a 1:1 complex, but this species obtained from the fragmentation of the 1:2 dimeric complex must present the same electronic spectrum as this one. Moreover, the formation of the 2:1 complex and the fragmentation of the 1:2 complex may simultaneously occur.

When followed by UV–visible spectroscopy, complexation of Al(III) by quercetin in a mixture methanol–water (90:10) in presence of HCl (pH 2.5) is shown by the decrease of the quercetin absorption band (λ_{max}=372 nm) and the simultaneous raising of an unique absorption band at λ_{max}=425 nm. An unique isobestic point, observed at 394 nm, demonstrates the presence of only one complex form, even for high Al/Q ratios (100), and so only one binding site of quercetin is involved in this complex. The molar ratio method used to determine the stoichiometry indicates the formation of Al(III):Q=1:1 complex.

These different approaches lead the conclusion that the first site occupied by Al(III) in quercetin in pure methanol solution is the 3-hydroxychromone group. Indeed, a great number of arguments clearly indicate that complex I involves the 3-hydroxyl-4-keto group: (i) among the 3HF, 5HF and 3′4′diOHF molecules, 3HF has the greatest chelation power, (ii) the complex I stoichiometry (1:2) is the same than the one obtained with 3HF in pure methanol, (iii) in presence of HCl, an unique complexed form of quercetin is observed, only 3HF has the same behaviour in acidic medium, whereas 5HF and especially 3′4′diOHF do not form a complex at very low pH, (iv) even if stoichiometries are different, the λ_{max} recorded for complex I and for the complex obtained in presence of HCl in the medium are very similar, (v) addition of HCl (10^{-2} M) to complex II observed in pure methanol regenerates the original spectrum of complex I, showing that only complex I is stable in very acidic medium.

Because of important steric hindrance induced by the first complexation in position 3, and also because of the presence of a strong intramolecular hydrogen bond between the hydroxyl in position 5 and the carbonyl group [17], it seems difficult for the 5-hydroxychromone group to take part to the formation of the second complex. So, it is obvious that the second chelating site involved in complex II is the catechol group. The destruction of this complex by addition of HCl confirms this hypothesis insofar as, among the three binding sites, only the 3′4′-dihydroxyl group has this comportment in very acidic medium. These observations lead us to suggest the complexation reaction scheme presented on Fig. 4, showing the two complex forms detected by electronic spectroscopy: $[AlQ_2^{(3)}]^+$ and $[Al_2Q^{(3,3',4')}R_4]^{3+}$ where R=CH_3OH, $Q^{(3)}$ and $Q^{(3,3',4')}$

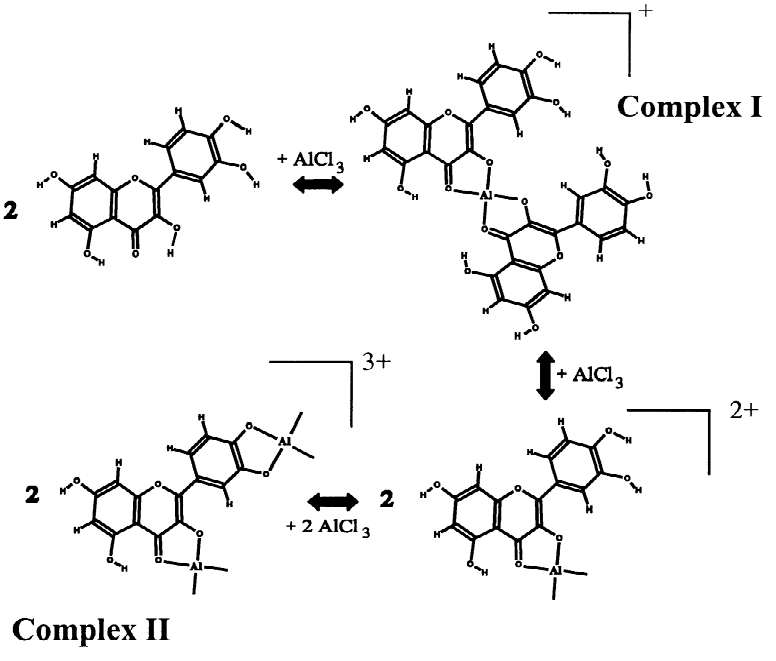

Fig. 4. Proposed mechanism for the complexation of quercetin by Al(III).

are quercetin molecules with hydroxyl groups deprotonated at the level of the chelating sites.

A great number of papers deal with the complex formation of quercetin with metal, and for purpose of comparison, we have examined the results obtained in various studies present in literature. Recently, Zhou and co-workers [24,25] have studied by fluorescence spectroscopy rare earth metal(III) complexes with quercetin; they found complexes of stoichiometry metal(III):Q 1:3 and proposed a chelation model involving the 3-hydroxychromone group. On the basis of IR and UV spectral investigations, Yuldashev et al. [26] have observed a complex 1:1 between Mo(VI) and quercetin and have suggested that quercetin was coordinated in bidentate fashion through the O2 and O3 oxygen atoms of the γ-pyrone ring. Both 1:1 and 1:2 types of complexes have been observed in the quercetin–copper(II) system [7,8,27–30]. In a first step, quercetin coordinates to copper at the 3-hydroxy and 4-carbonyl groups. The subsequent coordination step was assumed to occur at the catechol site, because the 5-hydroxy and 4-carbonyl site is not capable of chelate formation when 3-hydroxy and 4-carbonyl site is occupied. In opposition to these studies, the chelating behaviour of quercetin with iron(II) or iron(III) seems different. All results [31,32] indicate that the coordination to the catechol group is the strongest for iron and form a complex of stoichiometry 1:1, even at low value of pH.

Table 2
Bond lengths (in Å) calculated by AM1 method for quercetin, complex I and complex II

	Quercetin	Complex I	Complex II
O1C2	1.392	1.381	1.374
C2C3	1.363	1.384	1.390
C3C4	1.471	1.441	1.437
C4C10	1.451	1.415	1.400
C10C5	1.418	1.427	1.434
C5C6	1.403	1.397	1.394
C6C7	1.401	1.406	1.408
C7C8	1.403	1.408	1.421
C8C9	1.399	1.393	1.382
C9C10	1.412	1.426	1.436
C9O1	1.380	1.380	1.386
C2C1′	1.460	1.449	1.451
C1′C2′	1.402	1.407	1.412
C2′C3′	1.395	1.391	1.389
C3′C4′	1.412	1.417	1.432
C4′C5′	1.402	1.405	1.396
C5′C6′	1.390	1.387	1.391
C6′C1′	1.404	1.409	1.413
C4O2	1.248	1.320	1.347
C3O3	1.379	1.362	1.379
C5O5	1.381	1.358	1.356
C7O7	1.370	1.360	1.347
C4′O4′	1.371	1.363	1.361
C3′O3′	1.381	1.379	1.374
AlO3		1.768	1.752
AlO2		1.797	1.768
AlO3′			1.757
AlO4′			1.757

Then, the coordination of a second iron ion to quercetin is observed involving the carbonyl oxygen and the hydroxyl group either in position 5 or in position 3.

3.2. Conformational analysis of complexes of quercetin in methanol

Geometry optimisation of quercetin by AM1 semiempirical method has shown that the molecule, in an isolated state, adopts a staggered conformation with a θ (O1–C2–C1′–C6′) angle value of 26.7° [17]. The molecular structure of quercetin is stabilised by three intra-molecular hydrogen bonds: O2–H-O5: 2.004 Å, O2–H-O3: 2.221 Å and O3′–H-O4′: 2.260 Å. From energetic considerations, the fact the O2–H-O5 hydrogen bond is stronger than this one formed between O2 and H-O3 could partially explain that the complex formation is more favourable on the 3-hydroxy-4-carbonyl site than on the 5-hydroxy-4-carbonyl site. The main geometrical parameters of quercetin and of the two complexes, obtained after energy minimisation with the AM1 method, are presented in Tables 2 and 3.

In complex I, the two quercetin molecules have exactly identical geometries and the same electronic distribution. The structure of the ligand and essentially the γ-pyrone moiety is profoundly affected by coordination with Al(III) and complex I mainly involves a pyronium form (Fig. 5a). The structural modifications, concerning the A and B rings suggest a participation of a mesomeric cinnamoyl form (Fig. 5b) and a benzoyle form (Fig. 5c) to the resonance of complex I. Indeed, the presence of the hydroxyl groups in position 7 and 4′, conjugated with the carbonyl function, explain the contribution of these mesomeric forms. The extension of the π-system delocalisation up to the ring B conducts to reduce the θ angle to a value close to 8.8°. At the chelating site level, the coordination with Al(III) leads to the formation of a five-membered ring, coplanar with the chromone moiety.

For energy minimisation of complex II, two methanol molecules have been added to each aluminium in order to obtain a tetra-coordinated ion. So, the optimised structure corresponds to a system of type $[QAl_2(MeOH)_4]^{3+}$. The structural modifications of the ligand in complex II are similar to those observed in complex I. Concerning the geometrical parameters, the bond lengths calculated for complex II vary in the same way with higher magnitude than the variations observed for complex I compared to the free quercetin molecule. So, the three mesomeric forms involved in the first complex also contribute to the stability of complex II, and their participation to the resonance of the system is increased by the second chelation. The ligand adopts a quasi-planar structure. The molecular structure of the two complexed forms are not all that different, the implication of a second complexing site simply reinforces the structural modifications engendered by the first chelation.

Table 3
Angles (°) and main dihedral angles (°) calculated by AM1 method for quercetin, complex I and complex II

	Quercetin	Complex I	Complex II
O1C2C3	121.2	119.4	118.6
C2C3C4	122.5	121.1	121.1
C3C4C10	115.1	119.6	120.6
C3C4O2	120.1	116.3	116.0
C4C10C5	123.4	126.0	127.1
C4C10C9	119.1	116.6	116.2
C10C5C6	120.9	120.7	120.9
C5C6C7	119.2	119.6	120.0
C6C7C8	121.8	121.7	121.2
C7C8C9	117.8	117.8	118.0
C8C9O1	114.1	114.6	114.7
O1C2C1′	110.9	111.8	111.2
C2C1′C2′	120.6	120.4	121.2
C1′C2′C3′	120.3	119.5	118.3
C2′C3′C4′	120.3	120.8	121.0
C3′C4′C5′	119.6	119.3	120.6
C4′C5′C6′	119.9	119.7	118.2
C5′C6′C1′	120.1	121.1	121.9
C2C3O3	119.5	123.7	123.1
C10C5O5	124.2	123.8	123.7
C6C7O7	122.1	122.4	123.3
C2′C3′O3′	123.2	123.6	122.8
C3′C4′O4′	123.3	123.1	116.7
C3O3Al		107.0	105.4
C4O2Al		107.3	106.1
C3′O3′Al			104.5
C4′O4′Al			104.7
O1C2C1′C6′	26.7	8.8	2.4
C2C3O3Al		176.6	180.0
C2′C3′O3′Al			179.7

3.3. Validation of the structural models of complexes by spectroscopic techniques

In order to validate the proposed complex models obtained from AM1 calculations, electronic spectra were calculated and compared to the experimental ones. The simplest excited state theory we use is single excitation configuration interaction (CIS) [33]. The UV–visible spectra in methanol and the spectral positions of the calculated transitions for complexes I and II are represented in Fig. 6. The line height is relative to the value of calculated oscillator strengths. The agreement between theoretical and experimental wavelength are rather good assuming that solvent effects are not taken into account in the calculations. Moreover the calculated oscillator strengths are consistent with the absorption coefficients of the experimental spectra. The bathochromic shift of the band I observed on the experimental spectra by addition of Al(III) to the quercetin solution is fairly reproduced by calculations from the complexed forms models. Other

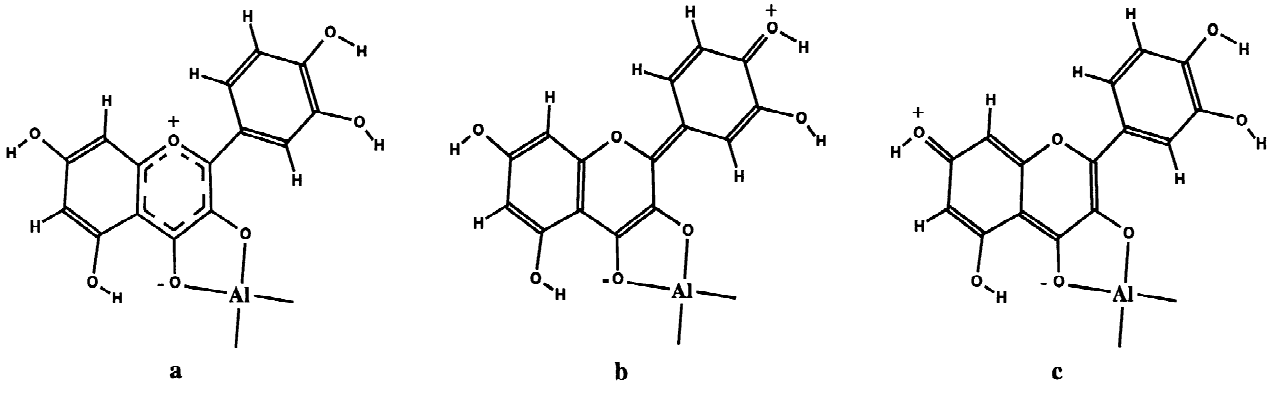

Fig. 5. Pyronium (a), cinnamoyl (b) and benzoyle (c) forms participating in the resonance of complex I. Only one ligand has been displayed.

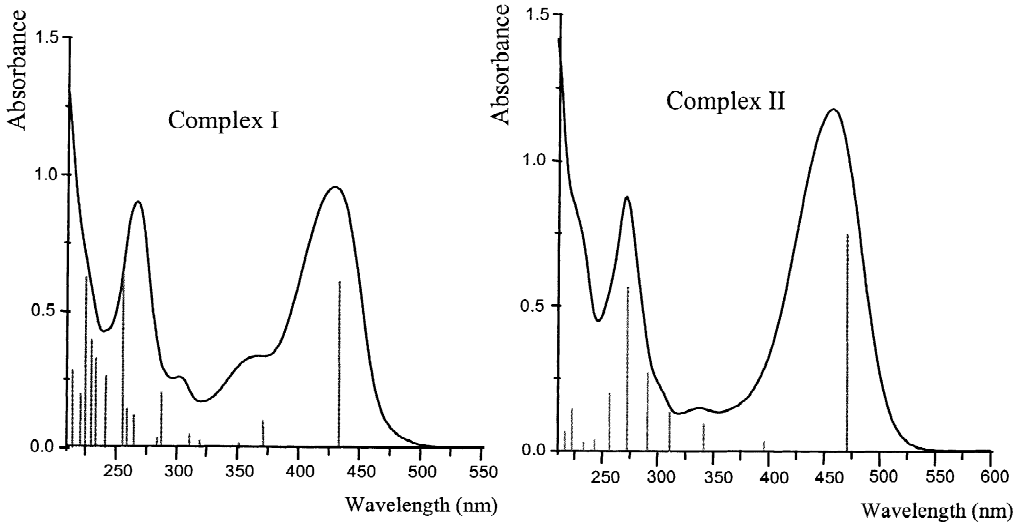

Fig. 6. Experimental UV–Vis spectra and theoretical positions of absorption bands calculated from optimised structures for complex I and complex II.

models of complexes implicating other complexing sites, like the 5-hydroxy-4-carbonyl group or the *ortho*-dihydroxyl group for the first complex, have been tested for quercetin. For these others possible complexed forms, the theoretical absorption spectra totally disagreed with the recorded spectra of complex I and II in methanol. These observations indicate that the chelating sites involved in complexes I and II are those presumed in the first part of this paper and so confirm our hypothesis.

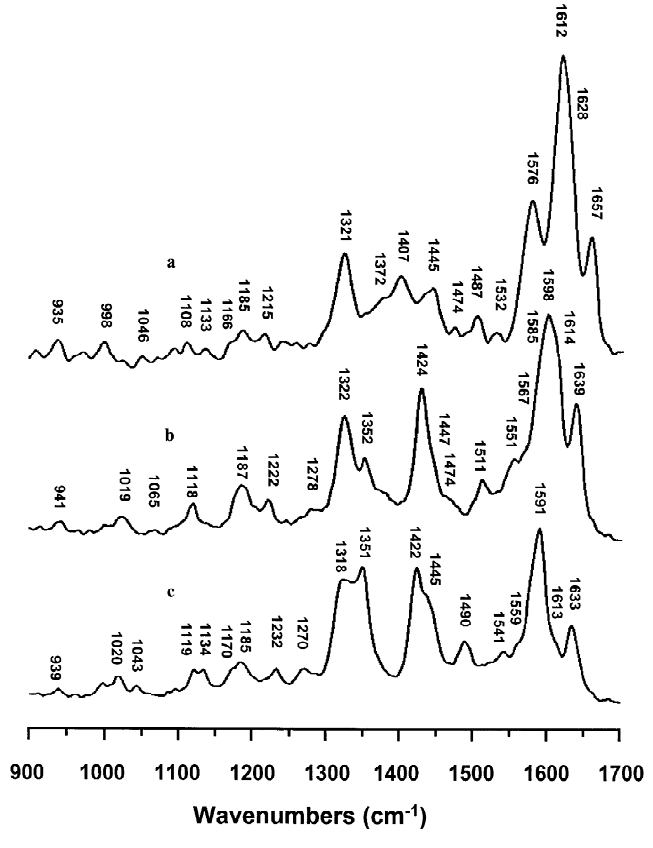

Fig. 7. FT-Raman spectra of quercetin (a), complex I (b) and complex II (c).

Raman spectroscopy has also been used to validate the proposed complex formation mechanism. The FT-Raman spectra of free quercetin and of complexes I and II are shown in Fig. 7a–c, respectively, in the spectral range 900–1700 cm^{-1} where the most intense lines appear. These spectra have been recorded with a quercetin concentration of 10^{-2} M in methanol. For the complexed forms, $[Q]/[AlCl_3]$ ratios of 2 and 0.1 have been used for complexes I and II, respectively. Whereas the Raman spectra of the two complexes are rather similar, the comparison of these spectra with this of quercetin show important spectral changes, notably in the 1500–1700 cm^{-1} range. This first observation well indicates that the major structural and electronic changes occur with the first step of complexation. For the free quercetin molecule, the two lines located at 1657 and 1628 cm^{-1} (shoulder) could be assigned to a mechanical coupling between the C4=O2 and C2=C3 stretching modes. The chelation on the 3-hydroxy-4-carbonyl group completely modifies the C4=O2 bond, and only the C2=C3 stretching mode is then observed in the high wavenumbers, at 1639 and 1633 cm^{-1} in the complexes I and II spectra, respectively. In complex I the increase of the C4=O2 bond length connected to an increase of C3–O3 lead to a coupling of vibrations of these two bonds. The new bands at 1551 and 1424 cm^{-1} in complex I spectrum could be considered to be associated with, respectively the anti-symmetric and symmetric C–O stretching modes at the chelating site level. For complex II, the corresponding bands appear at 1541 and 1422 cm^{-1}. The 8a and 8b modes (according to Wilson's notation for substituted benzene ring vibrations [34]) corresponding to ring C–C stretchings are also perturbed by the chelation. The massif formed by the two broad bands at 1612 and 1576 cm^{-1} in quercetin spectrum

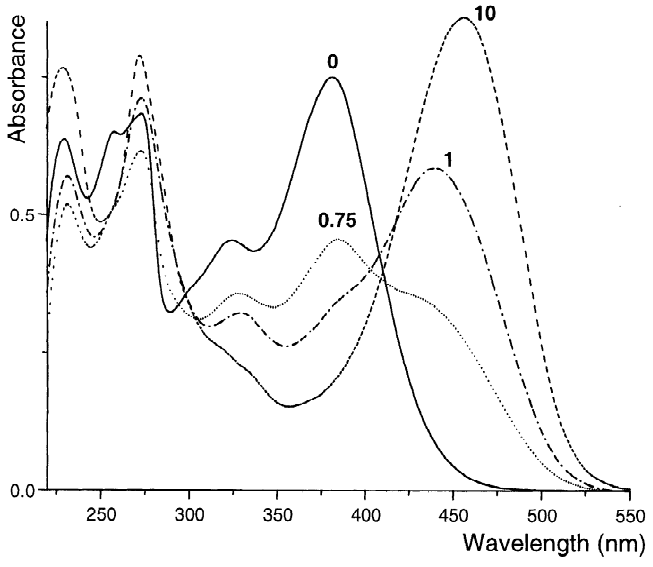

Fig. 8. Electronic absorption spectra of quercetin in methanol in alkaline medium and in presence of Al(III). The molar ratios $[AlCl_3]/[Q]$ are indicated on each spectrum.

is assigned to a mixture of the 8a and 8b modes of the two phenyl rings A and B of the molecule, this massif is profoundly modified in the complexes spectra showing that the structure of the two rings are modified by the chelation. The line of low intensity, observed at 1474 cm^{-1} in the quercetin and complex I spectra, could be assigned to the C–O stretching of the catechol group. The vanishing of this line in the complex II Raman spectrum once more confirms the involvement of the *ortho*-dihydroxyl group in the second chelate formation.

3.4. Chelating properties of quercetin in alkaline medium

Our previous studies concerning the complexes formed with 3HF, 5HF or 3′4′diOHF have shown that the pH conditions profoundly affect the ionization of acidic groups and thereby the stoichiometry of the complexed forms. For this reason the same experiments as those described in pure methanol have been undertaken in methanol in the presence of sodium acetate, 10^{-1} M. In this medium, the hydroxyl functions in position 7, 3 and 4′ are partially deprotonated [35], and the UV–Vis spectrum of quercetin in presence of AcO^- ions (band I: $\lambda_{max}=382$ nm) slightly differs from this of quercetin in pure methanol (band I: $\lambda_{max}=372$ nm). For more alkaline medium, a rapid oxidation of quercetin occurs and no complex formation is observed. AcO^- competes with the different chelating sites of quercetin and a more important amount of Al(III) is necessary to observe the complex formation of quercetin in this medium compared to pure methanol. The progressive addition of $AlCl_3$ in the methanoic solution of quercetin containing AcO^- was conducted to obtain a collection of UV–Vis spectra; to simplify the scheme, only some spectra have been reported in Fig. 8. From the recorded spectra, some observations could be made: (i) for $[AlCl_3]/[Q]<0.75$, the band I of the complex appears at 443 nm and an isobestic point is observed at 412 nm. (ii) For $0.75<[AlCl_3]/[Q]<1.85$, no isobestic point is observed and the band I shifts toward the long wavelength. (iii) For $[AlCl_3]/[Q]>1.85$, a second point isobestic point is observed at 456 nm and the absorption band of the final complex is shifted to 440 nm. The curve of the absorbance versus the molar ratio $[AlCl_3]/[Q]$ plot for different wavelengths show three inflection points, indicating the existence of three complex forms. The stoichiometry of these three complexes successively formed are Al(III):Q=1:2, 1:1 and then 2:1. The first complex with a $\lambda_{max}=443$ nm, is constituted of two quercetin molecules bound to an aluminium ion. The complexing site implied in this entity is without any doubt the catechol group. Indeed, our previous studies have shown that the *ortho*-dihydroxyl group presents a strong chelating power in alkaline medium in comparison with the 3-hydroxychromone or 5-hydroxychromone groups. The last formed complex corresponds to a molecule of quercetin coordinated to two Al(III), the second site of complexation is the 3-hydroxychromone group. In fact this complex form is the same that is observed in pure methanol, with the same position of the band I ($\lambda_{max}=456$ nm). Concerning the intermediate complex of stoichiometry 1:1, one can suppose that it results of a partial destruction of the first complex and consequently that only one Al(III) entity is bound to the catechol group.

Table 4
Stoichiometry and chelating sites successively involved in complexation of Al(III) with quercetin, in different media

Medium	Stoichiometry Al(III):Q	λ_{max} (nm)	Chelating sites
Pure methanol	1:2	428	3
	2:1	456	3 and 3′4′
Methanol+AcO^-	1:2	443	3′4′
	1:1		
	2:1	456	3′4′ and 3
Methanol+water+HCl	1:1	425	3

Chelating sites 3 and 3′4′ are relative to the 3-hydroxychromone and *ortho*-dihydroxyl groups, respectively. λ_{max} corresponds to the wavelength at the absorption maximum of the complex.

4. Conclusion

Utilising UV–visible and FT-Raman spectroscopies combined with quantum chemical calculations, this study examined the interaction of metal ion Al(III) with the quercetin molecule in methanoic solution. The experiments were performed under different pH conditions and Table 4 summarises the stoichiometry and the chelating sites involved in complexes obtained in the different media. In acidic medium and in pure methanol solution, the first site implicated in the complex formation process is the 3-hydroxychromone group, whereas in alkaline medium, the catechol group has the highest chelating power. For larger amounts of Al(III), with an Al(III)/Q ratio greater than 0.5, the same complex $[Al_2QR_4]^{3+}$ is formed in pure methanol and in alkaline solution. However, in acidic medium, the *ortho*-dihydroxyl group is never involved in complexation with Al(III).

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