Organic solvent–luteolin interactions studied by FT-Raman, Vis-Raman, UV-Raman spectroscopy and DFT calculations

Carla Marrassini [a,b,*], Abdenacer Idrissi [a], Isabelle De Waele [a], Khadidja Smail [c], Noureddine Tchouar [d], Myriam Moreau [a], Alberto Mezzetti [a,e,**]

[a] LASIR UMR 8516, Université Lille 1, France
[b] Cátedra de Farmacognosia, IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina
[c] Département de Biotechnologie, Faculté des Sciences de la Nature et de la Vie, Université des Sciences et de la Technologie d'Oran Mohamed Boudiaf (USTO-MB), BP 1505, Oran 31000, Algeria
[d] Laboratoire de Modélisation et Optimisation des Systèmes Industriels (LAMOSI), Département de Chimie Physique, Faculté de Chimie, USTO-MB, BP 1505, Oran 31000, Algeria
[e] Service de Bioénérgetique, Biologie Structurale et Mécanismes, UMR 8221, IBITeC-S, CEA-Saclay, France

ARTICLE INFO

ABSTRACT

The interactions of luteolin with three alcohols (methanol, 1-propanol, 1-butanol) and dimethylsulfoxide (DMSO) were studied by FT-Raman, Vis-Raman and UV-Raman spectroscopies, coupled to density functional theory calculations. No large shift was observed for the bands in the spectra of luteolin in alcohols or DMSO in the 1700–1550 cm^{-1} region. This is possibly related to the presence of a strong intramolecular hydrogen bond involving the 5-OH and the carbonyl of luteolin, as suggested by literature data [V. Exarchou, A. Troganis, I.P. Gerothanassis, M. Tsimidou, D. Boskou, Tetrahedron 2002, 58, 7423–7429] and DFT calculations. Furthermore, DFT calculations suggest that the C=O stretching of luteolin is implicated in several vibrational modes, whereas the most upshifted band in the 1700–1550 cm^{-1} can be interpreted as arising mainly from a 5-OH bending. The results are discussed in the framework of vibrational spectroscopy studies on flavonoids, of the photophysical properties of luteolin, and of the reported literature of vibrational spectra of luteolin under different conditions, in particular when interacting with biomolecules.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Flavonoids are natural phenolic compounds ubiquitous in plants and therefore found in a variety of vegetables, fruits, and beverages. Flavonoids possess several interesting biological properties which include radical scavenging ability [1], anti-inflammatory capacities, and cardioprotective, anti-carcinogenic [2], antiallergic, hepatoprotective, antithrombotic, and antiviral activities [3].

Luteolin (5,7,3′,4′,-tetrahydroxyflavone; Fig. 1) is one of the most common flavones and is found in different plants as celery, green pepper, perilla leaf, and chamomile [4]. Luteolin's most outstanding biological properties are its antioxidant ([5] and refs. therein), anti-inflammatory [4] and anti-tumor activities ([6] and refs. therein).

Luteolin has also a photoprotective and antioxidant role in plants [7,8] and in the skin [9]. Luteolin is also the main component of weld and is responsible for its characteristic yellow color. Weld has been used in the Middle Ages and in the Renaissance for dyeing textile fibers, and as a lake in paintings [10,11].

Vibrational spectroscopy is a powerful approach to investigate at an atomic level the interaction between organic molecules and their surrounding microenvironment, especially biomacromolecules. When dealing with naturally-occurring organic molecules, vibrational spectroscopy can provide detailed information on pigment–protein/ ligand–protein/cofactor–protein interactions, mechanism of ligand binding, localization of molecules in membranes, mechanism of biochemical reactions, etc. Several vibrational spectroscopy techniques are widely applied in molecular biophysics to investigate these issues, such as resonance Raman [12,13], non-resonant Raman [14], and FTIR difference spectroscopy [15]. The particularity of these techniques is that the spectral contributions arising from the organic molecule can be identified, shedding direct light on its interaction with the protein (or more broadly speaking with the biomacromolecule(s)). Molecular vibrations are extremely sensitive to structure and intermolecular interactions (local dielectric constant, presence of H-bonds, presence of nearby charged residues…), so that marker bands can provide precise information (e.g. the presence of a hydrogen bond between the molecule and the protein). In particular, when the organic molecule contains carbonyl moieties, the effect of intermolecular interactions on the position of their C=O stretching band is generally very strong. However, a prerequisite to rationalize how band position is influenced by the surrounding microenvironment, is a detailed characterization of the

* Correspondence to: C. Marrassini, Cátedra de Farmacognosia, IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.
** Correspondence to: A. Mezzetti, Service de Bioénérgetique, Biologie Structurale et Mécanismes, UMR 8221, IBITeC-S, CEA-Saclay, France.
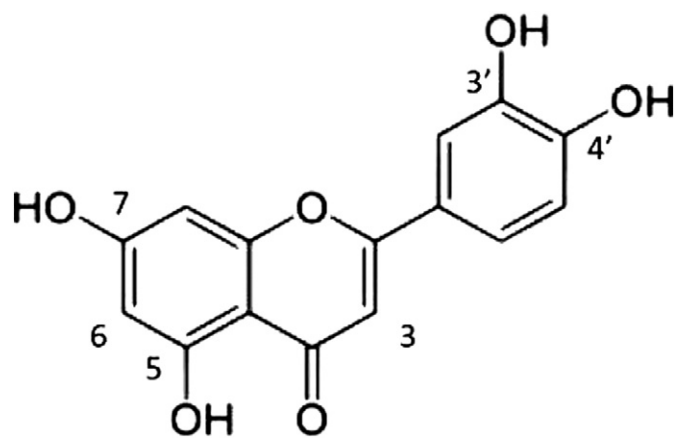
Fig. 1. Structural formula of luteolin.

target organic molecule in prototypical environments, and to this aim organic solvents (which are characterized by precise physical and chemical parameters) are widely used. Non-resonant Raman (using a differential "solution-minus-solvent" approach) and resonance Raman are particularly useful to characterize organic solute–organic solvent interactions because, compared to infrared spectroscopy, bands are narrower and the water bending contributions around 1630 cm^{-1} (either as moisture trace in the solvent or water vapor) do not interfere. Experimental data on organic solvents can be fully exploited when calculations (DFT, MD, QM/MM, etc.) are used to assign vibrational modes or to rationalize solute–solvent interactions (see for instance [16]).

In the last decade, a steadily increasing number of articles using vibrational spectroscopy to study flavonoids in different microenvironments, e.g. binding sites of biomacromolecules ([17–19] and refs. therein), organic nanoparticles [20], and nanocomposites [21], have been published. Concerning luteolin, vibrational spectroscopy has been used to characterize its interactions with oligosaccharides [22], with Human Serum Albumin (HSA) [23], with acetylcholinesterase [24], with α-glucosidase [25], with phospholipids [26], and with composites membranes [27], and to characterize co-crystals of luteolin with isonicotinamide [28]. A particular field of interest is the characterization of luteolin in ancient textiles ([29] and refs. therein), by Raman spectroscopy, which is indeed a technique of choice to identify organic colorants in art and archeological objects [30].

In the present work, we report the results of experimental spectra (of luteolin FT-Raman, Vis-Raman, and UV-Raman) in four different solvents (methanol, 1-propanol, 1-butanol, and DMSO) along with FTIR and FT-Raman spectra of solid-state luteolin and DFT calculations to attribute specific bands. Differently from other flavonoids [20,31], a weak solvent effect on band position in the 1700–1550 cm^{-1} region was observed. The results are interpreted in terms of the particular molecular structure of luteolin.

2. Material and methods

Luteolin (3′,4′,5,7-tetrahydroxyflavone, 98%) was purchased by Sigma Aldrich. Methanol, 1-propanol, and 1-butanol were purchased from Sigma Aldrich. DMSO was purchased from Acros Organics. All solvents were of spectroscopic grade. Solutions of 5 mM (FT-Raman and Vis-Raman) and 0.1 mM (UV-Raman) were prepared in the four solvents. These two concentrations were chosen according to the need of obtaining spectra with a good signal-to-noise ratio (for FT-Raman and Vis-Raman) and because of the need of minimizing the extremely intense luteolin signal (due to the Resonance Raman phenomenon) in UV Raman experiments.

FT-Raman experiments were performed on a Bruker RFS 100/S spectrometer. Radiation of 1064 nm from a Nd:YAG laser was used for the excitation. The spectral resolution was set to 4 cm^{-1} for solutions and

solvents, and to 2 cm^{-1} for solid luteolin. Spectra were obtained by averaging 36,000 scans for solutions and pure solvents, and 200 scans for solid luteolin.

FTIR spectra were recorded on a Bruker Tensor 27 equipped with a DTGS detector and an ATR accessory. 200 scans were averaged. Resolution was set to 2 cm^{-1}.

Vis-Raman spectra were recorded on a Visible HR LabRam system (Horiba Jobin Yvon) using 633 nm, 532 nm and 473 nm laser excitation wavelengths.

UV-Raman spectra were recorded on a UV HR LabRam system (Horiba Jobin Yvon) using a 325 nm laser excitation wavelength.

In order to have a rational assignment of the spectral contribution of the C=O and OH bending modes of luteolin, we carried out quantum calculations using Gaussian package. Beside classical implicit solvent calculations, the study of specific solute–solvent interactions for luteolin in alcohols was performed adding solvent molecules. Geometry optimization calculations were performed on the various configurations of luteolin that was surrounded by solvent molecules. The number of solvent molecules was chosen to be equal to the number of functional groups of luteolin (five). The solvent molecules were localized near the C=O and the OH groups of luteolin. We used the density functional theory (DFT), which incorporates Becke's three parameter exchange with the Lee, Yang and Parr correlation functional method (B3LYP) with the cc-PVTZ basis set. The optimized geometries were confirmed to be the minima on the potential energy surface by analyzing the vibrational frequencies, which were found to have no imaginary components.

3. Results and discussion

3.1. Experimental vibrational spectra and DFT calculations

FTIR and FT-Raman spectra of solid luteolin are reported in Fig. 2. The results differ from previously reported spectra [32–34], most probably
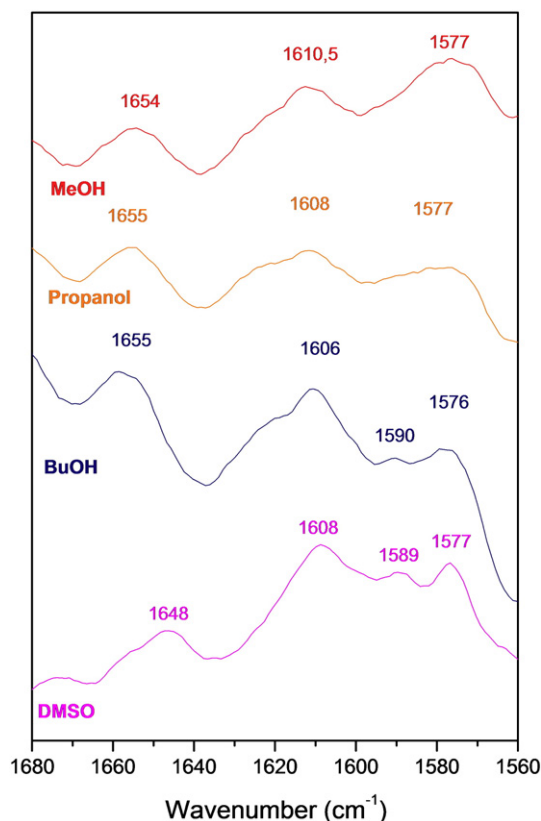

Fig. 2. FTIR and FT-Raman spectra on solid luteolin.

because of the higher spectral resolution and the better signal-to-noise ratio (SNR) of the present spectra, and new band were identified (for a comparison, see Table 2). In particular, new bands were identified at 1598 and 1576 cm^{-1} in the FTIR spectrum, and at 1630 cm^{-1} (as a shoulder), 1599 cm^{-1}, and 1579 cm^{-1} (as a shoulder) in the FT-Raman spectrum. In order to better understand these bands in term of molecular vibrations, a comparison with DFT calculation was made. The comparison with DFT calculations for luteolin in vacuum does not seem to be appropriate, as solid state luteolin is surrounded by a medium with a quite high dielectric constant. Conversely, comparison with DFT calculations performed with an implicit solvent with a relatively high dielectric constant (MeOH) is of particular interest (Table 1). Indeed, the FT-Raman spectrum of solid luteolin shows 6 bands in the 1680–1550 cm^{-1} region, in agreement with DFT calculations (showing that the vibrational modes n. 72–77 have a noticeable Raman intensity). Conversely, the FTIR spectrum of solid luteolin shows only 5 bands in the same spectral window, in agreement with DFT results in MeOH (very small IR intensity for vibrational mode n. 76).

Another interesting aspect of the FTIR spectrum is the lack of the characteristic and intense C=O stretching band, as observed, for instance, in flavone [35] or in 7,3′,4′-trihydroxyflavone (whose molecular structure differs from luteolin only for the absence of the 5-OH group) [36]. In fact DFT analysis shows that for luteolin it is very difficult to find a single vibrational mode which can be interpreted as a C=O stretching band; C=O stretching is indeed involved in all the vibrational modes from 72 to 77. This seems to be a particular feature of luteolin, most probably related to the strong intramolecular H-bond between the 5-OH and the C=O group. This issue will be discussed in more detail below.

FT-Raman spectra of luteolin as a solute in different solvents were obtained after solvent subtraction. In Fig. 3, the 1680–1550 cm^{-1} region is shown. This is the region where the most characteristic modes of flavones and flavonoids absorb, namely the C=O stretching, the C=C stretching and some of the ring A and B vibrations ([32–34] and refs. therein). As it can be observed, the position of the bands does not vary significantly, the most solvent-dependent bands being the one at the highest wavenumber (ranging from 1656 cm^{-1} in MeOH and propanol to 1652 cm^{-1} in DMSO) and the band whose position lies between 1609 cm^{-1} (in MeOH) and 1605 cm^{-1} (in DMSO). It should be noticed that experiments in other solvents were not possible, due to the low solubility of luteolin in several solvents and to the relatively high concentration required to record FT-Raman spectra. Band positions are also reported in Table 2, where the comparison with some literature data is also provided.

DFT calculations (with the implicit solvent), similar to DFT calculations in vacuum, show that it is difficult to describe the different normal modes in terms of vibrations of chemical groups. The most striking result is that – in agreement with experimental data – the bands in the 1700–1550 cm^{-1} region are almost insensitive to the kind of solvent; this means that their position does not depend on the dielectric constant around the molecule. To take into account the specific effect of hydrogen bonds between the alcoholic solvents and luteolin, DFT calculations in the presence of alcoholic solvent molecules (see Materials and methods section) were performed, and reported in Table 3. An example
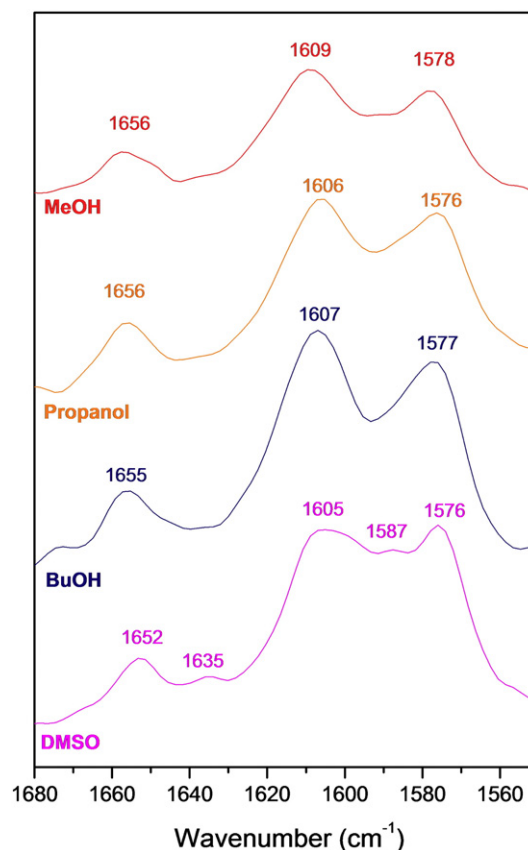

Fig. 3. FT-Raman spectra of luteolin in different solvents in the 1680–1550 cm^{-1} region. No baseline correction was applied.

of the minimized conformation of luteolin with 5 methanol molecules is shown in Fig. 4. The most noticeable effect is observed on the vibrational mode n. 77 which upshifts from ~1683 cm^{-1} to ~1698/1699 cm^{-1}. A noticeable upshift is also observed for mode n. 75 (from ~1637 cm^{-1} to 1644/1647 cm^{-1}), whereas mode 74 has a very small upshift (from 1627 to 1630/1632 cm^{-1}). The same DFT calculations also show that the most striking effect induced by the hydrogen bonding interactions with the alcoholic solvents (notably, the H-bonding involving the luteolin C=O and the 5-OH as acceptors) is observed for the 5-OH stretching of luteolin in methanol and in propanol (from 3126 cm^{-1} – implicit solvent DFT calculations – to 3172 cm^{-1}-luteolin interacting with 5 solvent molecules). Interestingly, the wavenumber of 5-OH stretching mode decreases strongly when the H-bonding solvent is 1-butanol (see Table 3). Given that the OH stretching region has not been explored experimentally, these DFT results will be only commented in relation to the behavior of the band mainly reflecting 5-OH bending (see below). It should be noticed that, due to the very strong intramolecular H-bond between the 5-OH and the C=O of luteolin, the 5-OH moiety interacts with the alcoholic solvent molecules

Table 1
Vibrational modes of luteolin. Calculated intensities are in arbitrary units, vibrational frequencies are in cm^{-1}.

Vibrational mode	77	76	75	74	73	72
FTIR solid state	1652		1608	1598	1576	1564
FT-Raman solid state	1658	1630sh	1610	1599	1579sh	1574
DFT calc. in vacuum (uncorrected values)	1700	1663	1653	1641	1630	1613
DFT calc. in vacuum (IR intensities, a.u.)	480	136	532	136	71	94
DFT calc. in vacuum (Raman intensities)	315	37	1147	14	42	799
DFT calc. in MeOH, implicit solvent (uncorrected values)	1683	1656	1637	1627	1610	1597
DFT calc. in MeOH, implicit solvent (IR intensities, a.u.)	804	9	500	845	252	212
DFT calc. in MeOH, implicit solvent (Raman intensities, a.u.)	612	804	2682	895	328	3366

Table 2
Raman bands of luteolin in the 1700–1550 cm^{-1} region. Data from the present work (FT-Raman, FTIR, Vis-Raman, UV-Raman) are compared with literature data. Laser wavelengths used in Raman experiments are: blue = 473 nm; green = 532 nm; red = 633 nm; and UV = 325 nm.

Vis-Raman in methanol	473 nm	5 mM	1657		1609		1578	
	532 nm	5 mM	1655		1612		1578	
	633 nm	5 mM	1654		1610.5		1577	
UV-Raman in methanol	325 nm	0.1 mM	1661		1611		1580	
FT-Raman in methanol		5 mM	1656		1609		1578	
Vis-Raman in 1-propanol	473 nm	5 mM	1656		1606		1576	
	532 nm	5 mM	1652		1606		1578	
	633 nm	5 mM	1655		1608		1577	
UV-Raman in 1-propanol	325 nm	0.1 mM	1660		1607		1577	
FT-Raman in 1-propanol		5 mM	1656		1606		1576	
Vis-Raman in 1-butanol	473 nm	5 mM	1658		1603		1578	
	532 nm	5 mM	1655		1601		1578	1556
	633 nm	5 mM	1655		1606	1590	1576	1554
UV-Raman in 1-butanol	325 nm	0.1 mM	1659		1607.5		1578	
FT-Raman in 1-butanol		5 mM	1655		1607		1577	
Vis-Raman in DMSO	473 nm	5 mM						
	532 nm	5 mM			1605		1576	
	633 nm	5 mM	1648		1608	1589	1577	
FT-Raman in DMSO		5 mM	1652	1635	1605	1587	1576	
FT-Raman solid state (this work)			1658	1630sh	1610	1599	1579sh	1574
FTIR solid state (this work)			1652		1608	1598	1576	1564
FT-Raman solid state [32]			1660		1612		1576	
SERS solid state [32]			1649		1616		1582	
Raman 514,5 solid state [33]			1652		1612		1575	
FTIR solid state [33]			1656		1612		1575	
SERS 514 solution 10–5 M [23]			1651		1618		1569	

just with the two electron doublet localized on the oxygen atom, i.e. acting as an H-bond acceptor.

DFT calculations (both with implicit and explicit solvents) suggest that the vibrational mode n. 77 can be interpreted as mainly due to the 5-OH bending. Indeed implicit solvent DFT calculations show that the dielectric constant of the solvent has no influence on the position of this vibrational band. Conversely, DFT calculations with explicit solvent show that the H-bonding between the alcoholic solvents (acting as H-bond donors) and the chemical moieties of luteolin (notably the C=O and the 5-OH groups, both acting as H-bond acceptors) has a small upshifting effect on vibrational mode n. 77 (which is consistent with the calculated downshift for the 5-OH stretching vibrational mode). This upshifting effect is also observed experimentally in FT-Raman spectra, where the highest energy band in the 1700–1550 cm^{-1} region shifts from 1652 cm^{-1} (in DMSO, no intermolecular H-bonds) to 1655/1656 cm^{-1} (alcoholic solvents, acting as H-bond donors). In Vis-Raman the effect is even more pronounced (1648 cm^{-1} in DMSO vs. 1654/1655 cm^{-1} in alcoholic solvents, see Fig. 5).

However, intermolecular interactions (and notably H-bonds) are known to have much stronger effects on the vibrational bands of flavonoids, especially on the C=O stretching of flavonoids devoid of the 5-OH group ([37]; see also [20,31]). We suggest that the presence of the strong intramolecular H-bond between the 5-OH group (donor) and the C=O moiety (acceptor), creating a 6-membered ring system, plays a key role in determining the vibrational properties of luteolin. It should be noted that the strength of the 5-OH–O=C intramolecular interaction is obtained by the X-ray structure of solid luteolin [38] and of luteolin co-crystals [28], as well as by NMR studies in solution [39]

which demonstrates that this interaction is maintained even in aqueous solutions.

Vis-Raman (λ_{exc} = 633 nm) and UV-Raman (λ_{exc} = 325 nm) spectra are shown in Figs. 5 and 6, respectively. These spectra show an apparently worse SNR compared to FT-Raman, but should be kept in mind that these spectra were recorded in minutes, whereas FT-Raman measurements required ~12 h. Table 2 shows also the data obtained for Vis-Raman using two other excitation wavelengths (λ_{exc} = 473 nm and 514 nm). The observed vibrational bands lie at a similar spectral position and are in agreement with those observed in FT-Raman. This is an interesting result, as a major concern in studying hydroxyflavones is the fact that organic solvents as DMSO and alcohols can induce deprotonation of OH groups in a fraction of molecules (see [40,41] and refs. therein). The formed anion has different absorption properties, absorbing at higher wavelength; it is therefore in principle possible that when exciting in the visible the Raman bands of the anions are more enhanced – by resonance or pre-resonance conditions – than those of the neutral molecule. This is apparently not the case for luteolin.

UV-Raman spectra (λ_{exc} = 325 nm) show a strong enhancement of bands, which required us to perform the measurements at a much lower concentration compared to Vis-Raman and FT-Raman experiments (0.1 mM vs 5 mM). Partial absorption at 325 nm by DMSO hampered the recording of the UV-Raman spectrum in this solvent. The position of the bands in the three alcohols is almost unchanged, and similar to Vis-Raman and FT-Raman data. This strongly suggests that in the more concentrated solutions used for the Vis-Raman and FT-Raman spectra, no aggregation phenomena take place.

Table 3
DFT calculations on luteolin in different conditions. Details are provided in the text.

| Vibrational mode	78	77	76	75	74	73	72
Molecular interpretation	5-OH stret.	5-OH bend.					
DFT in vacuum	3156	1700	1663	1653	1641	1630	1613
DFT in DMSO (implicit solvent)	3125	1683	1656	1637	1627	1609	1597
DFT in methanol (implicit solvent)	3126	1683	1656	1637	1627	1610	1597
DFT in 1-propanol (implicit solvent)	3126	1683	1656	1637	1627	1611	1598
DFT in 1-butanol (implicit solvent)	3127	1684	1656	1638	1627	1611	1598
DFT in methanol (with 5 solvent molecules)	3171	1698	1654	1647	1632	1612	1598
DFT in 1-propanol (with 5 solvent molecules)	3172	1699	1654	1646	1631	1611	1597
DFT in 1-butanol (with 5 solvent molecules)	3121	1697	1654	1644	1630	1610	1597
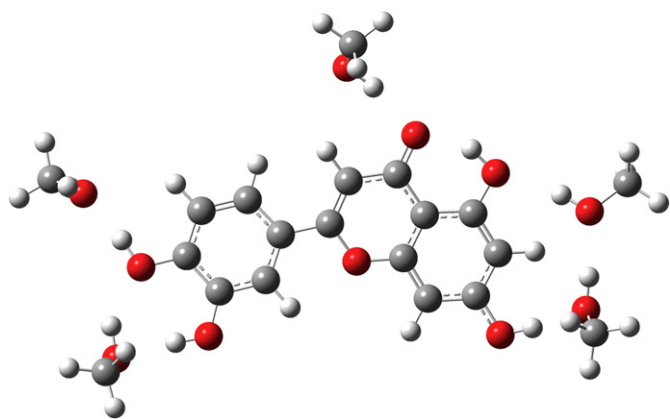
Fig. 4. Configuration of luteolin surrounded by MeOH molecules used in DFT calculations. See text for further details.

3.2. Relevance to biophysical studies on luteolin

As mentioned in the introduction, several studies report the use of vibrational spectroscopy to investigate the interaction between luteolin and biomolecules [22–28]. Among them, some are of particular interest as shifts of vibrational bands upon binding of luteolin to biomolecules were reported. Torreggiani and coworkers [23] used Surface-Enhanced Raman spectroscopy (SERS) to investigate the interaction between luteolin and HSA. Luteolin bound to HSA showed SERS bands at 1648 cm^{-1} and 1561 cm^{-1}, whereas the corresponding SERS bands of free luteolin in solution were at 1651 cm^{-1} and 1569 cm^{-1}. Both bands therefore downshift upon bonding to HSA, the highest effect being the one of 1569 cm^{-1} band. While the small downshift of the
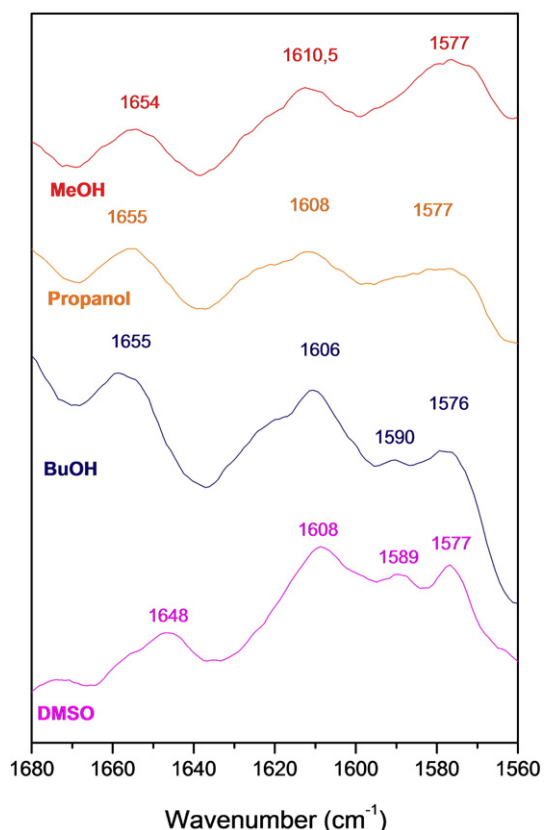

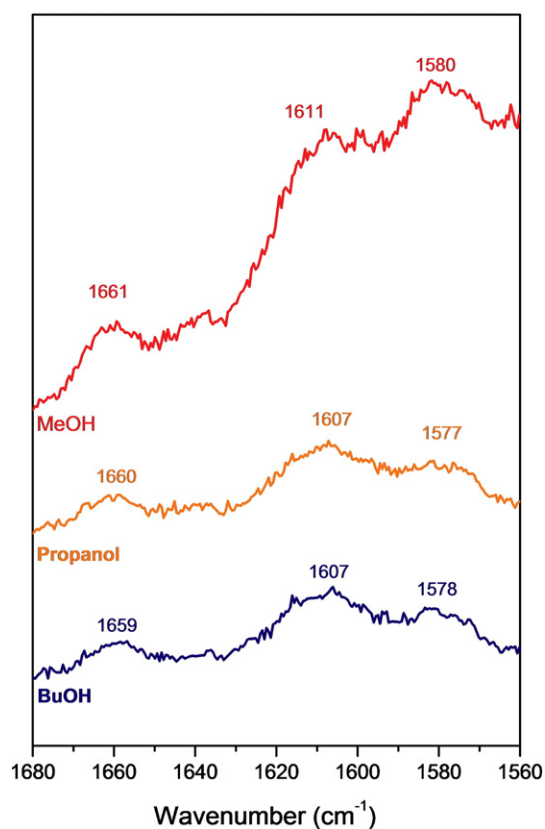**Fig. 6.** UV-Raman spectra of luteolin (λ_{exc} = 325 nm) in different solvents.

highest frequency band is fully consistent with the results reported here, the relatively strong downshift of the 1569 cm^{-1} seems to be related to a specific protein–luteolin interaction which cannot easily be modeled by the solvents explored in the present work. Jung and co-workers [22] used FTIR spectroscopy to study the interaction of luteolin with cyclosophorases, a class of oligosaccharides, and with cyclodextrins. While in the latter case no band shift was observed, in the former a strong upshift of the highest frequency band in the 1700–1550 cm^{-1} region was observed (from 1649 cm^{-1}, value reported for free luteolin, to 1660 cm^{-1}, value reported for luteolin interacting with cyclosophorases). A similar upshifting effect was also reported for the 1607 cm^{-1} band, which shifts to 1628 cm^{-1} when bound to cyclosophorases. This suggests that some specific interactions between luteolin and its molecular environment may also lead to quite strong shifts of these two vibrational bands.

Matczak-Jon and coworkers [28] have investigated co-crystals of luteolin and isonicotinamide. This study is of particular interest because 1) two polymorphic forms were identified and 2) in both cases the X-ray structure is known. The form I of the co-crystal shows Raman bands attributed to luteolin at 1652, 1609, and 1574 cm^{-1}. This form I is characterized by several H-bonds involving the hydroxyl groups as donor and/or acceptors, and the C=O as an acceptor. The form II of the co-crystal shows luteolin Raman bands at 1657, 1609, and 1568 cm^{-1}. Form II is also characterized by the presence of several H-bonds. Probably, a key difference between the two forms is that in form II the 5-OH is involved, as an acceptor, in an intermolecular H-bond with the NH$_2$ of isonicotinamide, whereas in form I the 5-OH is not involved in any intermolecular H-bond [28]. In addition, the inter-molecular H-bond involving the C=O is stronger in form II than in form I [28]. The simplest explanation is therefore that, similarly to what has been suggested by our DFT calculations of luteolin interacting with solvent molecules, the intermolecular hydrogen bonds tend to upshift the position of some vibrational modes in the 1700–1550 cm^{-1}, notably

Fig. 5. Vis-Raman spectra of luteolin (λ_{exc} = 633 nm) in different solvents.

the position of the highest energy band in this spectral region. It is worth noting that FT-Raman spectrum of solid luteolin (Fig. 2) provides a higher value for this band, being placed at 1658 cm^{-1}. The crystal structure of luteolin [38] reveals that the C=O group is involved in a strong intermolecular interaction with the 4′-OH group of a nearby molecule.

We can draw some general conclusion taking into account literature data and the results of the present work. When using vibrational spectroscopy to investigate the interaction between luteolin and its environment in ordered systems (protein binding pocket, co-crystals, complexes with oligosaccharides…) some key features emerge:

1) Differently from other flavonoids, and from other natural molecules, no band can be directly interpreted as arising mainly from a C=O stretching. This makes the interpretation of the spectra in terms of luteolin-molecular environment more difficult, and straightforward piece of information about the polarity of the environment surrounding the C=O cannot be derived, as done for other flavonoids interacting with specific environment or matrices ([18,20,42, 19] and refs. therein);

2) The effect of intermolecular interactions, especially H-bonds, on the vibrational bands is difficult to rationalize given the presence of the very strong intramolecular 5OH−O=C H-bond;

3) Some specific interactions (especially intermolecular H-bonds) due to the relative orientation of luteolin towards its molecular environment may have a quite pronounced effect on the position of some bands;

4) The parallelism between organic solvents and environment in ordered systems does not seem extremely appropriate to describe how luteolin vibrational bands are influenced by specific flavonoid-binding pocket (or flavonoid-partner molecule in co-crystals). In fact, the relative orientation, the strength of the H-bonds (or of other interactions) and other geometrical parameters seem to play a key role, which cannot be easily appraised studying solutions — which are dynamic and disordered systems;

5) DFT calculations can be very helpful in rationalizing the effect of specific intermolecular interactions. When crystallographic structures of luteolin–protein (or luteolin macromolecule, or luteolin–partner molecule) are known, or if at least some hypothesis on how luteolin interacts with its molecular environment can be formulated, DFT (or other theoretical approaches) can be extremely helpful to understand the effect of specific intermolecular interactions on luteolin vibrational bands.

3.3. Relevance to luteolin photophysics

We underlined before that one of the main point suggested by the present work is the strength of the intramolecular H-bond interaction 5OH−O=C, which does not seem to be perturbed in protic solvents, in agreement with previous NMR data. This peculiar interaction is believed to be the molecular basis of the photostability of luteolin [43, 44] and, more broadly speaking, of 5-OH substituted flavones [43–45]. It should in fact be noticed that ~85% of naturally-occurring flavonoids have an OH group in position 5 ([46]; see also [7]), and this feature appears to be a key point for their photoprotective role [45].

A more detailed analysis (experimental and theoretical) of the vibrational properties of a series of flavonoids is currently carried out in our laboratories, in order to further clarify solvent effects on the vibrational bands of flavones and to better understand the role of the 5-OH−O=C intramolecular H-bond. In parallel, we are also investigating the photostability of 5-OH substituted flavonoids, aiming to establish a correlation between these two particular aspects.

4. Conclusions

In this work, a first detailed study by vibrational spectroscopies and DFT calculations on the interactions of luteolin with organic solvents was carried out. The most striking feature was that no large solvent-induced shift was observed in the bands of the spectra in the 1700–1550 cm^{-1} region. In addition, differently from other flavonoids, DFT calculations and the FTIR spectrum did not identify clearly any band as given mainly by C=O stretching. These characteristics are possibly related to a strong intramolecular hydrogen bond involving the 5-OH and the carbonyl of luteolin. This interaction has most probably a key role in luteolin photostability and in its photoprotective role.

Acknowledgments

References

[1] B.H. Havsteen, Pharmacol. Ther. 96 (2002) 67–202.
[2] F.L. Juana, P.A.J. Angel, V.M. Manuel, in: R. Atta ur (Ed.), Studies in Natural Products Chemistry, Elsevier, 2012, pp. 115–134.
[3] E. Middleton Jr., C. Kandaswami, T.C. Theoharides, Pharmacol. Rev. 52 (2000) 673–751.
[4] M. Mizuno, Y. Nishitani, in: R.R. Watson, V.R. Preedy (Eds.), Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease, Academic Press, San Diego, 2013, pp. 227–234.
[5] Y.C. Zhang, F.F. Gan, S.B. Shelar, K.Y. Ng, E.H. Chew, Food Chem. Toxicol. 59 (2013) 272–280.
[6] Z. Tai, Y. Lin, Y. He, J. Huang, J. Guo, L. Yang, G. Zhang, F. Wang, Cell. Signal. 26 (2014) 619–662.
[7] J.B. Harborne, C.A. Williams, Phytochemistry 55 (2000) 481–504.
[8] G. Agati, M. Tattini, New Phytol. 186 (2010) 786–793.
[9] G. Seelinger, I. Merfort, C.M. Schempp, Planta Med. 74 (2008) 1667–1677.
[10] N. Eastaugh, V. Walsh, T. Chaplin, R. Siddall, Pigment Compendium: A Dictionary of Historical Pigments, Elsevier, 2004. 394–395.
[11] E.S.B. Ferreira, A.N. Hulme, H. McNab, A. Quye, Chem. Soc. Rev. 33 (2004) 329.
[12] B. Robert, Photosynth. Res. 101 (2009) 147.
[13] Y. Wang, H.E. Van Wart, Methods Enzymol. 226 (1993) 319.
[14] R. Callender, H. Deng, Annu. Rev. Biophys. Biomol. Struct. 23 (1994) 215–245.
[15] A. Barth, Biochim. Biophys. Acta Bioenerg. 1767 (2007) 1073.
[16] D. Bovi, A. Mezzetti, R. Vuilleumier, M.P. Gaigeot, B. Chazallon, R. Spezia, L. Guidoni, Phys. Chem. Chem. Phys. 13 (2011) 20954.
[17] F.-T. Liu, S.G. Agrawal, Z. Movasaghi, P.B. Wyatt, I.U. Rehman, J.G. Gribben, A.C. Newland, L. Jia, Blood 112 (2008) 3835–3846.
[18] K. Cieślik-Boczula, J. Maniewska, G. Grynkiewicz, W. Szeja, A. Koll, A.B. Hendrich, Vib. Spectrosc. 62 (2012) 64–69.
[19] S. Ionescu, I. Matei, C. Tablet, M. Hillebrand, Curr. Drug Metab. 14 (2013) 474.
[20] A. Barras, A. Mezzetti, A. Richard, S. Lazzaroni, S. Roux, P. Melnyk, D. Betbeder, N. Monfilliette-Dupont, Int. J. Pharm. 379 (2009) 270.
[21] G. Cirillo, O. Vittorio, S. Hampel, F. Iemma, P. Parchi, M. Cecchini, F. Puoci, N. Picci, Eur. J. Pharm. Sci. 49 (2013) 359–365.
[22] S. Lee, D. Seo, H. Park, Y. Choi, S. Jung, Antonie Van Leeuwenhoek 84 (2003) 201–207.
[23] Z. Jurasekova, G. Marconi, S. Sanchez-Cortes, A. Torreggiani, Biopolymers 91 (2009) 917.
[24] Pedro L.V. Falé, Lia Ascensão, M. Luisa Serralheiro, Parvez I. Haris, Food Funct. 3 (2012) 1176–1184.
[25] Jiakai Yan, Guowen Zhang, Junhui Pan, Yajie Wang, Int. J. Biol. Macromol. 64 (2014) 213–223.
[26] K. Xu, B. Liu, Y. Ma, J. Du, G. Li, H. Gao, Y. Zhang, Z. Ning, Molecules 14 (2009) 3486–3493.
[27] Y. Zhang, L. Xiang, Y. Zhang, X. Gao, Sep. Purif. Technol. 65 (2009) 130–136.
[28] M. Sowa, K. Slepokura, E. Matczak-Jon, CrystEngComm 15 (2013) 7696–7708.
[29] Z. Jurasekova, E. del Puerto, G. Bruno, J.V. Garcia-Ramos, S. Sanchez-Cortes, C. Domingo, J. Raman Spectrosc. 41 (2010) 1455–1461.
[30] F. Casadio, M. Leona, J.R. Lombardi, R. Van Duyne, Acc. Chem. Res. 43 (2010) 782–791.
[31] S. Protti, A. Mezzetti, C. Lapouge, J.P. Cornard, Photochem. Photobiol. Sci. 7 (2008) 109–119.
[32] C. Corredor, T. Teslova, M.V. Canamares, Z. Chen, J. Zhang, J.R. Lombardi, M. Leona, Vib. Spectrosc. 49 (2009) 190.
[33] N.F.L. Machado, L.A.E. Batista de Carvalho, J.C. Otero, M.P.M. Marques, Vib. Spectrosc. 49 (2009) 190–195.
[34] A. Rygula, T.P. Wrobel, J. Szklarzewicz, M. Baranska, Vib. Spectrosc. 64 (2013) 21–26.
[35] L. Vrielynck, J.P. Cornard, J.C. Merlin, M.F. Lautie, Spectrochim. Acta A 50 (1994) 2177–2188.
[36] M.G. de Carvalho, M.S.R. Gomes, M.C.C. de Oliveira, C.J. da Silva, A.G. de Carvalho, Braz. J. Pharmacogn. 21 (2011) 397–401.
[37] J.P. Cornard, L. Vrielynck, J.C. Merlin, J.C. Wallet, Spectrochim. Acta A 51 (1995) 913–923.

[38] P.J. Cox, Y. Kumarasamy, L. Nahar, S.D. Sarker, M. Shoeb, Acta Crystallogr. Sect. E 59 (2003) o975–o977.

[39] V. Exarchou, A. Troganis, I.P. Gerothanassis, M. Tsimidou, D. Boskou, Tetrahedron 58 (2002) 7423–7429.

[40] S. Protti, A. Mezzetti, J.P. Cornard, C. Lapouge, M. Fagnoni, Chem. Phys. Lett. 467 (2008) 88–93.

[41] A. Mezzetti, S. Protti, C. Lapouge, J.P. Cornard, Phys. Chem. Chem. Phys. 13 (2011) 6858–6864.

[42] S. Protti, K. Raulin, O. Cristini, C. Kinowski, S. Turrell, A. Mezzetti, J. Mol. Struct. 993 (2011) 485–490.

[43] G.J. Smith, S.J. Thomsen, K.R. Markham, C. Andary, D. Cardon, J. Photochem. Photobiol. A Chem. 136 (2000) 87–91.

[44] M. Kaneta, N. Sugiyama, Bull. Chem. Soc. Jpn. 4 (1971) 3211-3211.

[45] T. Matsuura, Afinidad 34 (1977) 48–56.

[46] T.K. Devon, A.I. Scott, Handbook of Naturally-Occurring Compounds, Acetogenins, Shikimates, and Carbohydrates, vol. 1, Academic Press, New York, 1975.