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Application of comparative vibrational spectroscopic and mechanistic studies in analysis of fisetin structure

Jasmina M. Dimitrić Marković[a,*], Zoran S. Marković[b], Dejan Milenković[c], Svetlana Jeremić[b]

[a] Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
[b] Department of Bio-Chemical and Medical Sciences, State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Serbia
[c] Bioengineering Research and Development Center, 34000 Kragujevac, Serbia

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ABSTRACT

This paper addresses experimental and theoretical research in fisetin (2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one) structure by means of experimental IR and Raman spectroscopies and mechanistic calculations. Density Functional Theory calculations, with M05-2X functional and the 6-311+G (2df, p) basis set implemented in the Gaussian 09 package, are performed with the aim to support molecular structure, vibrational bands' positions and their intensities. Potential energy distribution (PED) values and the description of the largest vibrational contributions to the normal modes are calculated. The most intense bands appear in the 1650–1500 cm^{-1} wavenumber region. This region involves a combination of the C=O, C2=C3 and C–C stretching vibrational modes. Most of the bands in the 1500–1000 cm^{-1} range involve C–C stretching, O–C stretching and in-plane C–C–H, C–O–H, C–C–O and C–C–C bending vibrations of the rings. The region below 1000 cm^{-1} is characteristic to the combination of in plane C–C–C–H, H–C–C–H, C–C–C–C, C–C–O–C and out of plane O–C–C–C, C–C–O–C, C–C–C–C torsional modes. The Raman spectra of baicalein and quercetin were used for qualitative comparison with fisetin spectrum and verification of band assignments. The applied detailed vibrational spectral analysis and the assignments of the bands, proposed on the basis of fundamentals, reproduced the experimental results with high degree of accuracy.

1. Introduction

Flavonoid family is the vast and very important group of low-molecular weight plant phenolics found ubiquitously in plants. Structurally they are benzo-γ-pyrone derivatives built upon a $C_6H_5(A)–C_3–C_6H_5(B)$ flavone skeleton in which oxygen is the heteroatom [1,2].

The unique electron-rich and highly conjugated chemical structure of flavonoids, especially flavones and flavonols, enable them to act as very good hydrogen and electron donors which are very important determinants in antioxidant activity. Their numerous beneficial actions, which include: antitumor activity, the ability to inhibit certain enzymes, detoxification of hydrogen peroxide through non-enzymatic defense mechanisms, pigmentation, nitrogen fixation, termination of the propagation phase in which hydro-peroxy lipids are formed, growth and reproduction, regulation of iron channels associated with phosphorylation, resistance to pathogens and predators, chemical defense and UV-protection of plants, are closely related to their pronounced antioxidant potential [2–19].

The ways to disclose the relationship between the structure, properties and performance of flavonoids as very important molecules are provided, more and more frequently, by combination of experimental measurements and the various tools of computational chemistry. This paper is a part of ongoing investigations of flavonoids' molecular structures [20–22] and it addresses experimental and theoretical research in fisetin structure by means of experimental IR and Raman spectroscopies and mechanistic calculations. Because of its specific structural features fisetin is considered as a potent antioxidant that is consequently arising great medical interest in it. Among its most striking and diverse collection of activities are stimulation of the signaling pathways that enhance long-term memory, neuroprotective role, modulation of the expression of more than twenty genes at transcription level, inhibition of the aggregation of the amyloid beta protein (Abeta) that may cause the progressive neuronal loss in Alzheimer's disease and induction of neuronal differentiation. Recently this has lead to the creation of a new type of therapeutic drug for Alzheimer's disease [23,24]. The applied combination of the experimental vibrational spectroscopic methods and theoretical calculations, could be generally applied in analyzing the structural changes of naturally

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[*] Corresponding author. Tel.: +381 11 333 6624; fax: +381 11 218 7133.
E-mail address: markovich@ffh.bg.ac.rs (J.M. Dimitrić Marković).

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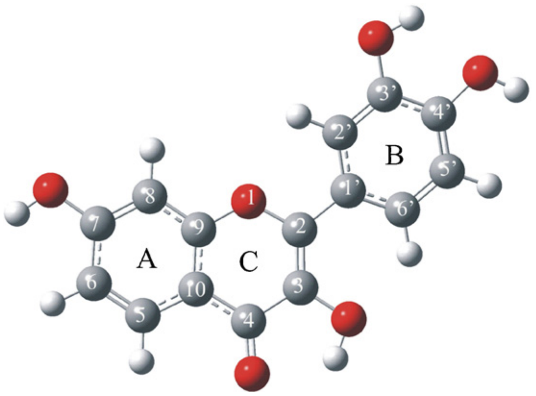

Fig. 1. Structure and atomic numbering of fisetin.

occurring flavonoids under physiologically relevant conditions, providing insight into the mechanism of their bioactivity.

2. Experimental

2.1. Computational methods

The conformations of the most stable fisetin form (Fig. 1) were fully optimized with M05-2X method developed by the Truhlar group [25] by using the 6-311+G (2df, p) basis set as implemented in the Gaussian 09 package [26]. This hybrid meta exchange–correlation functional is parameterized so that it includes both nonmetallic and metallic compounds. This functional yields satisfactory overall performance for the main-group thermochemistry and thermochemical kinetics, as well as organic, organometallic, biological and noncovalent interactions [25,27,28].

The vibrational frequencies were obtained from diagonalization of the corresponding M05-2X Hessian matrices. The nature of the stationary points was determined by analyzing the number of imaginary frequencies: 0 for minimum and 1 for transition state. Relative energies were calculated at 298 K.

The vibrational modes were assigned on the basis of PED analysis using VEDA 4 program with its visualization interface [29]. Normal coordinate analysis of fisetin was carried out to obtain a more complete description of the molecular motions involved in the fundamentals. The calculated vibrational wavenumbers were scaled with the scale factor of 0.9444 in order to obtain better match between calculated and experimental wavenumber values.

Potential energy surface was obtained in relation to the torsion angle τ, defined by the C3–C2–C1′–C2′ atoms, between the rings B and C. The natural bond orbital (NBO) analysis [30–33] of fiestin was also performed.

2.2. IR spectra

IR spectra were recorded on a Thermo Nicolet 6700 FT-IR Spectrometers with ATR. The spectra were recorded in the middle IR region, 4000–400 cm^{-1}, which is important for monitoring the structural changes of molecule. The spectral resolution was 2 cm^{-1}. Fisetin (Merck, USA) was studied in potassium bromide matrix with a ratio of 1 mg:150 mg (fisetin:KBr).

2.3. Raman spectra

Raman spectra were recorded on Thermo Scientific Nicolet Almega XR Raman spectrometer. A 532 nm laser was used for sample illumination, enabling a spectral resolution of 2 cm^{-1}. The spectrum of solid phase was recorded at room temperature by using the potassium bromide matrix (1 mg:150 mg; fisetin:KBr). Each spectrum was collected after 1 s of exposure. The number of exposures was ten. The fluorescence correction has been done.

The theoretical Raman intensities (I_i^R) were derived from the computed Raman scattering activities using the following equations:

$$I_i^R = C(\upsilon_0 - \upsilon_i)^4 \cdot \upsilon_i^{-1} \cdot B_i^{-1} \cdot S_i \qquad (1)$$

where B_i is a temperature factor which accounts for the intensity contribution of excited vibrational states, and is represented by the Boltzmann distribution:

$$B_i = 1 - \exp\left(-\frac{h\upsilon_i c}{kT}\right) \qquad (2)$$

In Eq. (1), υ_0 is the wavenumber of the laser excitation line (υ_0 = 18,797 cm^{-1} which corresponds to the wavelength of 532 nm), υ_i is the wavenumber of normal mode (cm^{-1}), while S_i is the Raman scattering activity of the normal mode Q_i. The theoretical Raman intensity, I_i^R, is given in arbitrary units (C is a constant equal to 10^{-12}). In Eq. (2) h, k, c, and T are the Planck and Boltzmann constants, speed of light and temperature in Kelvin, respectively. The value of factor B_i was assumed to be 1. Otherwise, the calculated Raman intensities for the bands below 300 cm^{-1} were extremely overestimated in comparison to the experiment [34].

3. Results and discussion

3.1. Conformational analysis

The obtained geometrical parameters of fisetin (bond lengths, bond angles, and torsional angles), calculated by using M052X/6-311+G (2df, p) level of theory, are presented in Table 1. In order to determine the preferred relative positions between the rings B and C the conformational space of the structure presented in Fig. 1 is explored as a function of torsional angle τ between those rings (Fig. 2). It is found that fisetin has a non planar geometry with the part of the molecule, containing cycles A and C, which has complete planarity. In order to examine potential energy surface, the torsion angle τ was scanned in steps of 10° without constraints on all other geometrical parameters. Removing constrain for the torsional angle τ the conformational absolute minimum is found at τ = 11.64°, followed by a relative minimum at τ = 168.82° with energy difference of only 0.38 kcal mol^{-1}. This result is in a good agreement with the result obtained by the PM3 method (166°) [35], while the results obtained by B3LYP/6-311++G (d, p) method point to planar molecular structure [20].

The maximum of the potential energy lies at τ = 90° and the introversion barrier between the two minima is about 4.76 kcal mol^{-1} (Fig. 2). This value is almost 1 kcal mol^{-1} lower than one obtained by the B3LYP method [20]. It is worth mentioning that in going from τ = 0° to ±30°, the potential energy curve is very flat with an energy variation of about 0.29 kcal mol^{-1} indicating the planar conformation as the one easily obtained, requiring a negligible amount of energy. This result is in good agreement with value obtained by PM6 method (0.17 kcal mol^{-1}) [35].

The results of NBO analysis of the most stable structure of fisetin indicate strongly localized double bonds at the C2–C3 and C4–O positions of the ring C. The bond order values suggest a highly independent electronic delocalization in the rings B and A, which is also one of the main structural features implicated in antioxidant activity of flavon-3-ols. Almost planar structure of fisetin (Fig. 1) enables such electronic delocalization. The C2–C1′ bond lies in the chromone plane, because the torsional angle τ is 168.8° and its length is 1.464 Å. The bond order of this bond, obtained by the NBO analysis, is close to 1 indicating very small conjugation across all the rings of the π system. The positions of the double bonds in the ring C around the carbonyl group indicate a crossconjugated system