Synergic application of spectroscopic and theoretical methods to the chlorogenic acid structure elucidation

Svetlana Marković [a,*], Jelena Tošović [a], Jasmina M. Dimitrić Marković [b]

[a] Faculty of Science, University of Kragujevac, 12 Radoja Domanovića, 34000 Kragujevac, Serbia
[b] Faculty of Physical Chemistry, University of Belgrade, 12-16 Studentski trg, 11000 Belgrade, Serbia

A B S T R A C T

Although chlorogenic acid (5-O-caffeoylquinic acid, **5CQA**) is a dietary polyphenol known for its pharmacological and nutritional properties, its structural features have not been completely elucidated. This is the first study whose aim is to contribute to clarification of the **5CQA** structure by comparing the experimental and simulated IR, Raman, ^{1}H NMR, ^{13}C NMR, and UV spectra. For this purpose, a comprehensive conformational analysis of **5CQA** was performed to reveal its most stable conformations in the gas-state and solution (DMSO and methanol). The lowest-energy conformers were used to predict the spectra at two levels of theory: B3LYP-D3/and M06-2X/6–311+G(d,p) in combination with the CPCM solvation model. Both methods provide very good agreement between all experimental and simulated spectra, thus indicating correct arrangement of the atoms in the **5CQA** molecule. The quinic moiety is characterized with directed hydrogen bonds, where the carboxylic hydrogen is not oriented towards the carbonyl oxygen of the carboxylic group, but towards the oxygen of the proximate hydroxyl group. In the gas-state the lowest-energy conformers are characterized with the O4—H4⋯O9′ hydrogen bond, whereas in the solvated state the structures with the O4—H4⋯O10′ hydrogen bond prevail. Knowing the fine structural details, i.e. the proper conformation of **5CQA**, provides a solid base for all further investigations related to this compound.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

It has been widely accepted that dietary antioxidants play a protective role against the development and progression of the pathological conditions related to oxidative stress. Epidemiological studies showed that consumption of fruits and vegetables is helpful in preventing some chronic diseases [1,2]. Fruits and vegetables are rich in natural polyphenols, whose antioxidative properties proved to contribute to the protective role of these foods [3]. An important class of polyphenols is phenolic acids. The interest in these compounds originates from their numerous biological roles [4]. One of the phenolic acids that is wide spread in human diet is chlorogenic acid (Fig. 1).

Chlorogenic acid (5-O-caffeoylquinic acid, **5CQA**) is an ester of the caffeic and quinic acids. It can be isolated from natural plants such as coffee, pears, plums, potatoes, tomatoes, carrots and oilseeds. Like other dietary polyphenols, chlorogenic acid shows pharmacological and nutritional properties, such as antitumor, antidiabetic, and

antihypertensive activities [5–7]. It has been recognized that **5CQA** can limit low-density lipoprotein oxidation, so it exhibits various antiatherosclerotic activities [8]. Furthermore, **5CQA** possesses anti-inflammatory, hypolipidemic, and antioxidative properties [9–12]. Theoretical investigations regarding antioxidative activity of **5CQA** have been initiated only recently [13,14]. Due to its high availability in the foods, the antioxidative potential of **5CQA** is very accessible. By scavenging alkylperoxyl radicals **5CQA** removes very toxic reactive species and prevents the damage they cause on DNA.

Chlorogenic acid exists in the form of white powder. Taking into account that the X-ray powder diffraction analysis for **5CQA** has not been carried out yet, the exact structure of solid **5CQA** is not known. There are few experimental studies devoted to the Raman and NMR spectra of **5CQA** [15–18]. In addition, UV–vis absorption spectroscopy has been used to investigate complex formation of some metal ions with **5CQA** [19,20]. The effects of solvent polarity on the UV–vis spectra of **5CQA** have been recently investigated [21]. It was shown in a recent work [18] that, in comparison to **5CQA**, (1S,3R,4S,5R)5-O-caffeoylquinic acid features an inverted configuration at the C4 atom. On the basis of the NMR study the preferential conformations of both isomers were
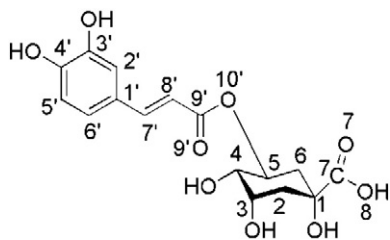
Fig. 1. Structural formula of 5-O-caffeoylquinic acid (**5CQA**). The atom labeling scheme is remained throughout the manuscript.

assumed. The aim of this study is to contribute to the **5CQA** structure elucidation by using different spectroscopic methods (IR, Raman, UV, ^{1}H and ^{13}C NMR) in conjunction with the corresponding quantum chemical calculations at two levels of theory: B3LYP-D3 and M06-2X.

2. Methods

2.1. Experimental

5CQA was purchased from a commercial source (Aldrich). The IR spectrum was recorded on a Thermo Nicolet 6700 FT-IR Spectrometer with the ATR and DTGS TEC detectors. The spectrum was recorded in the middle IR region, 4000–400 cm^{-1}. The spectral resolution was 2 cm^{-1}, the number of samples scans 512, the number of background scans 512. **5CQA** was studied in potassium bromide matrix with a ratio of (1: 200) mg (**5CQA**: KBr). The Raman spectrum was recorded on a Thermo Scientific DXR Raman microscope. The spectrum was excited with a diode pumped solid state high-brightness 532 nm laser. Objective lens was 50×. The scattered light was analyzed by the spectrograph with a 900 lines/mm grating. The spectrum was obtained in the region 3400–400 cm^{-1} directly from the pure powder samples. The laser output was kept at 10 mW. Acquisition time was 10 s with 10 scans. The fluorescence correction has been done. Thermo Scientific OMNIC software was used for data acquisition and data processing. The NMR spectra in DMSO-d_6 with TMS as the internal standard were recorded on a Varian Gemini 200 MHz NMR spectrometer (^{1}H at 200 and ^{13}C at 50 MHz). The UV spectrum in methanol was recorded on a Cary Series UV–vis spectrophotometer (Agilent Technologies) operating from 200 to 400 nm in a 1.0 cm quartz cell.

2.2. Theoretical

Two program packages were utilized within this study: Spartan'02 [22] and Gaussian 09 [23]. Spartan was exploited to search the conformational space of **5CQA**. For this purpose the MMFF (Merck Molecular Force Field) method [24] in combination with the Monte-Carlo routine was used. Gaussian was employed for all further computations. The **5CQA** rotamers resulting from the molecular mechanics calculations underwent a series of quantum mechanical calculations (see Section 3.1) to reveal the most stable conformers of the compound in the gas-state, as well as in the DMSO and methanol solutions (dielectric constants amount to 46.826 and 32.613, respectively). The structures of the lowest-energy rotamers in all three media were obtained by full optimizations, including frequency calculations which confirmed that all examined structures correspond to equilibrium geometries (no imaginary vibrations). For this purpose two DFT methods were applied: B3LYP-D3 and M06-2X, in combination with the 6–311+G(d,p) basis set and CPCM polarizable continuum solvation model [25]. B3LYP-D3 is a dispersion-corrected hybrid GGA (Generalized Gradient Approximation) functional which contains an additional term in the formula for the total energy [26,27], whereas M06-2X is a global-hybrid meta-GGA functional with 54% HF (Hartree-Fock) exchange [28]. The gas-phase geometry was used to predict the IR and Raman spectra, whereas

the conformers of **5CQA** in DMSO and methanol solutions were used to simulate the corresponding NMR and UV–vis spectra at both levels of theory.

The vibrational modes of **5CQA** were assigned on the basis of the PED (Potential Energy Distribution) analysis [29] using the VEDA 4 software [30]. A normal coordinate analysis of **5CQA** was performed to gain a more comprehensive description of the molecular motions involved in the fundamentals. The ^{13}C NMR and ^{1}H properties of **5CQA** in DMSO were predicted by means of the GIAO (Gauge Independent Atomic Orbital) method, as implemented in Gaussian 09.

To simulate the UV spectrum of **5CQA** in methanol the ground state geometry of the compound was subjected to the TDDFT (Time Dependent Density Functional Theory) [31] calculations in combination with both applied functionals. The B3LYP-D3 ground state geometry was used to perform the NBO (Natural Bond Orbital) analysis [32]. Using a strategy applied in a recent paper devoted to the UV–vis absorption of some phenolic compounds [33], the NLMO (Natural Localized Molecular Orbital) clusters of **5CQA** were constructed, and they were used to provide an informative description of electronic transitions. The NBO analysis was also used to gain insight into the favorable donor-acceptor interactions.

3. Results and discussion

3.1. Conformational analysis of 5CQA

Bearing in mind that **5CQA** can adopt numerous conformations, the first task of this work was to carry out conformational analysis of the compound, and thus determine its most stable conformers in the gas-state, and in the DMSO and methanol solutions. The MMFF based Monte-Carlo method constructed in total 1089 gas-phase rotamers of **5CQA**. These conformers were carefully inspected, and the duplicates excluded. In this way, a series comprising 224 conformers of **5CQA** was formed. To reorganize the stability order of the rotamers obtained by molecular mechanics (known for unreliable energy predictions), these 224 structures were subjected to quantum mechanical calculations. To avoid high computational cost, all the structures were optimized (frequency calculations were not performed) using the gas-phase B3LYP/6-31G(d) theoretical model. Twenty conformers of the lowest total energy were selected for further consideration. Their structures in the gas-phase, as well as in the DMSO and methanol solutions, were fully reoptimized, and frequency calculations were carried out. For this purpose, the B3LYP-D3/6–311+G(d,p) theoretical model was employed. Taking into account that **5CQA** contains many hydrogen bonds one can expect that this method, which has been designed for modeling short- and medium range interactions, will satisfactorily predict molecular properties, particularly conformational energies.

As expected, the order of rotamer stability in the gas-phase is notably different from that in solution, whereas the stability orders are almost identical in the two solvents of moderate polarity (Fig. S1). It is worth emphasizing that **5CQA** in the gas-state adopts three conformations whose differences in free energy do not exceed 1 kJ mol^{-1} (structures **1**, **2**, and **3** in Fig. S1). In solution, there are even nine such conformers (structures **8**, **12**, **1**, **9**, **14**, **11**, **2**, **13**, and **6**). Our calculations indicate that **5CQA** exists as a complex conformational mixture. In the gas-state the lowest-energy conformers are characterized with the O4—H4···O9′ hydrogen bond, whereas in the solvated state the structures with the O4—H4···O10′ hydrogen bond prevail.

We will now focus on the two most stable conformations of **5CQA**: **1** in the gas-phase, and **8** in solution. Their optimized geometries are depicted in Fig. 2, whereas the corresponding Cartesian coordinates are provided in Tables S1 and S2. Because the findings for the DMSO and methanol solutions are mutually very similar, this discussion will be based on the results for **8** solvated with DMSO. In both gas and solvated states the caffeic acid moiety of **5CQA** agrees with the crystal structure of caffeic acid [34]. **5CQA** has in total five intramolecular