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Quantum chemical calculations in the structural analysis of phloretin

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ABSTRACT

In this work, a conformational search on the molecule of phloretin [2′,4′,6′-Trihydroxy-3-(4-hydroxyphenyl)-propiophenone] has been performed. The molecule of phloretin has eight dihedral angles, four of them taking part in the carbon backbone and the other four, related with the orientation of the hydroxyl groups. A systematic search involving a random variation of the dihedral angles has been used to generate input structures for the quantum chemical calculations. Calculations at the DFT(B3LYP)/6-311++G(d,p) level of theory permitted the identification of 58 local minima belonging to the C_1 symmetry point group. The molecular structures of the conformers have been analyzed using hierarchical cluster analysis. This method allowed us to group conformers according to their similarities, and thus, to correlate the conformers' stability with structural parameters. The dendrogram obtained from the hierarchical cluster analysis depicted two main clusters. Cluster I included all the conformers with relative energies lower than 25 kJ mol^{-1} and cluster II, the remaining conformers. The possibility of forming intramolecular hydrogen bonds resulted the main factor contributing for the stability. Accordingly, all conformers depicting intramolecular H-bonds belong to cluster I. These conformations are clearly favored when the carbon backbone is as planar as possible.

The values of the *ν*C=O and *ν*OH vibrational modes were compared among all the conformers of phloretin. The redshifts associated with intramolecular H-bonds were correlated with the H-bonds distances and energies.

1. Introduction

Flavonoids are natural products derived from 2-phenylchromen-4-one (flavone). They are widely distributed in plants and fulfill many functions, including the production of yellow or red/blue pigmentation in flowers and their protection from attack by microbes and insects. The anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activity of flavonoids have attracted the attention of food manufacturers and consumers [1,2].

Phloretin [2′,4′,6′-trihydroxy-3-(4-hydroxyphenyl)-propiophenone or 3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-1-propanone] is a flavonoid known by its anti-oxidant activity and it is mainly present in apples. In this sense, the anti-oxidative properties of apples have been attributed to the phytochemicals present in the apple skin, namely phloretin [3,4]. The generation and abundance of reactive oxygen species is closely associated with the development and progression of atherosclerosis, and phloretin has an active role in avoiding the accumulation of these reactive oxygen species [5].

In its uncharged form, phloretin is known to be a powerful inhibitor of the glucose transport system in human red blood cell membrane [6–8]. In addition, it affects the membrane transport of glycerol, urea, chloride and a great number of other charged and neutral substances. It also acts as an uncoupler of the mitochondrial oxidative phosphorylation [9].

From a physical chemistry point of view, phloretin is known to adsorb to lipid surfaces and alter the dipole potential of lipid monolayers and bilayers [9–11]. In this respect, the effect of phloretin on lipid membranes can be ascribed to its strong interaction with the phosphate groups of lipids, as demonstrated by the pronounced downward shift of the asymmetric vibration frequencies of lipid phosphates in the FTIR spectra [9–11].

The interaction of phloretin with lipids can be investigated from two points of view: (a) considering the lipid changes induced by phloretin, thus focusing the study on the lipids or (b) considering the conformations that the flavonoid may adopt in the interaction, thus focusing the study on the phloretin structure. Up to our knowledge, only the first approach (a) has been used to deal with the phloretin–lipid interaction. On the contrary, no attempts to investigate the molecular structure of phloretin have been reported hitherto. In this sense, quantum chemical calculations constitute a powerful method to deal with this issue.

Taking into account the lack of fundamental information on phloretin, this work aims to shed light on the molecular structure of the compound, as a first step to understand its behavior and

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mechanisms of action in more complex environments (i.e., biological environments). In order to assess the molecular structure of phloretin, a systematic conformational study of the compound in the gaseous phase has been carried out. With its 34 atoms, phloretin is a large molecule rich in low-energy conformational minima. In this work, an effort was made to systematically search the most relevant conformers. This search involved a random variation of the relevant dihedral angles to generate more structures, which were subsequently subjected to minimization. The molecular structures of the located conformers have then been analyzed using hierarchical cluster analysis. This method allowed us to group conformers according to similarities, and thus, to correlate the conformers' stability, hydrogen bonding properties and νC=O and νOH vibrational frequencies.

2. Materials and methods

2.1. Computational methodology

The semi-empirical PM3 method was used to perform a systematic preliminary conformational search on the phloretin potential energies surfaces (PES). It provided a quick assessment of the energy conformers suitable for further analysis. This conformational search was carried out using HyperChem Conformational Search module (CyberChem, Inc. © 2004) [12]. Taking into account the high flexibility of the phloretin molecule, a random search appeared as the most appropriate way to perform a conformational analysis [13–15]. In this approach, the generation of new starting conformations for the energy minimization uses a random variation of the dihedral angles of previously found conformers [13,14]. The method searches on until all given starting geometries have been used or no new minima are generated.

In this work, eight dihedral angles defining conformational isomers of phloretin were considered in the random search: $C_2C_1C_{13}C_{16}$, $C_1C_{13}C_{16}C_{19}$, $C_{13}C_{16}C_{19}C_{21}$, $C_{16}C_{19}C_{21}C_{23}$, $C_{21}C_{22}O_{29}H_{30}$, $C_{25}C_{26}O_{31}H_{32}$, $C_{21}C_{23}O_{33}H_{34}$ and $C_3C_4O_{11}H_{12}$ (see Scheme 1). Conformations with energies lower than 75 kJ mol^{-1} were stored while higher-energy conformations or duplicate structures were discarded. The structures obtained from the random search served as start point for the construction of the input files later used in the quantum chemical calculations.

The quantum chemical calculations were performed with Gaussian 98 [16] at the DFT level of theory, using the 6-311++G(d,p) basis set [17]. The three-parameter density hybrid functional abbreviated as B3LYP, which includes Becke's gradient exchange correction [18] and the Lee, Yang and Parr [19] and Vosko, Wilk and Nusair correlation functionals, was selected for calculations [20].

Conformations were optimized using the Geometry Direct Inversion of the Invariant Subspace (GDIIS) method [21]. The optimized structures of all conformers were confirmed to correspond to true minimum energy conformations on the PES. The calculated frequencies were considered as obtained in calculation, without any further scaling.

2.2. Cluster analysis

After obtaining the optimized structures of the conformers of phloretin, a double entrance table was constructed as follows:

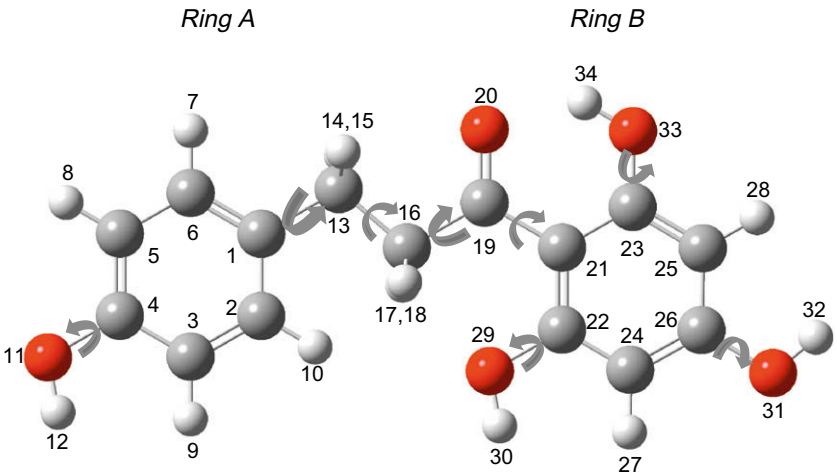
All dihedrals defining the conformers were considered for the analysis. For each dihedral angle, different columns corresponding to specific ranges of its possible values were built. The following ranges were considered:

(a) for $C_2C_1C_{13}C_{16}$: 80–95° and 50–55°,
(b) for $C_1C_{13}C_{16}C_{19}$: *ca.* 60°, *ca.* 90° and 180°,
(c) for $C_{13}C_{16}C_{19}C_{21}$: *ca.* −120°, *ca.* −140°, *ca.* −160°, *ca.* 45°, *ca.* 60°, *ca.* 75°, *ca.* 90°, *ca.* 120°, *ca.* 180°,
(d) for $C_{16}C_{19}C_{21}C_{23}$: *ca.* 0°, 40–50°, 55–75°, *ca.* 120°, *ca.* 155°, *ca.* 180°,
(e) for $C_{21}C_{22}O_{29}H_{30}$: *ca.* 0° and *ca.* 180°,
(f) for $C_{25}C_{26}O_{31}H_{32}$: *ca.* 0° and *ca.* 180°,
(g) for $C_{21}C_{23}O_{33}H_{34}$: *ca.* 0° and *ca.* 180°,
(h) for $C_3C_4O_{11}H_{12}$: *ca.* 0° and *ca.* 180°.

The ranges were defined according to the values obtained for the dihedrals considered in all the conformers. Symmetry related conformers were discarded.

Once defined the column ranges for all the dihedrals, the presence or absence of a given dihedral value was assigned as 1 and 0, respectively. For example, if the dihedral $C_{16}C_{19}C_{21}C_{23}$ is *ca.* 120°, 0 will be the value assigned to the columns corresponding to the ranges *ca.* 0°, 40–50°, 55–75°, *ca.* 155° and *ca.* 180°, and 1, the value assigned to the column corresponding to *ca.* 120°.

The comparison among conformers was carried out using the simple-matching coefficient (S_{sm}), which was calculated as



3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one

Scheme 1.