Influence of catecholic ring torsion on hydroxyflavones

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Abstract: Systematic quantum chemical investigation of quercetin and selected eight mono- and bi-hydroxyflavonols is presented. Structural analysis based on the Density Functional Theory showed that the energetically preferred conformation of flavonols substituted at the C5 and C3 atoms by a hydroxyl group is stabilised via intramolecular hydrogen bonds occurring between the (C4)O···HO(3 or 5) atomic pairs. Depending on the hydroxyl group positions, energetically preferred torsional orientation of the phenyl ring with respect to the planar benzo-γ-pyrone moiety changed from 0 to 180 degrees. Gas-phase electron transitions were investigated using the time-dependent DFT treatment. The dependence of maximal wavelengths on the torsional deformation of the phenyl ring is of a similar shape, i.e. minima observed for the perpendicular orientation and maxima for the planar one. Shape and energies of the Highest Occupied (HOMO) and Lowest Unoccupied (LUMO) Molecular Orbitals were compared. The obtained theoretical results were compared with available experimental data.

Keywords: BDE, hydroxyflavones, torsion

Introduction

Polyphenols are aromatic compounds with large systems of π-electron configurations. Thanks to this configuration they absorb visible light which makes them colourful. Their name derives from the Ancient Greek word πολύς (polus, meaning "many, much") and the word phenol which refers to a chemical structure formed by attaching of a hydroxyl (—OH) group to an aromatic benzenoid (phenyl) ring as it is found in alcohols (hence the -ol suffix, Quideau et al., 2011). The term polyphenol has been in use at least since 1894 (Merriam-webster). The number and characteristics of these phenol structures underlie the unique physical, chemical, toxic, metabolic or pharmaceutical properties of the class members (Williams et al., 2004). These compounds are not polymers of phenol. Although phenol can be polymerised by electrochemical oxidation, the formed products are not referred to as "polyphenols" (Mengoli et al., 1987). Polyphenols also possess significant binding affinity for proteins, which can lead to the formation of soluble and insoluble protein-polyphenol complexes (Papadopoulou et al., 2004).

Flavonoids are a specific group of naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables (Hollman et al., 2000). Physico-chemical properties of flavonoids are generally associated with three chemical features: (i) ortho-di-hydroxy structure in the B-ring, (ii) presence of a 2,3 double bond in the C-ring, and/or (iii) presence of a 4-oxo function in the C-ring (Figure 1). The various classes of flavonoids differ in the level of oxidation of the 'C' ring of the basic benzo-γ-pyrone structure. Common family members of flavonoids include flavones, flavanes, flavonols, catechins and anthocyanidins. Flavonols are flavonoids of particular importance because they have been found to possess antioxidant and free radical scavenging activity in foods (Shahidi et al., 1992). Some polyphenols are traditionally used as dyes. For example, 5-hydroxyflavonol and 3-hydroxylflavonol and their derivatives usually exhibit two strongly separated bands in their fluorescence spectrum due to the excited state intramolecular proton transfer, leading to two excited forms: normal N*, and tautomer T*. Fluorescence maxima positions and relative intensities depend on the solvent environment (Sengupta et al., 1979). This phenomenon is used in the construction of effective probes used in bio-structures' analysis (Klymchenko et al., 2002).

Excited states of flavonoids were experimentally studied as well as predicted by means of semiempirical, *ab initio*, and density functional theory (DFT) quantum mechanical methods (Tošović et al., 2017). The semiempirical ZINDO method combined with the configuration interaction, where singlet excitations are included, showed unpredictable accuracy (Kotzian et al., 1992). The more computing-demanding *ab initio* methods, e.g. complete active space self-consistent field (CASSCF) method (Andresson and Roos, 1995) or multi-reference configuration interaction (MRCI, Sherrill et al., 1999), are usually successful, but the quality of the obtained results is highly dependent on the selec-
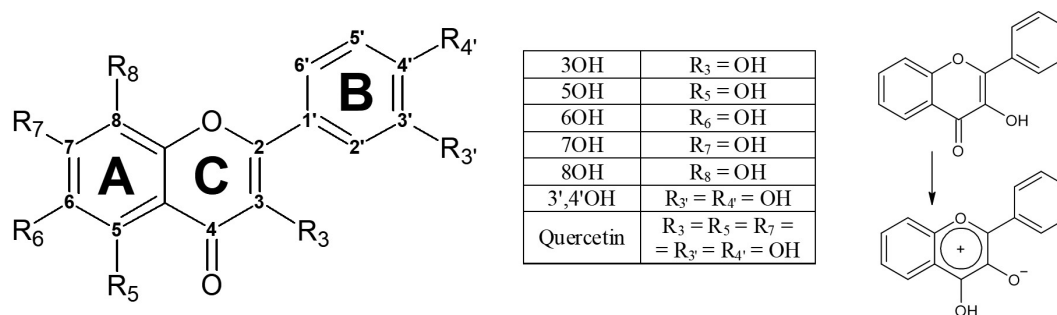
Fig. 1. General structure and numbering scheme of hydroxyflavones (R_x = H unless stated otherwise) and the reaction scheme of 3-hydroxyflavone **3OH** tautomerisation into **t3OH**.

tion of the created active space. In the last decade, the time-dependent density functional theory (TD-DFT) has been successfully applied to examine the excited states of some polyphenolic compounds (Gierschner et al., 2012).

Although many published works reported experimental and theoretical studies on the electronic structure and spectroscopic properties of a large variety of flavone and flavonol derivatives (Valle, 2006; Ash et al., 2010; Ahmed et al., 2017), systematic comparative studies on electron structure changes upon torsional deformation of the B ring are lacking. Therefore, a systematic theoretical analysis of selected flavonols is here presented (Fig. 1). Partial aims of this quantum chemical study are: (1) finding optimal structures of ground electron states and corresponding radicals formed after homolytic O—H group cleavage; (2) investigating the shape of gas-phase torsional potentials belonging to the B ring with respect to the hydroxyl group positions; (3) analyzing the torsional dependence of experimentally relevant vertical optical transitions and bond dissociation energies. Finally, the shapes and energies of the Highest Occupied (HOMO) and Lowest Unoccupied (LUMO) Molecular Orbitals were compared. Theoretical results were compared with available experimental data.

Methods

Gaussian program package at current revision 16 (Frisch et al., 2016) was used for all calculation done at the DFT level of theory. The B3LYP (Becke, 1988; Lee et al., 1988) hybrid functional and 6-31G* basis sets were employed for all atoms (Rassolov et al., 2001). To study the flexibility of neutral molecules in their singlet or doublet (for radicals after H• abstraction) electron ground state, a relaxed scan was done by optimizing the geometry with fixed C3—C2—C1'—C2' dihedral angle. Total of ten steps by 18 degrees were considered. First 3- excited states were calculated at each step by the TD-DFT method with the B3LYP functional.

Molecular orbitals were depicted using the Jsmol HTML5 web applet (Jmol 2019).

Based on the optimised structures, cleavage of the hydroxyl O—H bonds in terms of bond dissociation energies (BDE) was calculated following the equation

$$BDE = E(Ph—O^\bullet) + E(H^\bullet) – E(Ph—OH) \quad (1)$$

where $E(Ph—OH)$ represents the DFT energy of hydroxyflavone and $E(Ph—O^\bullet)$ is the DFT energy of its radical. Hydrogen atom energy $E(H^\bullet)$ in the gas phase is –0.500272784186 hartree.

Results

Within the selected theoretical method, most of the optimised structures are not planar with the exception of 3-hydroxyflavone (**3OH**). As a reference, predicted dihedral angles between rings AC and B (see Fig. 1) are compared in Tab. 1 with the experimental ones determined from crystal structures. Notable discrepancies can be found at first sight, but they can be argued quite easily. To begin with, the dihedral angle varies wildly even within the crystal structures of the same molecule (see e.g. quercetin), which is caused by solvent and crystal packing effects. Moreover, Fig. 2a indicates the barrier of less than 2 kJ mol⁻¹ for significant torsion for most of the moieties. In the solvent environment, this barrier is probably even lower; therefore, free rotations of at least ±20 ° are definitely enabled. From the symmetry point of view, quercetin and 3',4'-hydroxyflavone (**3',4'OH**) conformations with dihedral angles of 0 ° and 180 ° are not identical. While for the latter one, they are virtually the same from the energy point of view, in case of quercetin there is a small energy preference of around 2 kJ mol⁻¹ for the 0 ° angle (Fig. 2a). Given this negligible difference, only 180 ° torsion starting with the most stable conformations was considered in the following discussion.

Falantin et al. (2017) also found **3OH** with C_s symmetry with the strong intramolecular H-bond

Tab. 1. Theoretical angles and BDEs, angles from X-ray structures are in parenthesis.

	Dihedral angle C3—C2—C1'—C2'	BDE/kJ mol⁻¹
3OH	0 ° (5.5 ° − Etter, 1986) (4.7 ° − Schutte-Smith, 2019)	362
5OH	37 ° (5.8 ° − Shoja, 1990)	430
6OH	19 ° (9.8 ° − Seetharaman, 1992) (13 ° − Shoja, 1998)	363
7OH	19 ° (18.6 ° − Kumar, 1998)	371
8OH	19 °	352
3',4'OH	35 °	317(4'O•), 322(3'O•)
Quercetin	18 ° (1.0 ° − Domagała, 2011) (7 ° − Rossi, 1986) (6.7 ° − Jin, 1990) (32 ° − Vasisht, 2016)	321(3'O•) 314(4'O•) 346(3O•) 415(5O•) 377(7O•)
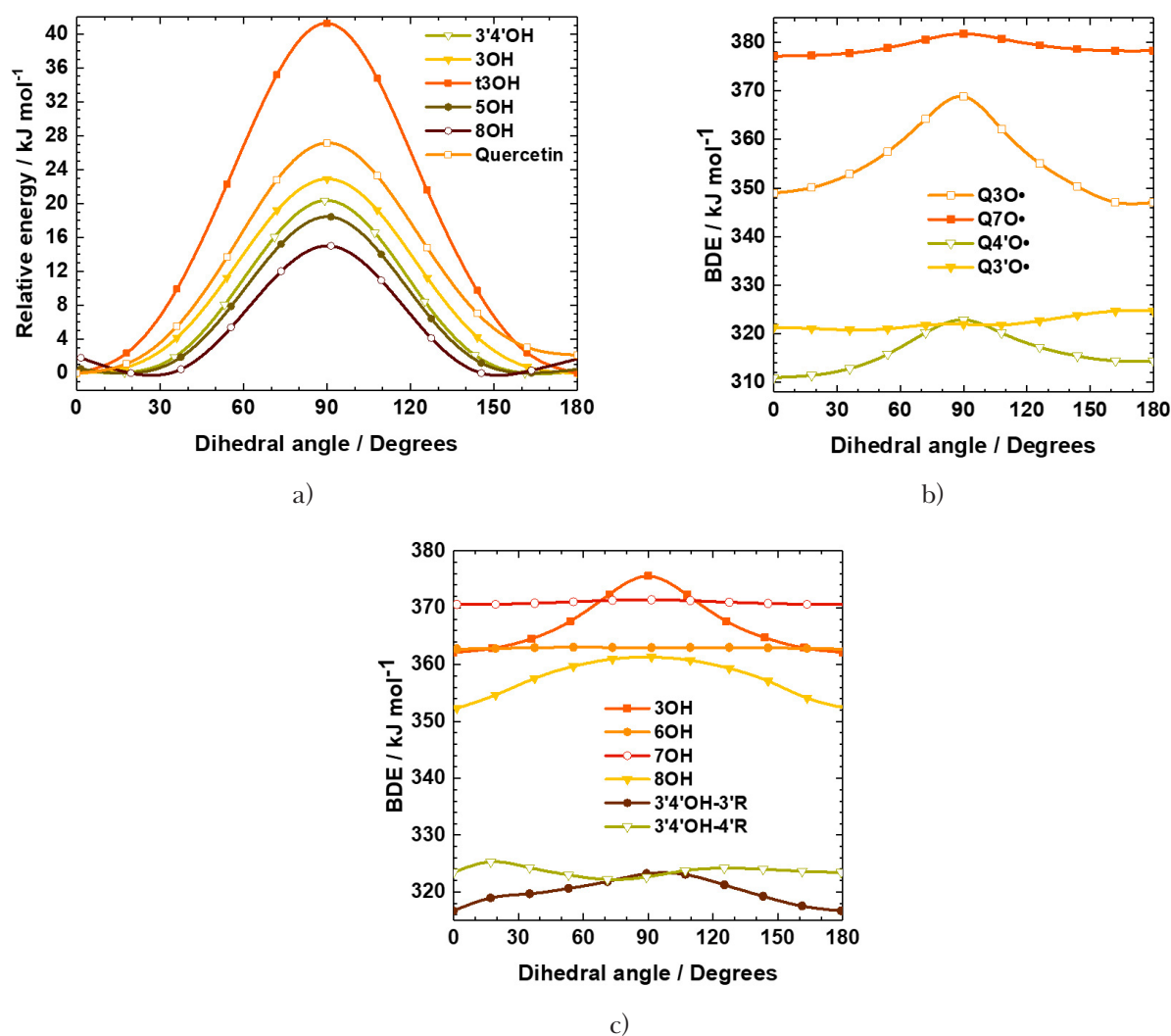
Fig. 2. Scan of catecholic ring torsion: a) neutral state energy (optimised geometry as reference), b) bond dissociation energy of quercetin, c) BDEs of other hydroxyflavones.

between the H3 atom of the hydroxyl group and the O4 atom. This H-bond also supports proton transfer, creating a tautomer (denoted by prefix **t**).

Multiple experimental results confirm the significance of the tautomeric form of 3-hydroxyflavone (**t3OH**, shown in Fig. 1) and 5-hydroxyflavone

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(**t5OH**). Incidentally, the highest rotational barrier shows **t3OH,** which can be explained by the conjugation of AC rings extended to the B ring. This is not the case in the **t5OH** tautomer, which was found to be very similar to **3'4'OH** in terms of torsion energy demands (not shown in Fig. 2). For other molecules, the findings are consistent with the single bond character of the C2—C1' bond. Position of the hydroxyl group in case of **5OH**, **6OH** and **7OH** seems to play a little to no role as almost identical curves were found in these cases; therefore, only one of them is shown in Fig. 2. The catecholic ring is the least restrained from the rotation in 8-hydroxyflavone. Apart from tautomers, quercetin has the most restrained B ring rotation.

Since flavonoids are often studied for their antioxidant properties, investigations of the change in dihedral angle on the energetics of the homolytic O—H bond cleavage should be considered essential. Despite employing somewhat small basis sets, our BDEs are in very good agreement with the very precise G4 BDEs (Alvareda 2016). Correlation coefficient between the data reached the value of 0.991 (not presented). Calculated BDEs of our studied hydroxyflavones have mostly the lowest values for ca. 0 ° and 180 ° angles and the highest values for 90 °. An exception is the 3'R radical formed from **3',4'OH** with a different type of dependence as seen in Fig. 2c: two maxima at 18 ° and at 126 °. Interestingly, when the quercetin
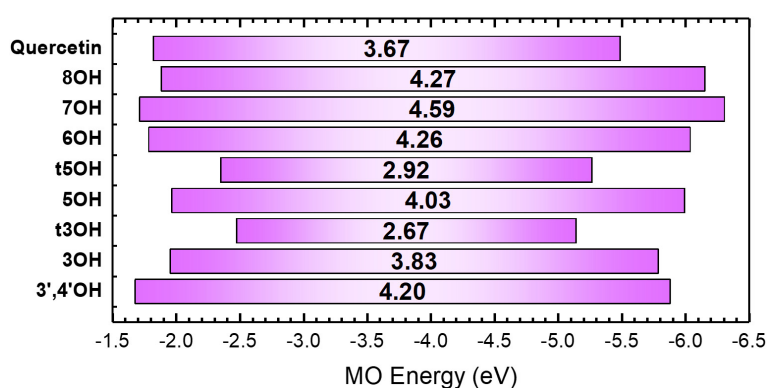

Fig. 3. Frontier MOs energy diagram. HOMO-LUMO energy gap is indicated by numbers.

Tab. 2. Electron transitions to the first 3- excited state of studied molecules with oscillator strengths f. Experimental absorption maxima are in parentheses (Wolfbeis et al., 1984; Dangleterre et al., 2008). H stands for HOMO and L for LUMO.

	λ_{abs} nm	E eV	f a.u.	Assignment			λ_{abs} nm	E eV	f a.u.	Assignment	
3',4'OH	348(342)	3.56	0.0055	H-1 → L	44 %		353	3.51	0.0006	H-2 → L	12 %
				H-1 → L+1	15 %					H-1 → L	35 %
	311(308)	3.99	0.3752	H → L	45 %	**6OH**	324(308)	3.83	0.0781	H → L	47 %
	283	4.38	0.0504	H-2 → L	42 %		285(266)	4.35	0.3869	H-2 → L	31 %
3OH	346(342)	3.58	0.3578	H → L	48 %					H-1 → L	11 %
	306	4.05	0.0000	H-3 → L	49 %		354(335)	3.51	0.0006	H-2 → L	18 %
	291	4.27	0.1051	H-1 → L	45 %	**7OH**				H-1 → L	25 %
t3OH	483	2.57	0.3398	H → L	50 %		298(309)	4.16	0.2627	H-2 → L	29 %
	377	3.29	0.0000	H-1 → L	50 %					H-1 → L	13 %
	331	3.75	0.0043	H-2 → L	47 %		290	4.27	0.0829	H → L	33 %
5OH	351(332)	3.53	0.0438	H → L	49 %		356	3.48	0.0005	H-1 → L	45 %
	316(298)	3.92	0.0030	H-3 → L	24 %	**8OH**	334(302)	3.72	0.0211	H → L	48 %
				H-2 → L	17 %		277(264)	4.48	0.0683	H-3 → L	38 %
	277(266)	4.47	0.0060	H → L+1	28 %	**Quercetin**	371(370)	3.34	0.4787	H → L	47 %
t5OH	522	2.37	0.0584	H → L	49 %		324	3.83	0.0722	H-1 → L	47 %
	386	3.21	0.0000	H-1 → L	48 %		293	4.23	0.0302	H-2 → L	43 %
	331	3.74	0.0880	H → L+1	48 %						

catecholic ring is rotated by 90 ° with respect to the plane defined by AC rings, difference in the energy demand for the cleavage of 4'O—H and 3'O—H bond becomes almost the same. In terms of O—H bond cleavage, monohydroxyflavone resembles quercetin. For example, Figs. 2b, 2c show BDE of 3-hydroxy group largely affected both in quercetin and in 3-hydroxyflavone in contrast to the very mildly changed quercetin BDEs of 7- and 5-hydroxy group or to those of **7OH** and **5OH** (not shown). BDE of **6OH** is also almost unaffected by the torsion.

Energy difference between the ground states of 3-hydroxyflavone and its tautomer is almost of 50 kJ mol^{-1} but ultrafast spectroscopy experiments confirmed that the first excited state of **3OH** spontaneously forms excited state tautomer followed by the decay to the tautomer ground state (Dzugan et al., 1986). Significant difference in energy of HOMO, LUMO as well as their energy gap can be seen comparing the ground state of **3OH** with **t3OH** (Fig. 3, also see Fig. 1 for chemical structure of **t3OH**). For the most hydroxyflavones, the first visible absorption band corresponds to the HOMO to LUMO $\pi \to \pi^*$ absorption, which is confirmed by the theoretical electron transitions to excited states compiled in Tab. 2.

Lower energy gap of the tautomers is projected in redshifted electron transition (e.g. 346 nm for **3OH** compared to 483 nm for **t3OH**). Flavonoids experimental absorption bands maxima often exhibit high sensitivity to solvent properties such as polarity, pH and temperature. Also, the UV-vis spectra are highly dependent on the number and position of substituents. Despite the calculations being done only in the gas phase, the predicted electron transitions, except for **7OH,** are in fair agreement with the experimental ones (R = 0.966). The typical π character of ground state HOMO and LUMO is illustrated in Fig. 4.
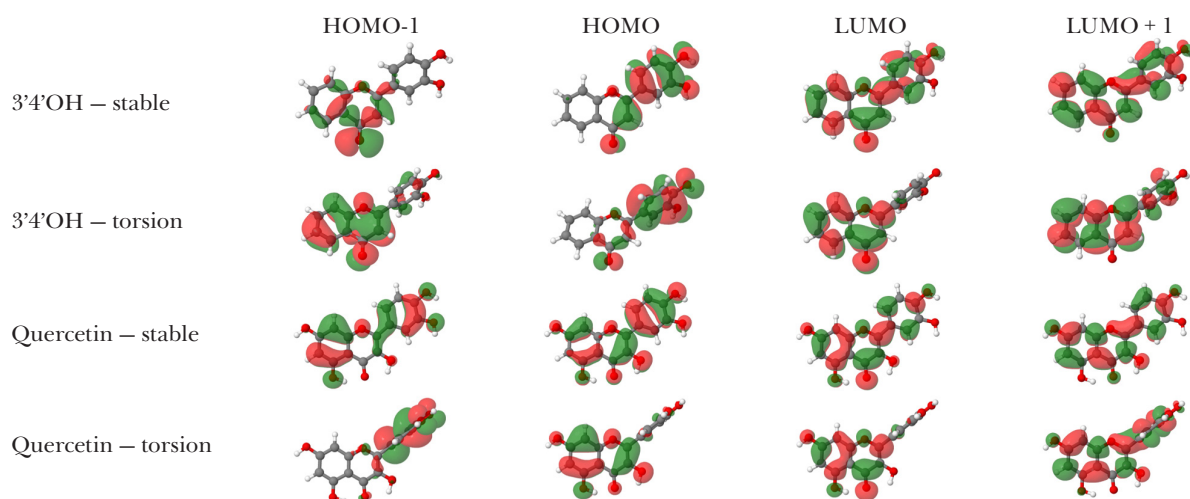

Fig. 4. Relevant molecular orbitals of stable geometries and after catecholic ring torsion (isosurface of 0.025 a.u.).
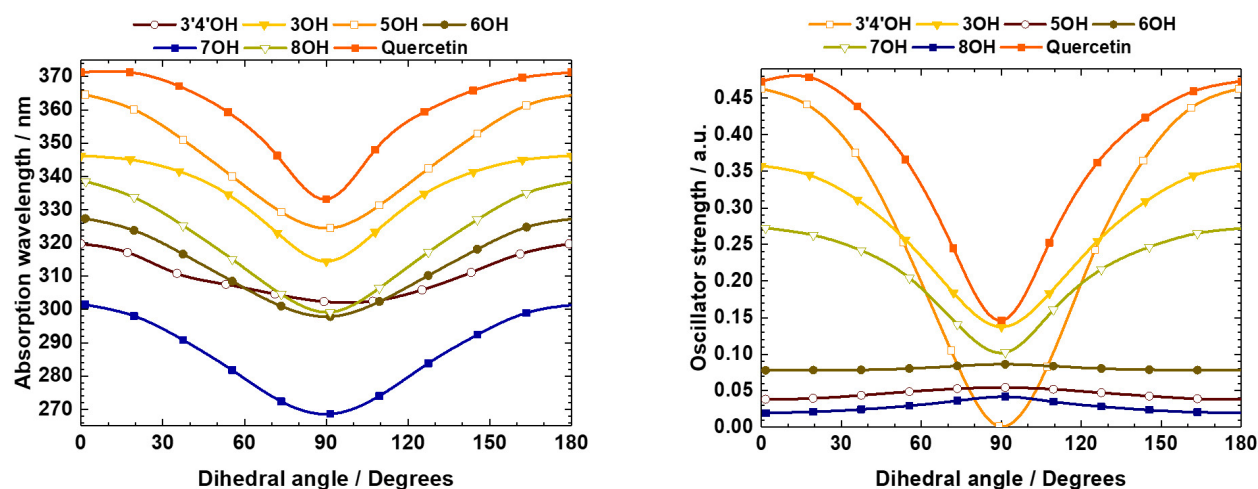

Fig. 5. Effect of dihedral C3—C2—C1'—C2' angle change on experimentally relevant excited state parameters.

On the other hand, HOMO-1 is of a non-bonding character yielding HOMO-1→LUMO transitions forbidden with almost zero predicted oscillator strength. Frontier molecular orbital shapes exhibit exceptional changes due to the torsional defects. In case of **3'4'OH,** visible transformation of π-type HOMO into non-bonding type orbital occurred while the oposite was observed for HOMO-1. Quercetin in particular has HOMO-1 of π character but the torsion shifts the electron delocalisation to the above catecholic ring only. In experimental spectra, these HOMO-1→LUMO transitions are slightly visible. Also, any change in dihedral C3—C2—C1'—C2' angles causes a blueshift in the excited states transitions. The largest blueshift accompanied by the largest drop in the oscillator strength was observed for the quercetin HOMO→LUMO transition (Fig. 5). Despite this, **5OH, 6OH** and **8OH** showed an increase in the oscillator strengths.

Conclusion

Knowledge of the rotation energy barrier around the C2—C1' bond is essential for the description of the dynamic behaviour of hydroxyflavones. Theoretical calculations based on density functional theory predict a small energy barrier permitting at least ±20 ° torsion of the catecholic ring with respect to the AC rings plane for most studied free molecules. Therefore, most hydroxyflavones are very far from a rigid molecule. For neutral molecules, the potential function is of a single-barrier type. The dihedral angle dependence of BDEs provided another useful information; 3-hydroxyflavonol showed the biggest difference between the highest and the lowest BDE values among mono-hydroxyflavones. Any deviation in dihedral C3—C2—C1'—C2' from the optimised geometry angle resulted in a blueshift of the excited states electron transitions. Since the effect of solvent and solid phase matrices is expected to be even more significant, application of molecular dynamics methods with multiple explicit molecules are planned for the future work.

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