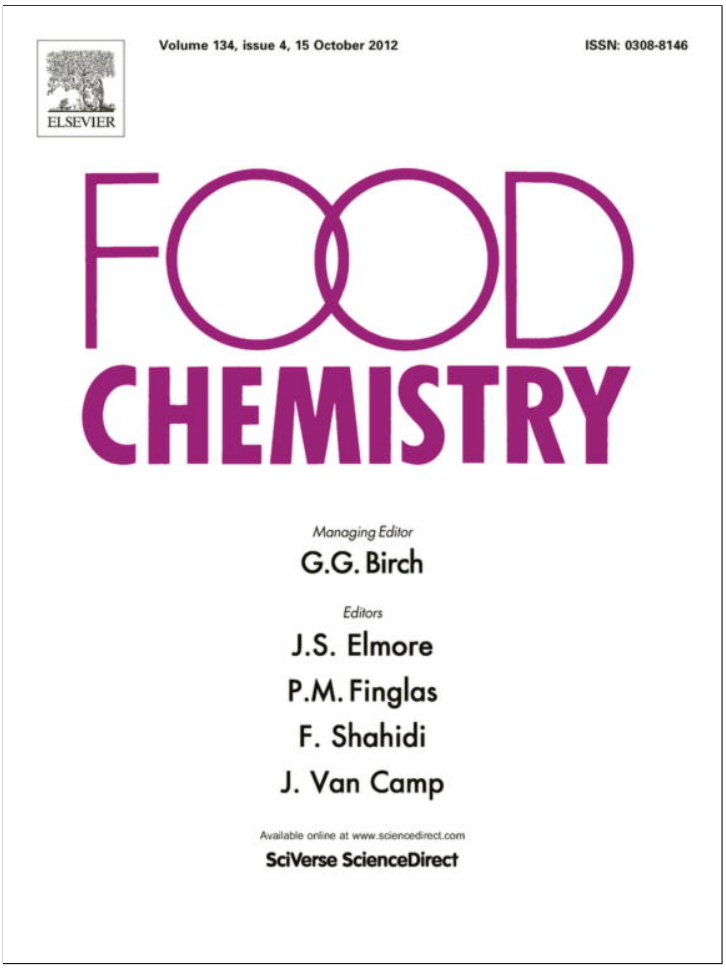

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PM6 and DFT study of free radical scavenging activity of morin

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ABSTRACT

Flavonoids have long been recognised for their general health-promoting properties, of which their antioxidant activity may play an important role. In this work, we have studied the properties of flavonoid morin using semiempirical and density functional theory (DFT) methods in order to validate the application of the recently developed parametric method 6 (PM6). Reaction enthalpies related to mechanisms of free radical scavenging by flavonoid morin were calculated by DFT and PM6 methods in gas-phase, water, DMSO and benzene. It has been shown that fast semiempirical PM6 method can mimic results obtained by means of more accurate time consuming DFT calculations. Thermodynamically favoured mechanism depends on reaction medium: SPLET (sequential proton loss electron transfer) is preferred in water and DMSO, and HAT (hydrogen atom transfer) is predominant in gas-phase. In benzene these two mechanisms are competitive.

1. Introduction

Morin (3,5,7,2′,4′-pentahydroxyflavone) is a yellow crystalline substance of acid properties extracted from wood of old fustic (*Chlorophora tinctoria*). It is widely distributed, biologically active, flavonol which occurs in the family Moraceae (e.g., in white mulberry (*Morus alba*) and fig (*Ficus carica*)), in almond (*Prunus dulcis*, family Rosaceae), in sweet chestnut (*Castanea sativa*, family Fagaceae) and many other herbs and fruits (Basile et al., 2000; Wijeratne, Abou-Zaid, & Shahidi, 2006).

Morin is an amphipathic molecule due to phenyl rings representing hydrophobic part of the molecule and the hydroxyl groups constituting the hydrophilic part. Polar hydroxyl groups can act as hydrogen bond donors and/or acceptors while oxygen atoms of the benzo-γ-pyrone segment can act as hydrogen bond acceptors. Due to its low polarity morin is only sparingly soluble (in the micromolar range) in aqueous media.

A very wide range of biological/pharmacological actions of morin including antioxidant properties (Caillet et al., 2007), xanthine oxidase inhibitory activity (Yu, Fong, & Cheng, 2006), anti-inflammatory properties (Galvez et al., 2001), anticancer activity (Manna, Aggarwal, Sethi, Aggarwal, & Ramesh, 2007), inhibitory activity of *Escherichia coli* ATP synthase (Chinnam et al., 2010), protective effect on DNA damage caused by free radicals (Choi et al., 2002) and prevention of low-density lipoprotein oxidation (Lian, Wang, Lo, Huang, & Wu, 2008) has been reported.

The mechanisms explaining biological activities of flavonoids and their metabolites are poorly understood and largely unknown, but it is possible that several different types of biochemical events precede them (Fraga, Galleano, Verstraeten, & Oteiza, 2010; Leopoldini, Russo, & Toscano, 2011). Antioxidation could be a result of direct scavenging of free radicals, sequestration of potential oxidants, altering the expression of multiple genes encoding enzymes with antioxidant function, and altering cell signalling (Prochazkova, Boušova, & Wilhelmova, 2011).

Morin was shown to be a potent scavenger of DPPH and ABTS free radicals (Burda & Oleszek, 2001; Rice-Evans, Miller, & Paganga, 1996). Despite the absence of catechol (*o*-dihydroxy) structure of

Abbreviations: HAT, hydrogen atom transfer; SET-PT, single electron transfer followed by proton transfer; SPLET, sequential proton loss electron transfer; BDE, bond dissociation enthalpy; IP, ionisation potential; PDE, proton dissociation enthalpy; PA, proton affinity; ETE, electron transfer enthalpy; DFT, density functional theory; PM6, parametric method 6.

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the B ring, which is recognised as the main prerequisite for high radical scavenging potency (Bors, Heller, Michel, & Saran, 1990), the activity of morin is comparable to flavonoids with 3′,4′-dihydroxy moiety on the B ring.

The scavenging of free radicals seems to play a notable part in the antioxidant activity of flavonoid compounds. Antiradical properties of flavonoids (Fl–OH) are related to their ability to transfer their phenolic H-atom to a free radical (e.g., alkoxyl radical, RO·). The formal H-atom abstraction from flavonoids described by:

$$\mathrm{Fl{-}OH} + \mathrm{RO^{\cdot}} \rightarrow \mathrm{Fl{-}O^{\cdot}} + \mathrm{ROH} \quad (1)$$

is known to involve complex processes. It has been recognised that this reaction proceeds via at least three different mechanisms (Klein, Lukeš, & Ilčin, 2007; Litwinienko & Ingold, 2007; Wright, Johnson, & DiLabio, 2001): single-step hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET). These mechanisms may co-exist, and depend on solvent properties and radical characters. The net result from all mechanisms is the same, that is, as it is given in reaction (1).

In the HAT mechanism, the hydrogen atom (proton together with the one of its two bonding electrons) is transferred to the free radical:

$$\mathrm{Fl{-}OH} \rightarrow \mathrm{Fl{-}O^{\cdot}} + \mathrm{H^{\cdot}} \quad (2)$$

The product of this reaction is flavonoid phenoxyl radical (Fl–O·). To be effective, Fl–O· must be a relatively stable free radical, so that it reacts slowly with a substrate but rapidly with RO·. HAT mechanism can be characterised by the homolytic bond dissociation enthalpy (BDE) of OH group. The O–H BDE can be calculated by the following equation:

$$\mathrm{BDE} = H(\mathrm{Fl{-}O^{\cdot}}) + H(\mathrm{H}) - H(\mathrm{Fl{-}OH}) \quad (3)$$

H(Fl–O·) is the enthalpy of the flavonoid phenoxyl radical generated after H· abstraction, H(H) is the enthalpy of the hydrogen atom, and H(Fl–OH) is the enthalpy of the parent flavonoid molecule. A lower BDE value, usually attributed to a greater ability to donate a hydrogen atom from the hydroxyl group, results in an easier free radical scavenging reaction. HAT is favoured for radicals with high H-atom affinity and is preferred in non-polar solvents because it does not involve charge separation.

In SET-PT mechanism first step is transfer of an electron by which the flavonoid radical cation Fl–OH·$^+$ is formed.

$$\mathrm{Fl{-}OH} \rightarrow \mathrm{Fl{-}OH^{\cdot +}} + e^{-} \quad (4)$$

This step can be characterised by the ionisation potential (IP). IP can be calculated as follows:

$$\mathrm{IP} = H(\mathrm{Fl{-}OH^{\cdot +}}) + H(\mathrm{e^{-}}) - H(\mathrm{Fl{-}OH}) \quad (5)$$

H(Fl–OH·$^+$) is the enthalpy of the flavonoid radical cation generated after electron abstraction and $H(e^-)$ is the enthalpy of electron. Second step is deprotonation of Fl–OH·$^+$:

$$\mathrm{Fl{-}OH^{\cdot +}} \rightarrow \mathrm{Fl{-}O^{\cdot}} + \mathrm{H^{+}} \quad (6)$$

It can be described by O–H proton dissociation enthalpy (PDE) which can be calculated by equation:

$$\mathrm{PDE} = H(\mathrm{Fl{-}O^{\cdot}}) + H(\mathrm{H^{+}}) - H(\mathrm{Fl{-}OH^{\cdot +}}) \quad (7)$$

H(H$^+$) is the enthalpy of proton. The net result of the SET-PT mechanism is the same as in the HAT mechanism.

Deprotonation of flavonoid molecule which results in formation of phenoxide anion Fl–O$^-$ is the first step in SPLET mechanism (Foti, Daquino, & Geraci, 2004; Litwinienko & Mulder, 2009):

$$\mathrm{Fl{-}OH} \rightarrow \mathrm{Fl{-}O^{-}} + \mathrm{H^{+}} \quad (8)$$

This step corresponds to the proton affinity (PA) of the phenoxide anion Fl–O$^-$. PA can be calculated by equation:

$$\mathrm{PA} = H(\mathrm{Fl{-}O^{-}}) + H(\mathrm{H^{+}}) - H(\mathrm{Fl{-}OH}) \quad (9)$$

H(Fl–O$^-$) is the enthalpy of the flavonoid anion generated after proton abstraction. In the second step electron transfer from Fl–O$^-$ takes place:

$$\mathrm{Fl{-}}O^{-} \rightarrow \mathrm{Fl{-}O^{\cdot}} + \mathrm{e^{-}} \quad (10)$$

It is related to electron transfer enthalpy (ETE). ETE can be determined by equation:

$$\mathrm{ETE} = H(\mathrm{Fl{-}O^{\cdot}}) + H(\mathrm{e^{-}}) - H(\mathrm{Fl{-}O^{-}}) \quad (11)$$

The net result of SPLET is again the same as in HAT and SET-PT – the formation of corresponding flavonoid radical. SET-PT and SPLET mechanisms are favoured in polar media because of charge separation involvement. They are preferred for radicals with high electron affinity.

The objective of the present work is to study radical scavenging activity of morin using theoretical DFT and PM6 calculations. Theoretical investigations of the physico-chemical properties of flavonoids can help in understanding of possible mechanisms of action. Reaction enthalpies of the individual steps of three free radical scavenging mechanisms (HAT, SET-PT and SPLET) may offer insight which mechanism is thermodynamically preferred.

2. Methods

DFT calculations were performed using the Gaussian 09 package (Frisch et al., 2009) and PM6 calculations using the MOPAC2009™ program package (MOPAC2009).

2.1. DFT calculations

The conformations of different morin forms (neutral, radical, radical-cation, and anion) are fully optimised with the new local density functional method (M05-2X), recently developed by the Truhlar group (Zhao, Schultz, & Truhlar, 2006), by using the 6-311++G (d,p) basis set as implemented in the Gaussian 09 package (Frisch et al., 2009). This new hybrid meta exchange–correlation functional is parameterised so that it includes both nonmetallic and metallic compounds. This functional also yields satisfactory overall performance for the main-group thermochemistry and thermochemical kinetics, as well as organic, organometallic, biological and noncovalent interactions. To calculate the thermodynamic properties in the solvent environment (water, benzene, and DMSO), calculation with SMD (Marenich, Cramer, & Truhlar, 2009), as implemented in Gaussian 09, was used in combination with DFT calculation at the M05-2X/6-311++G(d,p) level. The vibrational frequencies are obtained from diagonalisation of the corresponding M05-2X Hessian matrices. The nature of the stationary points is determined by analysing the number of imaginary frequencies: 0 for minimum and 1 for transition state. Relative enthalpies were calculated at 298 K.

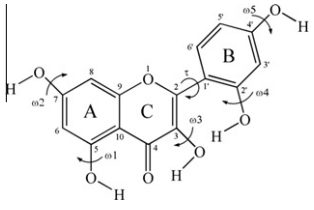

Fig. 1. Chemical structure of morin.

Table 1
Solvation enthalpies of H·, H^+ and e^- in kJ/mol, and relative permittivities ε_r of solvents.[a]

Solvent	$\Delta_{solv}H(H^{\cdot})$	$\Delta_{solv}H(H^+)$	$\Delta_{solv}H(e^-)$	ε_r
Water	−4	−1090	−236	78.39
Benzene	6.4	−894	−7	2.247
DMSO	5	−1115	−84	46.7

[a] Data were taken from Klein et al. (2007), and Rimarčik et al. (2010).

Potential energy surfaces are obtained in relation to the torsion angle τ between the rings B and C, defined by the O1–C2–C1′–C2′ atoms (Fig. 1). The torsion angle τ was scanned in steps of 10° without constrains on all other geometrical parameters. The effects of the following torsion angles rotations were also studied: ω1 (H–O5–C5–C6), ω2 (H–O7–C7–C8), ω3 (H–O3–C3–C2), ω4 (H–O2′–C2′–C1′), and ω5 (H–O4′–C4′–C3′). Afterwards, the structures were further optimised without any constrain around each potential minimum. Particular attention was devoted to the DFT interpretation of the reactivity of OH groups of morin.

2.2. MOPAC calculations

The geometries of morin molecule, radical cation, radicals, and anions were optimised using PM6 method, according to the procedure described above. Eigenvector following (EF) optimisation procedure was carried out with a final gradient norm under 0.01 kcal mol^{-1} $Å^{-1}$. Solvent contribution to the enthalpies of formation of morin species was computed employing COSMO (Conductor-like Screening Model) calculations implemented in MOPAC2009™. This approach was used for the parent molecule, radicals and ionic structures. The solvation enthalpies of hydrogen atom (H·), proton (H^+) and electron (e^-) in different solvents, along with the relative permittivities ε_r of the solvents, are collected in Table 1.

3. Results and discussion

Previously published studies indicate PM6 method as one which reproduces DFT results well (Amić & Lučić, 2010; Puzyn, Suzuki, Haranczyk, & Rak, 2008). In the present work the PM6 method is used to calculate reaction enthalpies related to the free radical scavenging mechanisms. As a starting point the DFT results obtained by Klein et al. (2007), dealing with antioxidant action energetics of tocopherols and chromans, are used with the intention to check if the PM6 method results match the DFT ones. The results of the performed PM6 and DFT calculations, in gas-phase and water for α-tocopherol and chroman A, are presented in Supplementary material (Table S1), along with the results of Klein et al. (2007). As can be seen from Table S1, compatibility of PM6 and DFT results in gas-phase is excellent. In water, matching of results is somewhat weaker. It could be noted that PM6 calculations are performed in a significantly shorter time than more accurate DFT methods. This simple test justifies the using of the PM6 method in studying the phenolic free radical scavenging energetic and point to PM6 method as the one valid alternative to the DFT, at least for the systems under study.

Since the behaviour of the different OH groups in polyphenolic compounds is largely influenced by electronic effects of the neighbouring groups and the overall geometry of the molecule, the conformation can be regarded as the first parameter of importance in analysing the antioxidant capacity of any polyphenolic molecule, morin in this case. A detailed conformational analysis of morin in both, gas and solvents (with SMD and COSMO models), is performed with the most important results presented in sections that follow. Analysis of the geometries of morin rotamers shows that all of them are non-planar, implying that the dihedral angle between the bicycle AC and the ring B is significantly different from zero. The most stable rotamer of morin is presented in Fig. 1.

To determine the preferred relative positions of the rings B and C, conformational space of morin structure (Fig. 2) is investigated as a function of torsional angle τ (O1–C2–C1′–C2′) between those rings. The minimisation procedure for the morin structure, performed at the M05-2X level with the 6-311++G** basis set and PM6 method, yields a non planar conformation as the more stable one. By removal of the torsional angle constraint, the conformational absolute minimum is found at $\tau = -38.52°$ followed by a relative minimum at $\tau = -149.35°$, with the energy difference of 9.6 kJ mol^{-1}. The potential energy maximum lies at $\tau = -92°$ and the interconversion barrier between the two minima is about 25.95 kJ mol^{-1}, which is somewhat higher then corresponding values for quercetin (Leopoldini, Marino, Russo, & Toscano, 2004) and fisetin (Marković, Mentus, & Dimitrić Marković, 2009). PM6 method gives a lower value for the dihedral angle $\tau = -32.72°$ (both DFT and semiempirical values are in good agreement with experimental data (Cody & Luft, 1994), see Table S1). Also, the corresponding values for a local minimum and interconversion barrier are lower then values obtained by DFT method, and amount 2.61 and 14.12 kJ mol^{-1}, respectively.

It is worth mentioning that in going from $\tau = 0°$ to ±40°, potential curve is not flat, and has maximum at $\tau = 0°$, by both methods. The energy barrier for this rotation is much lower then in previous case, and amounts 14.79 kJ mol^{-1} for the DFT and 8.79 kJ mol^{-1} for the PM6 method. This means that the rotation on this side requires a negligible amount of energy. Non-planarity can be caused by the steric repulsion between 3-OH and 2′-OH groups.

All species necessary to study reaction enthalpies related to the three mechanisms of free radical scavenging action (HAT, SET-PT and SPLET) are generated from the most stable conformation of morin. DFT and PM6 calculations in gas-phase, water, benzene and DMSO are performed. The geometries of all calculated species are given in Supplementary data. Water and benzene are chosen as the typical polar and non-polar solvent. Water is the main constituent of all physiological liquids. Poor solubility in water and amphipathic nature of morin allows its partition into lipid bilayers and by increasing its local concentration can express antiradical activity. Possible place of action is also at water/lipid interfaces. BDE of phenolic OH group represents the reaction enthalpy of HAT. IP is related to the first step of SET-PT mechanism. PA of phenoxide anion (abstraction of proton from the molecule) is related to the first step of SPLET mechanism. Therefore, BDE, IP and PA may be used to determine thermodynamically preferred reaction pathway (Klein et al., 2007; Rimarčik, Lukeš, Klein, & Ilčin, 2010).

Reaction enthalpies, calculated by DFT and PM6 methods for the reactions in different media involved in three mechanisms of antiradical activity of morin, are presented in Table 2. Corresponding ΔBDE, ΔIP, and ΔPDE values are presented in Table 3.

3.1. Bond dissociation enthalpy, proton affinity and ionisation potential

When analysing the computational thermochemical data presented in Table 2, i.e., BDE values obtained by both theoretical methods, it can be noted that among five OH groups in the structure of morin, the 3-OH, 4′-OH and 2′-OH of the cinnamoyl system (Fig. 1) have the greatest ability to donate H-atom. It can be suggested that the semiquinones formed during the reaction are derived from these three groups, which coincides with that reported in other works (Janeiro & Oliveira-Brett, 2005; Mendoza-Wilson, Santacruz-Ortega, & Balandran-Quintana, 2011; Musialik, Kuzmicz, Pawlowski, & Litwinienko, 2009). The 3-OH has the lowest BDE value in all

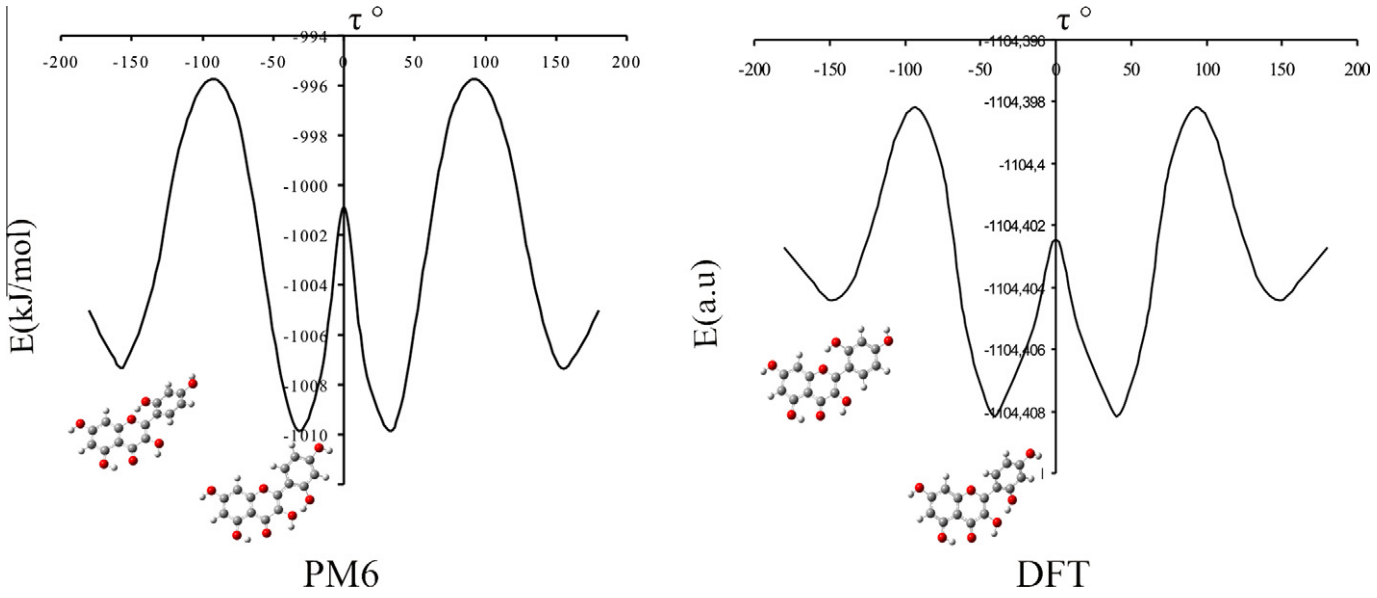

Fig. 2. PM6 and DFT energies profile for rotation around the C2–C1′ bond of morin in the gas-phase.

Table 2
DFT and PM6 calculated parameters of free radical scavenging activity for morin (in kJ/mol).

	DFT					PM6				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
Gas-phase		757					761			
3-OH	337		902	1334	324	322		883	1294	349
5-OH	416		981	1405	333	407		968	1331	398
7-OH	387		952	1349	360	392		952	1291	422
2′-OH	384		949	1403	303	350		910	1294	377
4′-OH	369		934	1372	319	353		914	1306	369
Water		351					381			
3-OH	325		−26	64	261	318		−64	12	305
5-OH	382		31	108	273	377		−5	23	353
7-OH	384		33	92	293	386		4	13	372
2′-OH	365		14	106	259	338		−44	47	291
4′-OH	366		15	105	261	347		−35	27	320
DMSO		514					527			
3-OH	330		−66	60	388	324		−86	−12	453
5-OH	400		4	124	394	385		−24	1	502
7-OH	390		−6	88	420	395		−15	−11	523
2′-OH	377		−19	120	375	345		−64	23	440
4′-OH	371		−24	105	384	351		−58	2	467
Benzene		659					684			
3-OH	337		92	353	399	329		58	317	426
5-OH	413		168	420	407	405		134	344	475
7-OH	393		148	375	432	399		129	315	498
2′-OH	385		140	417	382	353		83	317	450
4′-OH	374		129	396	393	359		88	331	442

Table 3
ΔBDE, ΔIP, and ΔPDE values of morin OH groups (with regard to phenol).[a]

	DFT			PM6		
	ΔBDE	ΔIP	ΔPDE	ΔBDE	ΔIP	ΔPDE
Gas-phase		−65			−57	
3-OH	−22		43	−13		45
5-OH	57		122	72		130
7-OH	28		93	57		114
2′-OH	25		90	15		72
4′-OH	10		75	18		76
Water		−11			8	
3-OH	−31		−20	−8		−17
5-OH	26		37	51		42
7-OH	28		39	60		51
2′-OH	9		20	12		3
4′-OH	10		21	21		12
DMSO		−7			−2	
3-OH	−31		−24	−11		−12
5-OH	39		46	50		50
7-OH	29		36	60		59
2′-OH	16		23	10		10
4′-OH	10		18	16		16
Benzene		−31			−30	
3-OH	−27		4	−11		18
5-OH	49		80	65		94
7-OH	29		60	59		89
2′-OH	21		52	13		43
4′-OH	10		41	19		48

[a] All theoretical values refer to phenol calculated with the same method.

solvents, so it represents the first site that can donate its H-atom, followed by the 4′-OH by DFT method and 2′-OH by PM6 method. There are controversial results regarding the first oxidation site of morin. In agreement with some authors (Jovanovic, Steenken, Tosic, Marjanovic, & Simic, 1994; Mendoza-Wilson et al., 2011; Panhwar, Memon, & Bhanger, 2010) our results show that such site corresponds to the 3-OH group, whereas others propose the 2′-OH group (Janeiro & Oliveira-Brett, 2005; Musialik et al., 2009).

Calculated PA values of all present OH groups, give the following sequence: 3-OH < 7-OH < 4′-OH, indicating proton transfer from 3-OH group as easier comparing to other four OH groups. In contrast to DFT calculations, PM6 method suggests that proton transfer in gas-phase and benzene is slightly easier from the 7-OH then from 3-OH group, while in water and DMSO 3-OH group is slightly more reactive than 7-OH group. We supposed that these differences are result of weakness of PM6 method in describing of ions. PA values calculated for different solvents are several times lower then corresponding values in the gas-phase (Table 2). This is a consequence of the interactions of every OH group and O anion with the solvent molecules.

The IP value for the most stable structure of morin was calculated also in the gas-phase, aqueous phase, DMSO, and benzene

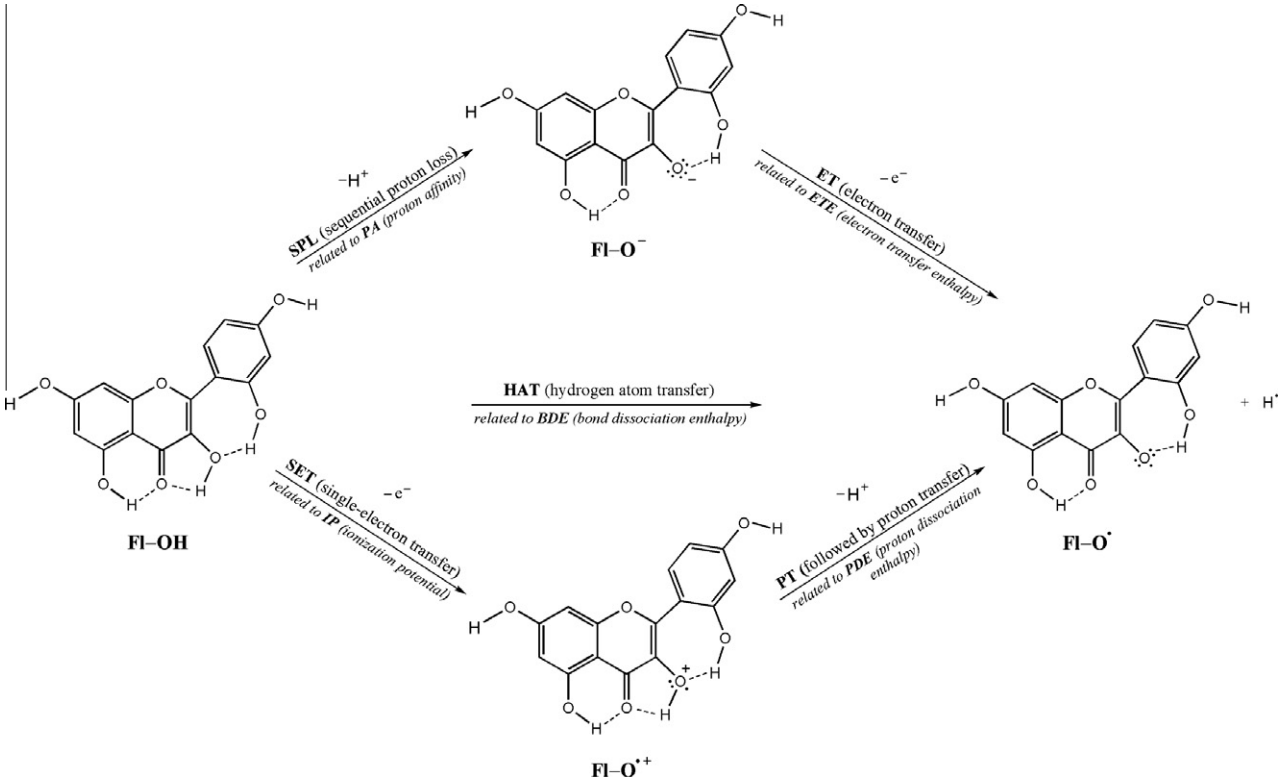

Fig. 3. Simplified presentation of HAT, SET-PT and SPLET mechanisms.

as the difference between the enthalpy of morin radical cation and parent molecule. The IP value of morin is somewhat higher in gas-phase than those of widely used synthetic food additives such as butylated hydroxyanisole, propyl gallate and dihydroguaiaretic acid (639, 702 and 672 kJ mol^{-1}, respectively) (Mandado, Graña, & Mosquera, 2004), or the naturally occurring polyphenolic flavonoid epigallocatechin-3-gallate (618 kJ mol^{-1}), which is considered as one of the most active antioxidants obtained from green tea (Mandado et al., 2004; Rice-Evans et al., 1996). Because of the stabilisation experienced by charged systems in polar solvents the IP's values obtained from SMD calculations are significantly lower, especially in water then in the gas-phase.

3.2. Antioxidant mechanisms

Preferred mechanism of antioxidant activity of a certain flavonoid can be concluded from the relative ΔBDE and ΔIP values. These values are calculated as the difference between BDE and IP values of flavonoid compound (morin in this case) and phenol as a reference compound. The BDE, IP, and PDE values computed for phenol, at the M05-2X/6-311++G(d,p) and PM6 levels in all media under investigations were presented in Table S2. According to Wright et al. (2001), for ΔIP $\leqslant -150$ kJ mol^{-1} and for ΔBDE around -42 kJ mol^{-1} HAT is considered as dominant mechanism, whereas for ΔIP > -188 kJ mol^{-1} predominant mechanism is SET-PT. From Table 3, it can be seen that the most active position susceptible to HAT mechanism is that with the most negative value of ΔBDE. SET-PT mechanism could be related to the most negative value of ΔIP, as well as to the smallest value of ΔPDE. For both mechanisms in all media this is the 3-OH position. The results concerning gas-phase showed ΔIP value of -65 kJ mol^{-1} and ΔBDE value of -22 kJ mol^{-1}. According to Wright conditions (Wright et al., 2001) the SPLET and SET-PT mechanisms, which have been quoted by several authors (Foti et al., 2004; Litwinienko & Mulder, 2009; Rimarčik et al., 2010), could be discarded as possible for morin in all solvents under investigation (Table 3) as the HAT mechanism seems to be the only practical or the preferred one in all media.

However, it should be kept in mind that the relative importance of HAT, SET-PT and SPLET mechanism is not only determined by micro environmental features (lipid phase and aqueous phase), but also governed by the characteristics of the scavenged radical species. It is important to note that besides structural properties, it is necessary to consider how the electron affinity and H atom affinity of the radical species, which potentially could react with morin, affect these three mechanisms. Also, it is very important to analyse how the electron densities of both compounds change along the reaction coordinate (Estevez & Mosquera, 2008).

On the other hand, if we neglect Wright's rules, on the basis of inspection of data from Table 2, it is clear that the HAT mechanism is dominant in gas-phase, because BDE values of OH groups are significantly lower than corresponding IP and PA values. In water and polar aprotic solvent DMSO, PAs of OH groups of morin are significantly lower than corresponding BDE values. This indicates that SPLET mechanism thermodynamically represents the most probable reaction pathway in polar solvents. On the basis of obtained values for BDE, PA and PDE, it is clear that 3-OH group should be more reactive OH group of morin. In non-polar solvent benzene, HAT and SPLET are competitive mechanism, because the differences between PAs and BDEs are not so pronounced as in the gas-phase. In all media, SET-PT mechanism is not the preferred one, because IP of morin molecule is always higher than BDEs and PAs. Fig. 3 depicts simplified presentation of HAT, SET-PT and SPLET mechanisms.

Mainly due to the large enthalpy of H^+ solvation in all studied solvents, PAs are significantly lower than gas-phase values. In benzene calculated PAs are noticeably higher than in water and DMSO. On the other hand, there is no such pronounced difference between BDEs in the studied environments.

In all studied media, the most abstractable hydrogen atom of morin molecule is that from 3-OH group (lowest BDE value). It is reasonable to expect that HAT mechanism proceed at that group and results in formation of 3-O· phenoxyl radical (Fig. 3). The lowest PA value is also characteristic of 3-OH group: this indicates that SPLET mechanism starts with deprotonation of the 3-OH group and also results in formation of 3-O· phenoxyl radical.

It should be noted that theoretical predictions, based on calculated reaction enthalpies, of thermodynamically preferred scavenging mechanism of morin in various solvents, are in accordance with experimentally determined kinetic solvent effects on the free radical scavenging ability of phenolic compounds (Litwinienko & Mulder, 2009), as well as with DFT predicted mechanisms from the thermodynamic point of view (Klein et al., 2007; Rimarčik et al., 2010).

Flavonoids, besides above mentioned mechanisms, can also chelate potentially toxic transition metal ions such as Fe^{2+}, Fe^{3+}, and Cu^{2+}, preventing the metal-catalysed free radical generation reactions (Prochazkova et al., 2011). During the Fenton reaction hydroxyl radicals are produced from hydrogen peroxide in the presence of a metal in a low oxidation state ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$), which are very short-lived and consequently highly reactive ones. In this, "metal chelation" mechanism, the loss of a proton by the flavonoid molecule is crucial for its antioxidant ability, because the cation's chelation often involves at deprotonated ligand. Therefore, the acidity of these compounds is an important parameter to be taken into account, since the smaller the OH group proton affinity, the easier deprotonation and the metal chelation. On the basis of obtained values for PA (Table 2), it appears that 3-OH and 7-OH groups could be more reactive in reaction with metal ions in all media. It is important to note that deprotonation of 3-OH group, due to the intramolecular H-atom transfer, results in formation of 2′-O^- anion (Marković et al., unpublished results). Consequently, 2′-O^- moiety could be involved in transition metal ion chelation.

4. Conclusions

In this work, conformational analysis, as well as the phenolic OH bond dissociation enthalpies, proton affinities, and ionisation potential, related to HAT, SPLET, and SET-PT mechanisms of flavonoid morin were studied.

The results obtained using the M05-2X/6-311++G(d,p) and PM6 levels of theory imply non-planar structure of morin as the most stable one. This most stable form is significantly distorted from planarity in all solvents and additionally stabilised by three internal hydrogen bonds (IHBs), while the other rotamers are stabilised by one or two IHBs.

The 3-OH group is the most favoured site for homolytic and heterolytic O–H breaking, by both methods, in all solvents. On the other hand, the presented DFT results indicate that the 5-OH group is not involved in the antioxidant mechanism due to the highest BDE and PA values. The main reason justifying this assumption lies in the fact that the hydrogen atom of the 5-OH group forms a strong hydrogen bond with O4 atom in parent molecule. In aqueous solution, both methods indicate that the 7-OH group is not the preferred site for radical inactivation.

It was found that IPs and PAs depend significantly on the solvent because of the stabilisation of charged species by polar solvents. Thus, the IPs of morin become significantly lower if water is used as solvent, while the PAs values are the lowest in DMSO and water. Based on the obtained results, both methods predict that the HAT mechanism is dominant in the gas-phase. The SPLET mechanism represents thermodynamically preferred reaction pathway in water, where PAs of OH groups are considerably lower than corresponding BDEs. In the non-polar solvent benzene HAT and SPLET are competitive mechanisms, while in the case of DMSO the most probably pathway is SPLET.

The present study revealed that both DFT and PM6 methods could be useful tools in studying energetics of free radical scavenging action of flavonoids. The results indicate that not only gas-phase calculations should be taken under consideration. This means that it is very important to perform calculations in polar and non-polar solvents to elucidate the preferred mechanism of free radical scavenging action of flavonoids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.03.124.

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