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Structure–antioxidant activity relationships of flavonoids isolated from the resinous exudate of *Heliotropium sinuatum*

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Abstract—Relationships between the structural characteristics of flavonoids isolated from the resinous exudate of *Heliotropium sinuatum* and their antioxidant activity were studied. Radical formation energies, ΔH of dehydrogenation and spin densities were calculated using DFT methods (B3LYP/6-31G*). Results show that studied flavonoids can be divided into two sets according to their activity. It has been found that antioxidant activity depends both on substitution pattern of hydroxyl groups of the flavonoid skeleton and the presence of an unsaturation at the C2–C3 bond. A good tendency between ΔH of dehydrogenation and antioxidant activity was established.

Flavonoids belong to a group of naturally occurring compounds with a number of biological activities, such as antibacterial, antimutagenic, cytotoxic and anticarcinogenic[1–4] however, antioxidant activity is one of the most studied.[5–7] Antioxidant activity arises from flavonoids ability to scavenge free radicals and thus eliminate reactive oxygen species.[8,9]

Most plants synthesize and accumulate flavonoids induced by abiotic and biotic stresses, for example, ultra violet radiation, low temperatures, wounding, low nutrients, lack of water and pathogen attack.[10] Species of the genus *Heliotropium* produce a resinous exudate covering their leaves and stems that is constituted mainly by flavonoids.[11–13] Considering these data and the interest in the pharmacological and therapeutical effects of flavonoids against free radical mediated diseases, we have previously studied the total antioxidant potential of resinous exudates from *Heliotropium* species and their pure compounds.[14] In that work we evaluated the antioxidant activity of flavonoids by measuring the bleaching of stable free radicals employing 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) derived radicals and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

The present study extends our previous research and establishes structure–activity relationships for the flavonoids **1**–**8** (Fig. 1) obtained from the resinous exudate of *Heliotropium sinuatum* and their antioxidant activity. Theoretical parameters were calculated by DFT methods and correlated with experimental parameters of antioxidant activity.

Theoretical calculations were done by using ab initio density functional theory (DFT) with an hybrid

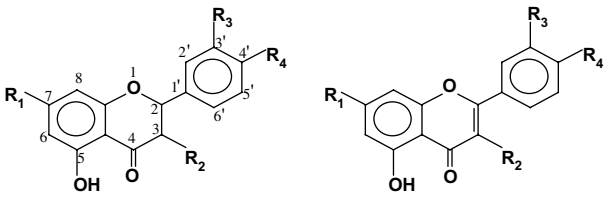

Figure 1. Structures of flavonoids isolated from the resinous exudate of *Heliotropium sinuatum*. **1** $R_1 = OCH_3$; $R_2 = H$; $R_3 = R_4 = OH$, **2** $R_1 = R_4 = OH$; $R_2 = R_3 = OCH_3$, **5** $R_1 = R_3 = OH$; $R_2 = H$; $R_4 = OCH_3$, **3** $R_1 = OH$; $R_2 = OCH_3$; $R_3 = R_4 = H$, **6** $R_1 = R_4 = OH$; $R_2 = R_3 = H$, **4** $R_1 = R_2 = R_3 = OCH_3$; $R_4 = OH$, **7** $R_1 = OH$; $R_2 = OAc$; $R_3 = R_4 = H$, **8** $R_1 = OH$; $R_2 = R_3 = R_4 = H$.

Keywords: Antioxidant activity; Flavonoids; Structure–activity relationships; Radical stability; Theoretical parameters.
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functional including a mixture of Hartree–Fock exchange with DFT exchange correlation. All quantum chemistry calculations were performed with Gaussian 98 W and B3LYP/6-31G*.[15] Initially, an optimization was carried out using a semi-empirical method (RHF, Steepest Descent, AM1, Hyperchem V7.1)[16] for each parent molecules. Calculations were carried out in UHF mode for radical structures. ΔH of dehydrogenation were determined by calculating the heat of formation differences between radicals and their parent flavonoids (bond dissociation energy). Radicals were constructed by an abstraction of a hydrogen atom from the corresponding hydroxyl moiety.

Stabilization energy values for each radical that eventually could be formed by dehydrogenation of each flavonoid at any of the phenolic hydroxyl groups are presented in Table 1. According to these values it is possible to establish the following relative stability scale for each flavonoid radical at the specified positions: C-4′ < C-3′ < C-5′ < C-7. As it can be seen in Figure 1, compounds **1**, **2**, **4**, **5**, and **6** can generate radicals at C-4′ and C-3′ positions. On the other hand, ΔE values, which represent the stabilization energy differences between the parent flavonoid energy and the most stable appropriate radical energy show that while stabilization energy decreases, antioxidant activity increases.

In Table 2, it is observed that compounds having the possibility of generating radicals at positions C-4′ and/or C-3′ are the most active ones in agreement with other experimental data.[17] Additionally it is observed that compounds **7** and **8** having no hydroxyl group on ring B, are those with the lowest antioxidant activity. However despite these observations, it is possible that this factor is not the only one affecting antioxidant activity, since compound **3**, which has one of the highest activities, has no possibility of forming radicals at C-4′ or C-3′. Also, in Table 2 values of ΔH of dehydrogenation as well as formation enthalpies, for the most stable radical generated from each parent flavonoid are presented. Experimental values of the antioxidant activity and theoretical enthalpy values concentrate mainly around two groups of activities, classifying the flavonoids into two sets, a very active one including compounds **1**, **2**, and **3**, and the other, less active, including compounds **4**, **5**, **6**, **7**, and **8.** ΔH of dehydrogenation for both sets of compounds show an inverse relationship with the activity, that is, the lower dissociation enthalpy and therefore the easier H-abstraction from hydroxyl groups in all the tested flavonoids was found for most active compounds.

Accordingly, the biggest antioxidant activity of compounds **1** and **2** is due to the radical generation at position C-4′ as shown by the theoretical energy values. Compound **1** behaves as the best antioxidant because of a better stabilization of the radical formed by H-abstraction at position C-4′. In this position could be a chance of forming a hydrogen bond with the phenolic hydroxyl group at C-3′.

Table 1. Stabilization energies for possible radical species formed from flavonoids

Compound	Energy (cal/mol)	ΔE [a] (cal/mol)
Flavonoid **1**	−4011.4059	74.4455
4′ Radical from flavonoid **1**	−3936.9604	
3′ Radical from flavonoid **1**	−3936.8100	
5 Radical from flavonoid **1**	−3923.9713	
Flavonoid **2**	−4251.0775	76.8531
4′ Radical from flavonoid **2**	−4174.2244	
7 Radical from flavonoid **2**	−4167.8636	
5 Radical from flavonoid **2**	−4163.6435	
Flavonoid **3**	−3774.7328	83.8254
7 Radical from flavonoid **3**	−3690.9074	
5 Radical from flavonoid **3**	−3686.7956	
Flavonoid **4**	−4519.9322	76.7685
4′ Radical from flavonoid **4**	−4443.1637	
5 Radical from flavonoid **4**	−4432.7028	
Flavonoid **5**	−4011.3787	78.0792
3′ Radical from flavonoid **5**	−3933.2995	
7 Radical from flavonoid **5**	−3927.9475	
5 Radical from flavonoid **5**	−3923.8267	
Flavonoid **6**	−3639.4853	79.3044
4′ Radical from flavonoid **6**	−3560.1809	
7 Radical from flavonoid **6**	−3555.6144	
5 Radical from flavonoid **6**	−3551.8235	
Flavonoid **7**	−4171.2918	84.5991
7 Radical from flavonoid **7**	−4086.6927	
5 Radical from flavonoid **7**	−4083.7815	
Flavonoid **8**	−3535.6448	83.8501
7 Radical from flavonoid **8**	−3451.7947	
5 Radical from flavonoid **8**	−3447.9617	

Structural optimization for each flavonoid and its radicals were determined by calculating the minimum energy conformation by using the semi-empirical Hamiltonian AM1.

[a] ΔE = Most stable radical energy − parent molecule energy.

Table 2. Antioxidant activity of flavonoid compounds and their ΔH of dehydrogenation

Test compd	R[a]	Formation enthalpy of parent molecule (kcal/mol)	Formation enthalpy of most stable radical (kcal/mol)	ΔH of dehydrogenation (kcal/mol)[b]
1	3.4	−1069.464813	−1068.843812	0.6210006
2	6.6	−1182.775789	−1182.139975	0.6358140
3	6.8	−993.0339708	−992.3889784	0.6449924
4	125	−1222.082336	−1221.446651	0.6356857
5	126	−1069.466189	−1068.828324	0.6378650
6	143	−954.9418287	−954.3039422	0.6378865
7	160	−1107.588135	−1106.939115	0.6490204
8	170	−879.7254852	−879.0782611	0.6472241

[a] R: ratio between antioxidant activities determined employing ABTS and DPPH methods (Data Ref. 14). This value could provide an insight regarding the experimental average activity of the flavonoids present in the resinous exudate.

[b] ΔH of dehydrogenation was calculated by DFT method for energy optimized species. These values may represent the relative stability of a radical with respect to its parent compound.

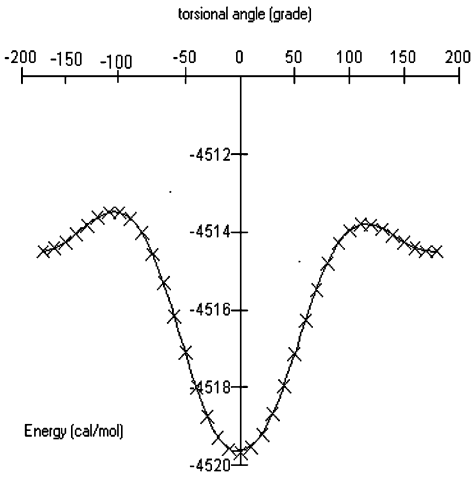

Figure 2. Rotational barrier of the 4′-OH group at flavonoid **1**. The rotational barrier of the torsional angle C4′–C3′–O3′–H3′ is observed to be near 6 kcal/mol and was calculated using the semi-empirical method AM1.

To analyze the formation of H-bond between the hydroxyl groups of the C3′ and C4′ the rotational barrier for the torsional angle C4′–C3′–O3′–H3′ was calculated using the semi-empirical method AM1. As shown in Figure 2, the rotational barrier has the lowest energy at 0°, forming an H-bond between O3′–H3′···O4′. This H-bond helps to break the O4′-H4′ bond and, in some way, stabilizes the radical in the O4′. Moreover, it could be possible to establish an H-bond O4′-H4′···O3′, but O3′ radical has a poor conjugation.

On the other hand, a careful analysis of the spin density map of most stable radical for each flavonoid (Fig. 3) allows us to establish that the resonance stabilization or conjugation factor is indeed an important parameter affecting radical stability and therefore, antioxidant activity of the studied compounds. For instance compound **3**, one of the most active ones shows an important conjugation that involves participation of at least 11 atoms. Spin density map of compound **3** shows a clear difference respect to the corresponding maps for compounds **7** and **8**, where no extended conjugation is observed involving the atoms 2 and 3. This was an expected point, since those atoms are sp^3 hybridized and the same atoms in compound **3** are sp^2 hybridized. Within the set of flavonoids having the lowest activity, compound **4** is the most active. This compound is significantly less active than compound **2** due to methylation in position 7. Last fact contributes to decreasing the number of hydroxyl groups that could form any radicals. Finally, among compounds **5**, **6**, **7**, and **8,** all of which are saturated at C2–C3, compounds **5** and **6** having hydroxyl groups at ring B are more reactive than compounds **7** and **8** according to the theoretical formation energy values of the corresponding radicals. Compound **5** presents a slightly superior activity than compound **6** due to the contribution of methoxyl group at position 4′ to the stabilization of the radical generated by H-abstraction from hydroxyl group at position 3′.

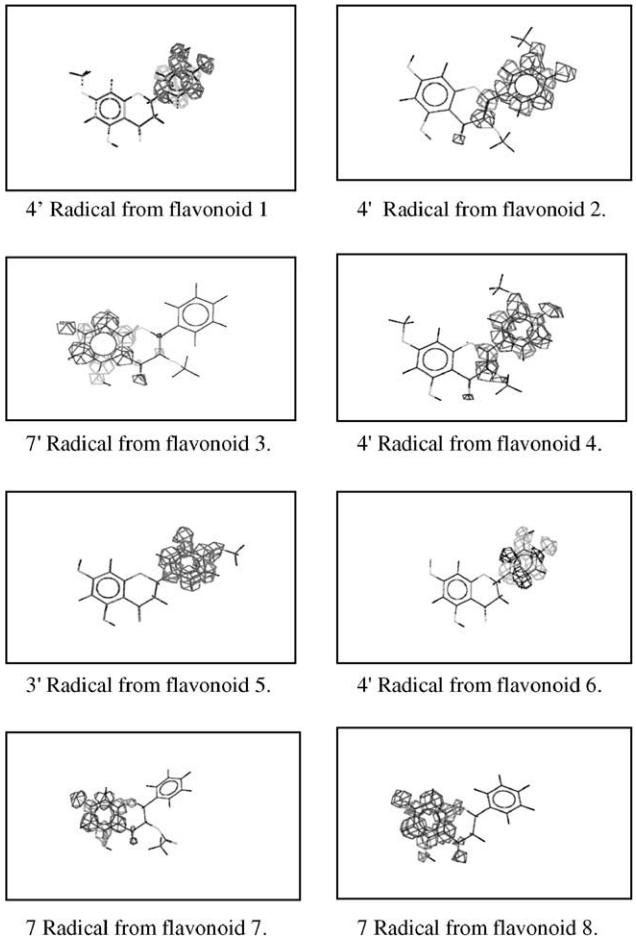

Figure 3. Spin density maps for the most stable radical from each of the parent flavonoids.

In brief all of the calculations using both semi-empirical and ab initio methods, and also the experimental data, allow us to separate the studied flavonoids into two sets of compounds, the first one being highly active and the second one having a low antioxidant activity. Based on the reasoning described above it is possible to conclude that in both sets of compounds, the antioxidant activity would depend mainly on two factors: (1) the number and location of the phenolic hydroxyl groups present in the flavonoid skeleton where the most active compounds would be those having hydroxyl groups at C-4′ and/or C-3′, for which the lowest ΔH values of dehydrogenation were obtained; and (2) the presence of unsaturation at C2–C3 allowing for the resonance stabilization of formed radicals according to the analysis of spin density maps. Therefore it is not possible to set forth a unique descriptor for correlating antioxidant activity. Nevertheless, a fairly good qualitative tendency between experimental and calculated values for flavonoid antioxidant activity behavior was found.

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