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Silybin and 2,3-Dehydrosilybin Flavonolignans as Free Radical Scavengers

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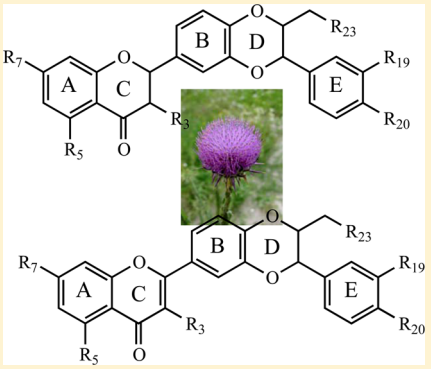
Supporting Information

ABSTRACT: The electronic properties of six derivatives of silybin (characterized by the absence of the 2,3 double bond) and six derivatives of 2,3-dehydrosilybin (characterized by the presence of the 2,3 double bond) have been studied by applying density functional theory to fully understand the free radical scavenger's mechanism for action and the relationship between reactivity and chemical structure. Optimized geometries, Raman spectra, and λ_{max} values are reported, enabling us to characterize the systems. These spectra may be useful for monitoring the oxidation between silybin and 2,3-dehydrosilybin, thus providing important experimental information. The relative abundance of deprotonated species under physiological conditions is also reported. Under physiological conditions (pH 7.4), ∼70% of silybin is protonated, but 60% of 2,3-dehydrosilybin is deprotonated. The free radical scavenger capacity is analyzed in terms of two mechanisms: electron transfer and adduct formation. Deprotonated molecules are better electron donors and worse electron acceptors than non-deprotonated species. The conclusions derived from this investigation completely concur with previous experimental results. The free radical scavenging activity of 2,3-dehydrosilybin derivatives is higher than that for silybin derivatives. What was not previously considered was the importance of the deprotonated species, which is remarkable and may be important for future experiments.



INTRODUCTION

Herbal drugs have been used for a long time for the treatment of a number of diseases, especially in Eastern medicine.[1] One of these medicinal plants, consumed for decades, is *Silybum marianum* (L.) Gaertn. (Asteraceae).[2] This is due to its capacity to prevent hepatic disorders[3] in the treatment of alcoholic cirrhosis[4] because it acts as an antidote to acute mushroom poisoning[4] and imposes potent antiinflammatory activity.[5] The active component of this plant is extracted from the seeds and is known as silymarin.[6] The antioxidant properties of silymarin have been reported in vitro and in animal studies.[3−8] Silymarin is able to scavenge free radicals and manifests antitumor activity against human tumors (for example, prostate and ovary)[9] and may also help to protect against nicotine-induced cytotoxicity.[10] Silymarin represents a unique mixture of flavonoids, with silybin constituting the principal active component (Figure 1)

It is already known that flavonoids and related molecules represent good free radical scavengers.[11−22] The relationship between the structure and the capacity to scavenge free radicals of several flavonoids has been established. In particular, for silybin and 2,3-dehydrosilybin (Figure 1), as for some derivatives, the mechanism to act as radical scavengers has been extensively studied, both experimentally and theoretically by Trouillas et al.[14] Authors reveal the importance of OH groups and the differences in free radical scavenger capacity for silybin and 2,3-dehydrosilybin. They conclude for silybin and its derivatives that the hydrogen atom transfer mechanism is less effective and the electron-transfer mechanism is predominant. Contrarily, the main mechanism for scavenging free radicals in the case of 2,3-dehydrisilybin and its derivatives is the hydrogen

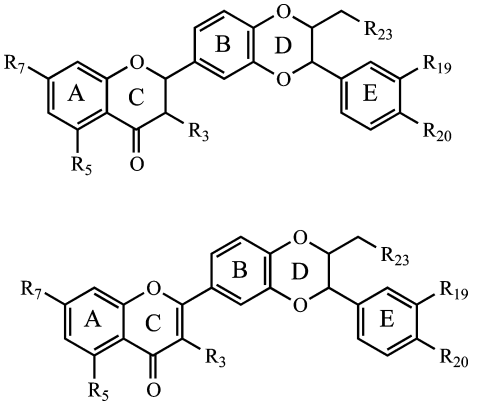


Figure 1. Chemical structures for compounds under study are shown. Numbers are for further reference of the position of the methoxy groups. For silybin and 2,3-dehydrosilybin, R_3, R_5, R_7, R_{20}, and R_{23} are OH and R_{19} is OCH_3. Nor-silybin and nor-dehydrosilybin correspond to the structures with OH at the positions R_3, R_5, R_7, R_{19}, R_{20}, and R_{23}.

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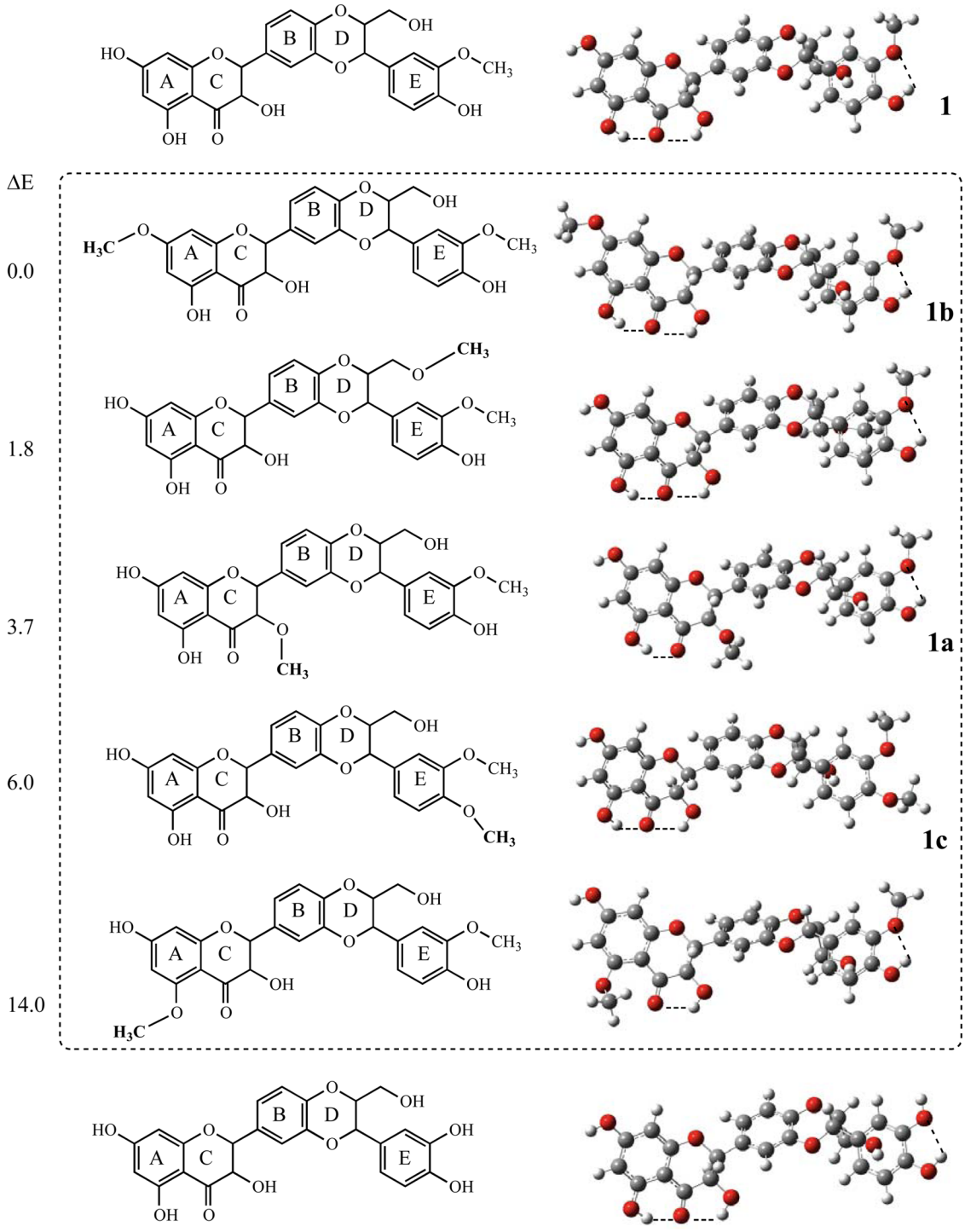

Figure 2. Schematic representation and optimized geometries of silybin and its derivatives are reported. Molecules within the dashed square represent isomers. Labels comply with previous results.[14] ΔE is in kilocalories per mole and corresponds to the energy differences between the isomers compared to the most stable.

atom transfer. Despite all of these results, there are other important considerations that have not been taken into account. The most important is the study of these molecules under physiological conditions (pH 7.4). The importance of pK_a values and the deprotonated molecules was previously reported[20−22] from the experimental point of view, but there are no theoretical reports, nor do results exist concerning another important mechanism for scavenging a free radical, which is adduct formation.

To fully understand the mechanism of action implemented by free radical scavengers and its relationship with the chemical structure, we report results for six derivatives of silybin (characterized by the absence of the 2,3 double bond) and six derivatives of 2,3-dehydrosilybin (characterized by the presence of the 2,3 double bond). (See Figures 1−3.)

Optimized geometries, Raman spectra, and λ_{max} values enable characterization and could be very useful for experiments. The analysis of the intramolecular hydrogen bonds gives us the

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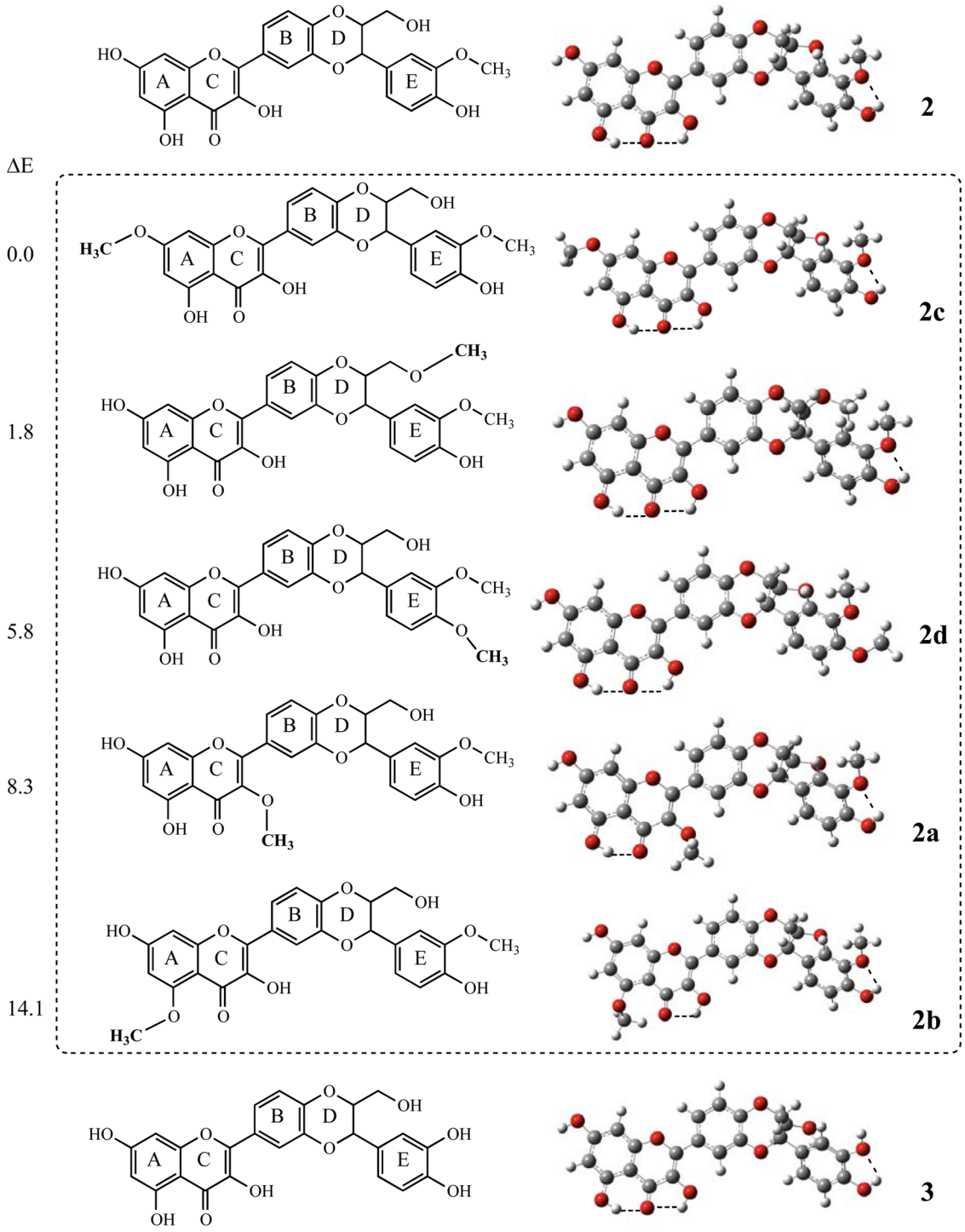

Figure 3. Schematic representation and optimized geometries of 2,3-dehydrosilybin and its derivatives are reported. Molecules within the dashed square are isomers. Labels comply with previous results.[14] ΔE (in kcal/mol) corresponds to the energy differences between the isomers compared with the most stable.

opportunity to highlight their importance in terms of the stability and reactivity of these molecules. We also determine the predominant species under physiological conditions (pH 7.4) and analyze the reactivity of these species. The electron-transfer mechanism is investigated in two directions: as electron donors and also as electron acceptors. Finally, we present results concerning the adduct formation of silybin, 2,3-dehydrosilybin, and some of the derivatives with •OOH.

COMPUTATIONAL DETAILS

Gaussian 09 implementation[23] was used to calculate geometry optimization and electronic properties of six derivatives of silybine and six derivatives of 2,3-dehydrosilybin. Initial geometries were fully optimized at M06/6-31+G(d) level of theory.[24−28] This methodology was used before to study organic molecules, the good performance of this functional even with relative small basis sets was reported.[29−31] Harmonic analyses were performed and local minima were identified (zero

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imaginary frequencies) to verify optimized minima. Marvin Sketch (version 14.10.27)[32] was used to obtain the pK_a at 298 K (pK_a min: 0; pK_a max: 14). The λ_{max} values had been obtained by applying time-dependent density functional theory (TDDFT) at M06/6-31+G(d) level of theory.

To investigate the single electron-transfer mechanism, vertical ionization energy (I) and vertical electron affinity (A) were obtained from single point calculations of cationic and anionic molecules using the optimized structure of the neutrals and the 6-311+g(d,p) basis set. Water and DMSO were included to mimic polar and nonpolar environments. A useful tool defined previously is the electron full electron donor−acceptor map (FEDAM).[33,34] In this map (see Figure 4) I and

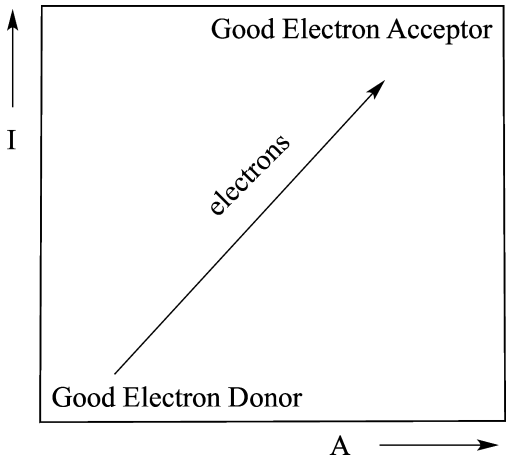

Figure 4. Full electron donor−acceptor map.

A are plotted and allow us to classify substances as either donors or acceptors of electrons. Electrons will be transferred from molecules located down to the left of the map (good electron donors) to those molecules that are up to the right (good electron acceptors).

RESULTS AND DISCUSSION

Geometry Optimization and Hydrogen Bonds. Figure 2 presents a schematic representation and the optimized structures of silybin and its derivatives. Structures of silybin (1), *3-O-Me*-silybin (1a), *7-O-Me*-Silybin (1b), and *20-O-Me*-silybin (1c) are similar to those reported previously.[14] Structures within the dashed square represent the monomethylated derivatives (isomers). Three of these structures present similar stability. The two most stable isomers have three intramolecular hydrogen bonds (dashed lines). The localization of OCH_3 on R_7 or R_{23} produces structures with practically the same stability (ΔE = 1.8 kcal/mol), and it is possible for both to coexist in an experiment. An energy difference smaller than 5 kcal/mol allows us to conclude that two isomers may coexist in an experiment. For the structure located at 3.7 kcal/mol, R_3 is OCH_3; therefore, one hydrogen bond is missing. At 6.0 kcal/mol, there is a structure with OCH_3 at R_{20}, and also there are only two hydrogen bonds. Apparently, the hydrogen bond formed between R_{19} and R_{20} is less important for stabilization than the hydrogen bond on R_3. In the less stable structure (within the dashed square), R_5 is OCH_3, and there is no hydrogen bond at this position. This destabilizes the structure by 14.0 kcal/mol, indicating that the hydrogen bond at R_5 position is the most important for the stabilization of these isomers. In Figure 2, it is possible to find another structure, not previously reported. This molecule, named nor-silybin, does not contain OCH_3 and has three intramolecular hydrogen bonds that stabilize the structure. The three structures (presented in Figure 2) that were not published previously and therefore do not have labels, are important for the total characterization of the derivatives of silybin. The methylation on R_5 indicates the importance of the hydrogen bond formed at this position, and nor-silybin (the molecule without OCH_3) enables us to investigate the role of the OH groups in terms of the free radical scavenging capacity of this system.

Figure 3 presents the schematic representation and optimized structures of 2,3-dehydrosilybin and its derivatives. Labels concur with previous reports. Results are similar to those previously reported[14] and also to the results for silybin. In this case, only one structure was not previously reported. It is 1.8 kcal/mol less stable than the ground-state monomethylated derivative and may therefore coexist in an experiment.

The conformational analysis of silybin and 2,3-dehydrosilybin derivatives was previously reported[14] and indicates the importance of H bonding. It is clear that for silybin and 2,3-dehydrosilybin derivatives intramolecular hydrogen bonds are crucial for stabilization. To add information to that previously reported and to analyze the influence of the hydrogen bonds in terms of the stability of these molecules, Figures 5 and 6 present a schematic representation of the optimized structures of silybin and 2,3-dehydrosilybin, with different hydrogen bonds as indicated in the Figures. For both molecules, the most stable structure presents three hydrogen bonds, and the least stable structure has no intramolecular hydrogen bonds. There are two structures of silybin that are less stable by 4.5 kcal/mol than the ground state. Both have two hydrogen bonds, one of them formed with the H atom of OH on R_5. The difference is the localization of the second hydrogen bond; in one structure it is between the H atom of the OH (on R_3) and the O atom of C═O, and in the other it is between the H atom of OH and the O atom of OCH_3 (on R_{23}). In this case, the stabilization due to the presence of this second H bond is the same. For 2,3-dehydrosilybin, there are two structures with two hydrogen bonds, one on R_5 that presents different stability. One of these is similar to silybin, with two H bonds connected to C═O. The other structure is less stable by 9.1 kcal/mol. Because of the 2,3-double bond, the structure of 2,3-dehydrosilybin is more rigid than in the case of silybin. This increases the steric impediment of the hydrogen atom on R_3 and the B-ring. For this reason, the structure with the H atom on R_3 turned to ring B is less stable by 9.1 kcal/mol. A stabilizing effect is observed by the hydrogen bond between the H atom of OH and the O atom of the methoxy group, but this stabilization is less than that produced by the stabilization due to the H bonding between the H atom of the hydroxy groups on R_3 and R_5 and the O atom of the C═O group. The hydrogen bond on R_5 is missing in the case of less stable structures. This corroborates the importance of this hydrogen bond for the stabilization of these systems. This is important for further investigations, for example, focusing on silybin as a metal chelating agent.[35]

The structural characterization of silybin and 2,3-dehydrosilybin derivatives is possible with the Raman spectra and λ_{max} values. Figure 7 and Table 1 present the Raman spectra and the λ_{max} values, respectively, for the optimized structures for silybin, 2,3-dehydrosilybin, the most stable structures of the monomethylated derivatives, and those molecules without OCH_3 (nor-silybin and nor-dehydrosilybin). We include only the most

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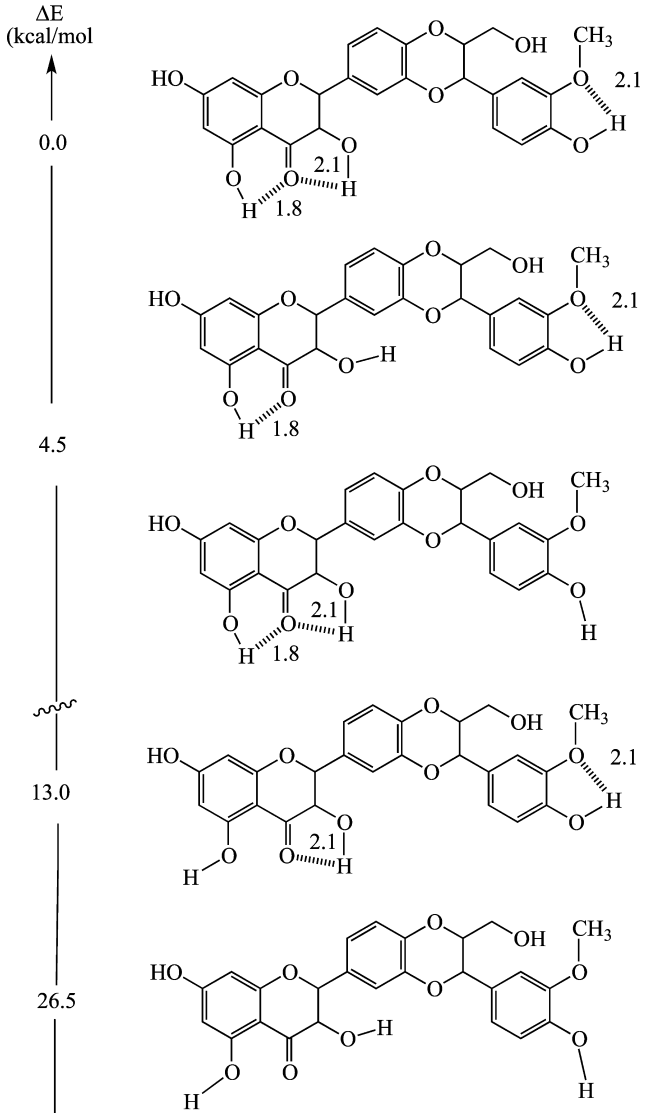

Figure 5. Schematic representation of silybin indicating hydrogen bond lengths (in angstroms).

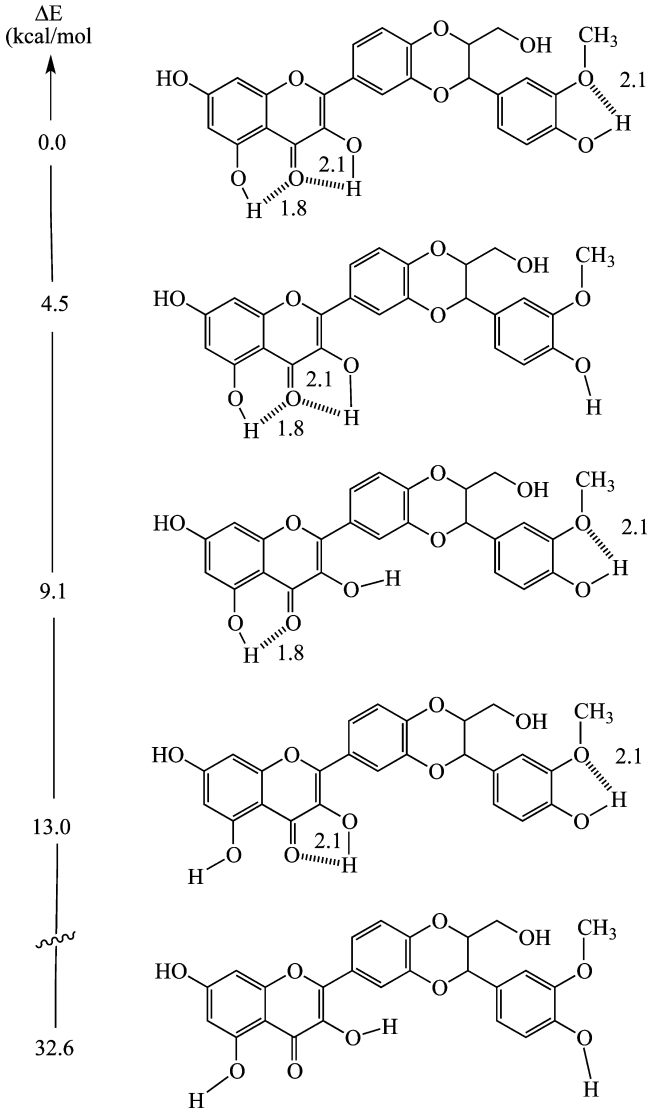

Figure 6. Schematic representation of 2,3-dehydrosilybin. Hydrogen bond lengths are indicated in angstroms.

stable monomethylated derivatives, as the spectra for all of the isomers are similar. Comparing the Raman spectra of silybin and 2,3-dehydrosilybin, there are important differences (indicated by stars). On the basis of this result, the oxidation of silybin into 2,3-dehydrosilybin may be followed with the Raman spectra. For the other spectra, the main difference is the presence of the OCH_3 signals for the monomethylated derivatives, as indicated in Figure 7. Therefore, the most important conclusion, concerning the Raman spectra, is the difference between silybin and 2,3-dehydrosilybin, as this can be useful for monitoring the reaction between them. As apparent in Table 1, λ_{max} corresponds to the UV region and is larger for 2,3-dehydrosilybin derivatives than for silybin. The λ_{max} values make it possible to distinguish between both molecules and may also be useful for following the oxidation between silybin and 2,3-dehydrosilybin. These spectra are very helpful as a reference for further experiments. After this structural characterization, it is possible to analyze the antiradical capacity of these molecules. The most stable structures will be used in what follows for this purpose.

Antiradical Capacity. *Electron-Transfer Mechanism.* The FEDAMs of the molecules being studied are reported in Figure 8, considering water and DMSO to mimic polar and nonpolar environment. Astaxanthina (Asta), a well-known antiradical molecule, is included for comparison. DPPH was used previously to measure free radical scavenging activity experimentally,[14] and for this reason we include this molecule on the maps. As previously reported,[14] *I* values are lower for the 2,3-dehydrosilybin derivatives than for silybin derivatives, but for both types of compounds *I* values are high. Their localization on the map indicates that these molecules were unable to transfer electrons to DPPH. As previously reported,[33,34] for the purpose of scavenging free radicals, molecules can either donate or accept electrons. Analyzing the capacity to accept electrons, a factor not previously considered, the *A* values indicate that the molecules being studied represent good electron acceptors and that 2,3-dehydrosilybin derivatives are better electron acceptors than silybin derivatives; however, Asta is a better electron acceptor than silybin and 2,3-dehydrosilybin derivatives, and according to their position on the map, DPPH is the best electron acceptor. Asta is able to

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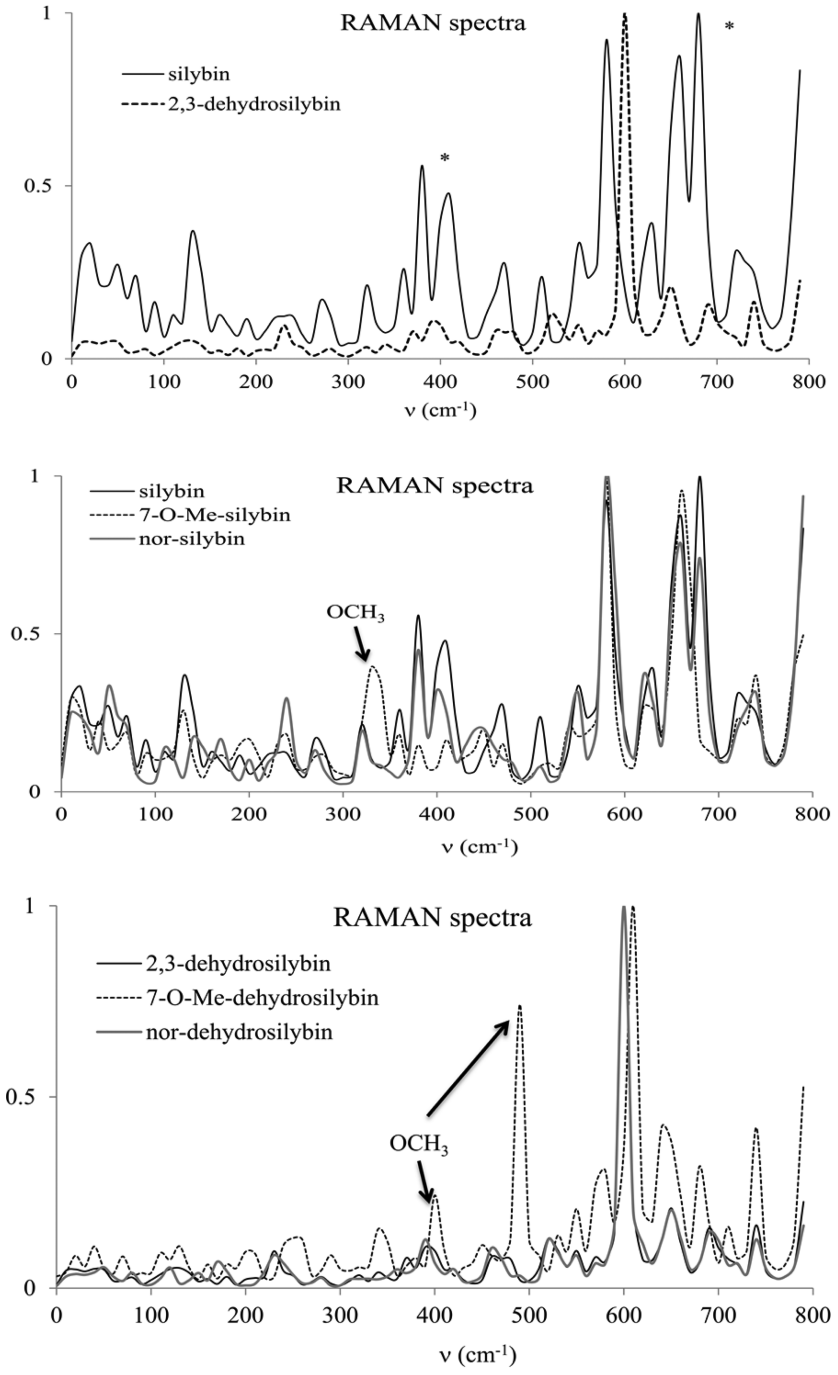

Figure 7. Raman spectra of the ground-state structures: silybin, 2,3-dehydrosilybin, the most stable monomethylated derivatives, nor-silybin, and nor-dehydrosilybin.

donate electrons to DPPH because it is localized down to the left with respect to DPPH, but silybin and 2,3-dehydrosilybin can neither accept nor donate electrons to DPPH. Concurring with previous results,[14] we can conclude from these values that the electron-transfer mechanism between these molecules and DPPH is not favorable.

It is important to study these molecules under physiological conditions (pH 7.4) to completely analyze antiradical capacity. For this purpose, the pK_a values for each H atom were calculated and also the relative abundance of each species (see Supporting Information, Table 1S). At pH 7.4, ∼70% of silybin is protonated but 60% of 2,3-dehydrosilybin is deprotonated. This is an important difference between these two molecules and means that deprotonated 2,3-dehydrosilybin will be present under physiological conditions and also under the experimental conditions in previous reports.[14] The pK_a values suggest that an important amount of deprotonated species is to be expected in the experiment. Therefore, it is crucial to analyze the electron donor−acceptor properties of the deprotonated molecules.

Figure 8 reports results for the deprotonated species (SIL-H and 2,3-DEHY-H, deprotonated silybin, and 2,3-dehydrosilybin derivatives, respectively). Deprotonated molecules are better electron donors and worse electron acceptors than the nondeprotonated species, and deprotonated 2,3-dehydrosilybin derivatives are better electron donors than the corresponding silybin derivatives. The position on the map indicates that 2,3-

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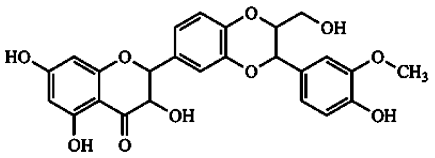
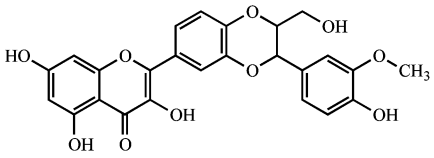
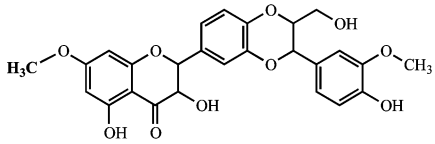
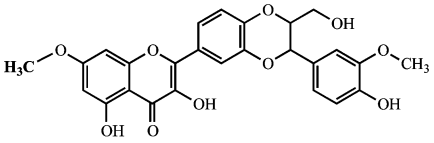
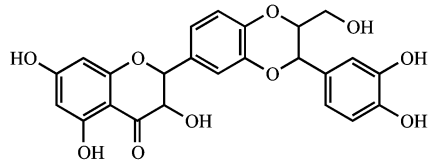
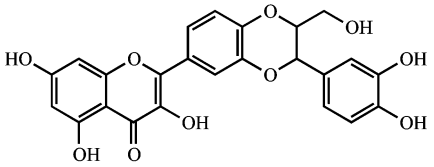
Table 1. Values of λ_{max} for the Most Stable Structures of Silybin, 2,3-Dehydrosilybin, the Most Stable Structures of the Monomethylated Derivatives (7-O-Me-Silybin and 7-O-Me-Dehydrosilybin), and Molecules without OCH_3 (Nor-Silybin and Nor-Dehydrosilybin)

Silybin and its derivatives	λ_{max} (nm)	2,3-dehydrosilybin and its derivatives	λ_{max} (nm)
	269		362
	274		365
	270		362

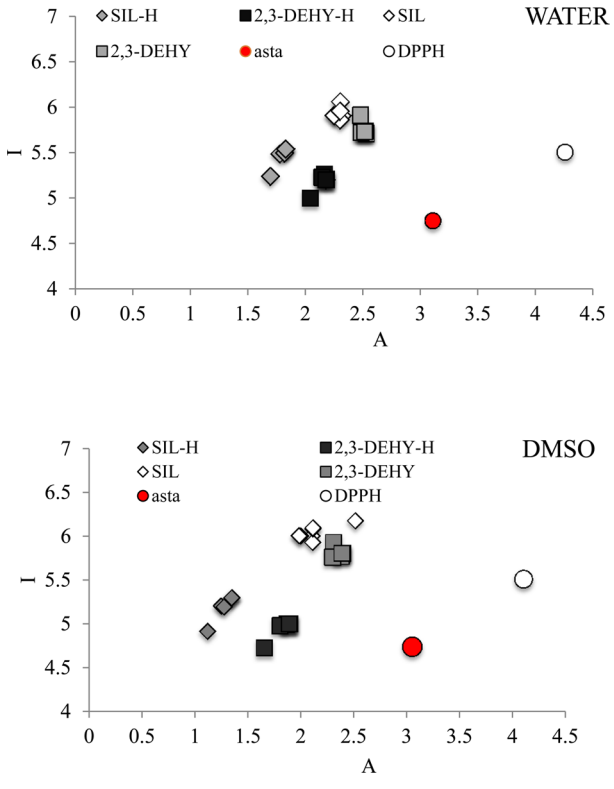


Figure 8. Full electron donor−acceptor map for silybin and its derivatives (SIL), 2,3-dehydrosilybin and its derivatives (2,3-DEHY), their deprotonated species (SIL-H and 2,3-DEHY-H, respectively), and DPPH. Astaxanthina (Asta) is also included for comparison.

DEHY-H molecules are down to the left with respect to DPPH, and they are therefore able to transfer electrons to DPPH. For SIL-H, the position on the map indicates that they are to the left of DPPH (are worse electron acceptors than DPPH), but the values for *I* are similar, and consequently the electron transfer will be less effective. Likewise, under experimental conditions, silybin derivatives are mainly not deprotonated and 2,3-dehydrosilybine are deprotonated. Comparing *I* values of silybin (5.91 and 6.18 eV, in water and DMSO, respectively), they are higher than the corresponding values for deprotonated 2,3-dehydrosilibin (5.20 and 5.00 eV, in water and DMSO, respectively). The latter is expected to be a better free radical scavenger than the first, in complete agreement with the experimental results previously reported that conclude that the DPPH scavenging activity of 2,3-dehydrosilybin derivatives is higher than that of the silybin derivatives.[14] This is an important result, as the electron-transfer mechanism is expected to be the fastest in terms of scavenging free radicals. The conclusion is thus a little different from that previously reported,[14] as the electron-transfer mechanism is favorable under physiological conditions, given that 2,3-dehydrosilybin derivatives are mainly deprotonated.

Adduct Formation Mechanism. Experimental results[14] indicate that silybin is less effective as a superoxide scavenger than 2,3-dehydrosilybin and nor-dehydrosilybin, with the last of these being the best scavenger. (See table 1 of ref 14.) The electron-transfer mechanism provides a partial explanation for these results, but it is necessary to analyze other possibilities to fully understand these experiments. To this end, the adduct formation mechanism is analyzed using •OOH as free radical and three molecules as free radical scavengers (those that were reported previously in ref 14): silybin, 2,3-dehydrosilybin, and nor-dehydrosilybin. (For the latter two molecules we used both protonated and deprotonated systems.) The free radical was added at all C−C double bonds or where hydrogen bonds were formed with different oxygen atoms. All systems were fully optimized. Tables 2−6 present the results of the dissociation energies (ΔE) for each free radical adduct molecule. Labels on the schematic representations indicate the position of the addition. Positive values correspond to adduct products that are

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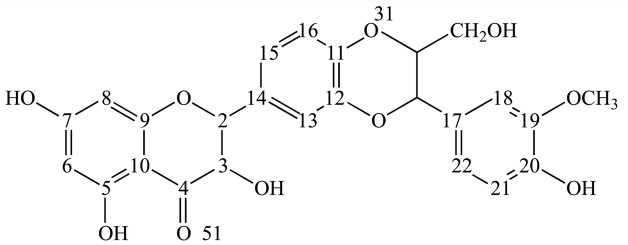
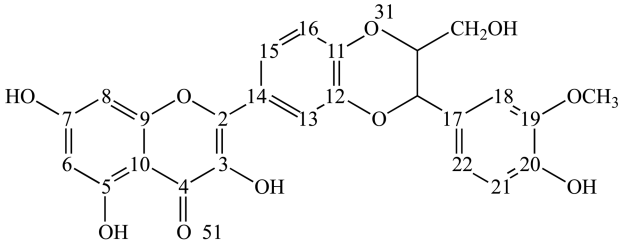
Table 2. Dissociation Energies (in kcal/mol) Corresponding to the Following Reaction



Silybin

C4	-15.9	C11	0.5	C17	-1.3
C5	-5.3	C12	8.7	C18	-1.6
C6	-1.7	C13	0.8	C19	7.7
C7	-6.4	C14	9.2	C20	6.8
C8	-3.9	C15	-1.5	C21	-1.9
C9	-2.5	C16	-1.7	C22	-4.2
C10	11.7				
				O31	8.4
				O51	8.2

Table 3. Dissociation Energies (in kcal/mol) Corresponding to the Following Reaction: [2,3-Dehydrosilybin-OOH]• → 2,3-Dehydrosilybin + •OOH[a]



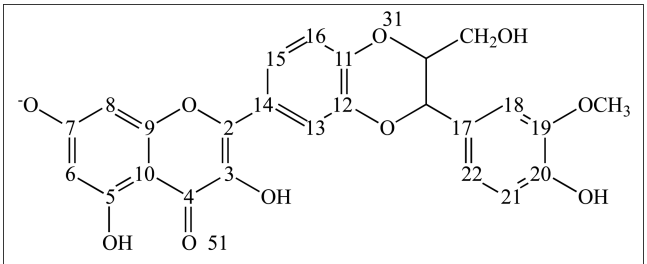
2,3-dehydrosilybin

C2	14.1	C11	4.3	C17	-0.6
C3	8.6	C12	-0.05	C18	-1.5
C4	10.7	C13	3.4	C19	2.7
C5	-3.5	C14	-8.7	C20	1.6
C6	-0.1	C15	1.8	C21	-1.9
C7	-4.5	C16	-3.0	C22	-4.4
C8	0.3				
C9	-6.2			O31	8.1
C10	-15.3			O51	12.7

[a]The position where the free radical is bonded is as indicated in the schematic formula.

more stable than the corresponding dissociated molecules. The atoms with positive ΔE will be named in this report "reactive atoms". Higher positive values indicate a stronger bond between the free radical scavenger and •OOH. For all of the compounds presented in Tables 2–6, on O31 and O51, the •OOH molecule forms hydrogen bonds that stabilize the products by >8 kcal/mol. Comparing these two reactive atoms, O51 presents more positive values than O31; therefore, this position is more suitable for the reaction. Silybin has nine reactive atoms that form adducts with positive values for ΔE. The most reactive atom is C10 with ΔE of 11.7 kcal/mol. Compared with 2,3-dehydrosilybin, this has 11 reactive atoms that form adducts with positive values for ΔE. In this case, the most stable adduct corresponds to •OOH bonded to C2, with ΔE equal to 14.1 kcal/mol. Moreover, we can also analyze the deprotonated 2,3-dehydrosilybin, considering that its relative abundance under physiological conditions is 60%. Results for deprotonated 2,3-dehydrosilybin are presented in Table 4. For

Table 4. Dissociation Energies (in kcal/mol) Corresponding to the Following Reaction: [2,3-Dehydrosilybin-OOH]•− → [2,3-Dehydrosilybin]− + •OOH[a]

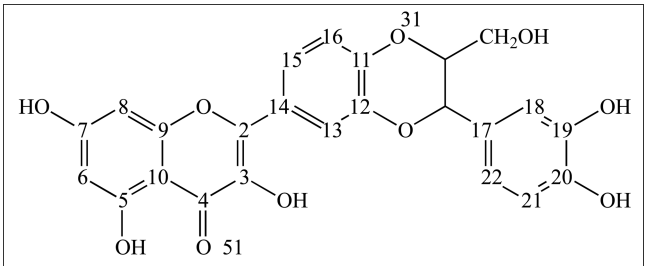


2,3-dehydrosilybin de-protonated

C2	19.6	C11	9.3	C17	-0.1
C3	16.0	C12	3.1	C18	-0.7
C4	7.3	C13	8.4	C19	3.3
C5	1.0	C14	-9.7	C20	2.7
C6	14.6	C15	6.1	C21	-1.8
C7	-18.9	C16	-0.3	C22	-5.3
C8	11.0				
C9	2.7			O31	10.2
C10	-2.2			O51	17.3

[a]The position where the free radical is bonded is as indicated in the schematic formula.

Table 5. Dissociation Energies (in kcal/mol) Corresponding to the Following Reaction: [Nor-dehydrosilybin-OOH]• → Nor-dehydrosilybin + •OOH[a]



nor-dehydrosilybin

C2	14.1	C11	4.3	C17	-1.2
C3	8.5	C12	-0.1	C18	0.9
C4	10.6	C13	3.4	C19	2.0
C5	-3.5	C14	-8.8	C20	0.8
C6	-0.2	C15	1.8	C21	-1.6
C7	-4.6	C16	-3.1	C22	-4.6
C8	0.3				
C9	-6.2			O31	8.0
C10	-15.4			O51	12.6

[a]The position where the free radical is bonded is as indicated in the schematic formula.

this molecule there are 15 reactive atoms, with positive values for ΔE. The most reactive atoms are C2, C3, C6, and O51, and the highest dissociation energy is 19.6 kcal/mol. This comparison reveals that the reactivity of 2,3-dehydrosilybin (protonated and deprotonated) is higher than for silybin, as the latter have more reactive atoms and the dissociation energies are superior for 2,3-dehydrosilybin (protonated and deprotonated) than for silybin. This is in complete agreement with previous experimental results,[14] which conclude that 2,3-dehydrosilybin is a better free radical scavenger than silybin.

Previous experiments[14] suggest that nor-dehydrosilybin is the best free radical scavenger of all systems being studied. Comparing the results for protonated and deprotonated 2,3-

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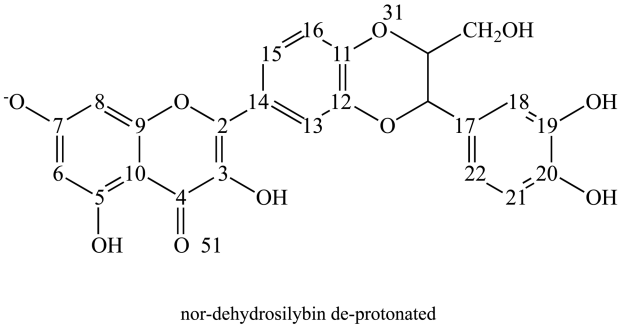
J. Phys. Chem. B 2015, 119, 11597−11606

Table 6. Dissociation Energies (in kcal/mol) Corresponding to the Following Reaction: [Nor-dehydrosilybin-OOH]$^{\bullet-}$ → [Nor-dehydrosilybin]$^{-}$ + $^{\bullet}$OOH[a]



nor-dehydrosilybin de-protonated

C2	19.6	C11	9.4	C17	-0.5
C3	15.9	C12	3.0	C18	1.9
C4	7.2	C13	8.4	C19	3.0
C5	1.0	C14	-9.7	C20	1.9
C6	14.6	C15	6.1	C21	-1.7
C7	-19.0	C16	-0.3	C22	-5.5
C8	11.0				
C9	2.7			O31	10.1
C10	-2.2			O51	17.2

[a]The position where the free radical is bonded is as indicated in the schematic formula.

dehydrosilybin and nor-dehydrosilybin (Tables 3−6), it is clear that ΔE values are very similar. Both nor-dehydrosilybin molecules have one more reactive atom (C18) than 2,3-dehydrosilybin molecules, but ΔE is very small, meaning that •OOH is not strongly bonded to that position. The differences between 2,3-dehydrosilybon and nor-dehydrosilybin are minimal, and it is evident that the substitution of OCH_3 by OH on 2,3-dehydrosilybin does not affect the free radical scavenger capacity when the adduct formation mechanism is analyzed. This does not concur with experimental results that suggest that nor-dehydrosilybin represents the best antiradical. Probably the main mechanism for action in nor-dehydrosilybin is the hydrogen atom transfer mechanism, as previously reported.[14] Despite these results, the conclusions reported here mainly concur with the experiments.

CONCLUSIONS

For silybin and 2,3-dehydrosilybin derivatives, intramolecular hydrogen bonds are crucial for stabilization. The most stable structure presents three hydrogen bonds, and the least stable structure has no intramolecular hydrogen bonds. Hydrogen bond on R_5 is the most important for stabilizing structures.

Raman spectra are very useful for characterizing these systems. Moreover, the oxidation of silybin into 2,3-dehydrosilybin can be followed with the Raman spectra. The main difference on the spectra, between nonmethylated and monomethylated derivatives, is the presence of OCH_3. The λ_{max} values allow us to distinguish between both molecules and may also be useful for monitoring oxidation between silybin and 2,3-dehydrosilybin. The λ_{max} values correspond to the UV region and are larger in the case of 2,3-dehydrosilybin derivatives than in the case of silybin.

The electron-transfer mechanism between all molecules being studied and DPPH is not favorable. Under physiological conditions (pH 7.4), ∼70% of silybin is protonated, but 60% of 2,3-dehydrosilybin is deprotonated. This is an important difference between these two molecules, implying that deprotonated 2,3-dehydrosilybin will be present under physiological conditions and also under the experimental conditions of previous reports. Deprotonated molecules represent better electron donors and worse electron acceptors than the nondeprotonated species, and deprotonated 2,3-dehydrosilybin derivatives are better electron donors than the corresponding silybin derivatives. The latter are expected to be worse free radical scavengers than the first.

Concerning the adduct formation mechanism, the reactivity of 2,3-dehydrosilybin (protonated and deprotonated) is higher than that for silybin, as the latter have more reactive atoms. Dissociation energies are also superior for 2,3-dehydrosilybin (protonated and deprotonated) than for silybin.

The conclusions reported in this investigation are in complete agreement with previous experimental results. The free radical scavenging activity of 2,3-dehydrosilybin derivatives is higher than that for silybin derivatives. What was not previously considered was the remarkable importance of the deprotonated species, which may thus be important for future experiments.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b06448.

Optimized structures, pK_a values, relative abundance of each species, and complete references with more than 10 authors. (PDF)

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Notes

The authors declare no competing financial interest.

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