Donator Acceptor Map of Psittacofulvins and Anthocyanins: Are They Good Antioxidant Substances?

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Psittacofulvins represent an unusual class of pigments (noncarotenoid lipochromes), which are found only in the red, orange, and yellow plumage of parrots. Anthocyanins are flavonoids, and they are one of the primary types of colorants found in plants. Blue butterflies acquire blue and UV hues on their wings, owing to the presence of flavonoids. It is assumed that these natural pigments are valuable antioxidants because they are able to scavenge free radicals. The aim of this investigation is to rationalize the scavenging activity of psittacofulvins and anthocyanins, in terms of the one electron transfer mechanism, taking into account that to prevent oxidative stress, substances must either donate or accept electrons. Density functional approximation calculations are used to obtain ionization potentials, electron affinities, electrodonating, and electroaccepting power indexes. Taking these values, a donator acceptor map (DAM) was constructed, indicating that anthocyanins are good electron donors, whereas psittacofulvins are good electron acceptors. Anthocyanins and vitamins are antioxidants, whereas psittacofulvins and carotenoids are antireductants (oxidants). In terms of solvent effects, animal pigments (carotenoids, psittacofulvins, and anthocyanins) are much better electron acceptors in water than in either the gas phase or benzene. Solvent effects do not alter the electron donor capacity of vitamins, but anthocyanins become effective electron acceptors in water, rather than effective electron donors. The information presented here may also be valuable for the design and analysis of further experiments.

Introduction

For many years, the idea has existed that pigmentation in animals may indicate antioxidant status.[1−3] Animal pigments are termed antioxidants because these molecules scavenge free radicals and thus limit oxidative stress. Animals may face a tradeoff when allocating pigments (acquired from the diet or elsewhere), using them either for physiological or coloration purposes. It is assumed that higher-quality individuals (those who are able to obtain more pigments or are in a better state of health) are able to devote more of the acquired substances to coloration, which in turn appears to be important for sexual advertisement and ultimately reproduction and species survival. Coloration thus reveals individual quality and becomes the target of sexual selection. If animal pigments are possible indicators of antioxidant status, it is important to analyze their antioxidant properties. In a previous work,[4] we made an analysis of the antioxidant (antiradical) capacity of carotenoids (CAR), melatonin, and vitamins. In this paper, results for other important pigments, namely psittacofulvins and anthocyanins (shown in Figure 1), will be described.

Psittacofulvins represent an unusual class of pigments that are manifested in the red, orange, and yellow plumage of parrots.[2,3,6−8] Apparently, these noncarotenoid lipochromes are found only in parrot feathers. The antioxidant action of psittacofulvins was studied using electron paramagnetic resonance to investigate the capacity of these molecules to scavenge free radicals in vitro.[9] The authors found that only one of these pigments (octadecaoctenal or octatrienal) can act as a potent inhibitor of hydroxyl radical formation. To date, it is not clear whether psittacofulvins represent good antioxidant (antiradical) substances or not, and to the best of my knowledge, there are no theoretical studies concerning these molecules.

Anthocyanins are flavonoids and constitute one of the primary types of colorants in plants.[3,6,10−12] Blue butterflies acquire blue and UV hues on their wings, as a result of the presence of flavonoids. It is assumed that these pigments constitute valuable antioxidants in plant foods,[10−18] and a considerable quantity of literature is devoted to describing the antiradical properties of these natural pigments (reviewed in refs 10, 13, and 18). A number of theoretical studies also exist that discuss the antioxidant properties of flavonoids.[14−16]

Anthocyanins represent one of the most widely distributed classes of flavonoids and are considered to comprise one of the most important families of natural antioxidants. These are polyphenolic substances because they contain at least one hydroxyl group attached to a benzene ring. According to the literature,[16,19−22] there are at least three mechanisms by which phenolic antioxidants are able to scavenge free radicals: electron transfer, H atom transfer (HAT), and sequential proton loss-electron transfer. The electron transfer mechanism offers a very important reaction for neutralizing free radicals, which was also observed in the case of several carotenoids (see ref 4 and references therein). Additionally, it was reported that the electron transfer mechanism of antioxidants (*anti*), when scavenging free radicals (R), occurs as indicated in the following equation:

$$anti + R \rightarrow anti(+) + R(-)$$

assuming that the antioxidants must either lose or donate an electron in order to neutralize the free radical. Previous reports

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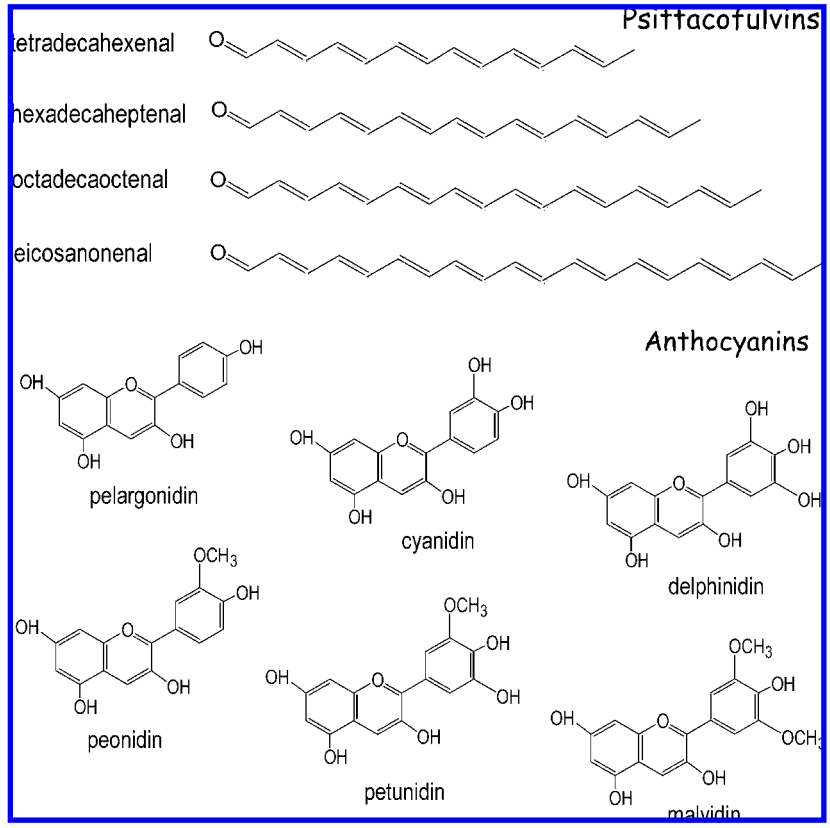

Figure 1. Psittacofulvins and anthocyanins pigments. Schematic representation of the molecular structure of psittacofulvins found in the feathers of parrots and anthocyanins, one of the most widely distributed types of flavonoid. Studied anthocyanins are neutral and cationic, since it was suggested that anthocyanins should also be stable carbocations.

analyze the relative antioxidant efficiency of carotenoids in terms of one electron transfer reaction,[4,23] as well as the antioxidant properties of flavonoids.[14−16,24,25] In all of these studies, the single electron transfer reaction of carotenoids and flavonoids is analyzed in terms of their ionization potential (IP). The best antioxidants present low IP values, because the lower the IP, the easier the electron abstraction. However previously,[4] we proposed an alternative mechanism for carotenoids to scavenge free radicals, that is, antiradicals might act either donating or accepting electrons, meaning that the following reaction is also possible.

$$anti + R \rightarrow anti(-) + R(+)$$

Hence, to understand the antiradical capacity of different molecules, it is important to study the electron transfer process, also taking into account their capacity to accept electrons. For this purpose, it is necessary to assess electron affinity (EA). Therefore subsequently, quantum chemical calculations were made for several carotenoids and some colorless antioxidants, such as melatonin and for vitamins A, C, and E and electron acceptance (Ra) and electron donation (Rd) indexes were defined, using fluor and sodium as references. A plot of Rd vs Ra provides a donator acceptor map (DAM, see Figure 2), useful for classifying any substance L in terms of its electron donating-accepting capacity. As the electron transfer reaction represents one of the mechanisms employed for radical scavenging that is discussed in the literature, the DAM may be regarded as a powerful representation, helping to reveal the antiradical capacity of any substance. The DAM is a useful tool when making a qualitative comparison between substances, as any molecule can be classified in terms of its electron donating-accepting

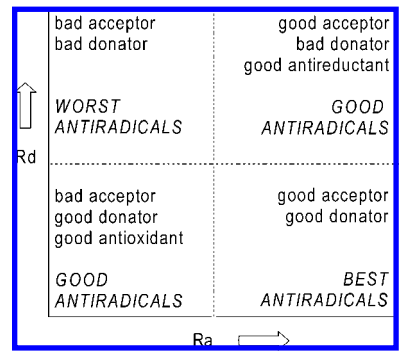

Figure 2. Donator acceptor map (DAM). Four regions are distinguished as described in detail in the text. Dash lines separating regions are only indicative, to clarify the image.

capacity (with respect to F and Na). In this context, the aim of this investigation is to rationalize the scavenging activity of psittacofulvins and anthocyanins, in terms of one electron transfer mechanism.

Computational Details and Construction of the DAM

Density functional approximation[26−28] as implemented in Gaussian 03[29] was used for all calculations. Two different functionals (BPW91[30,31] and B3LYP[32]) and two basis sets[33−36] (D5DV and 6−311G**) were employed in the calculations of complete optimizations, without symmetry constraints. Harmonic frequency analyses permitted us to verify optimized minima.

In order to evaluate oxidation capacity, it has been demonstrated[4,23] that relative antioxidant efficiency is determined by vertical IP (*I*). Compounds that have low *I* values are the most

easily oxidized substances and as a result, they represent the most efficient antiradicals, in terms of their electron donating capacity. To evaluate reduction capacity, it is necessary to estimate electron acceptance. This can be achieved by assessing vertical EA (A), which is a good indicator of the electron attraction force. Substances with high and positive A values have a greater capacity for accepting electrons. Compounds with high positive A values are the most easily reduced substances and thus they represent the most efficient antiradicals, expressed in terms of their electron accepting capacity. To compute I and A, further single-point calculations were necessary. I is calculated as the difference between the energy of the cation and the neutral molecule, assuming that both of these have the ground-state nuclear configuration of the neutral molecule. A is also calculated as vertical and represents the energy difference between the neutral and the anion, calculated using the ground-state nuclear configuration of the neutral molecule. Solvent effects were included, using the polarizable continuum model (PCM),[37,38] with water and benzene representing the solvents for polar and nonpolar environments, respectively.

Another useful way of measuring electrodonating and electroaccepting power has recently been described by Gázquez et al.[39] They established a simple charge-transfer model and analyzed the global response of a molecule immersed in an idealized environment that may either withdraw or donate charge. An alternative quadratic interpolation for the energy as a function of the number of electrons was proposed, to evaluate the response of a molecule to charge acceptance or withdrawal in terms of its electron affinity and ionization potential. Referring to this approximation, these authors conclude that the propensity to donate charge, or electrodonating power, may be defined as:

$$\omega^- = \frac{(3I + A)^2}{16(I - A)} \tag{1}$$

whereas the propensity to accept charge, or electroaccepting power, may be defined as

$$\omega^+ = \frac{(I + 3A)^2}{16(I - A)} \tag{2}$$

In the case of electrodonating power, lower values imply a greater capacity for donating charge. In the case of electroaccepting power, higher values imply a greater capacity for accepting charge. It is important to note that I and A refer to donating or accepting a single, whole electron, whereas $\omega-$ and $\omega+$ refer to fractional charges. In this way, the electrodonating and electroaccepting powers are based on a simple charge transfer model, expressed in terms of chemical potential and hardness. Chemical potential measures the charge flow direction, together with the capacity to donate or accept charge, assigning more emphasis to ionization potential than to electron affinity in the context of the charge donation process. Contrarily, electroaccepting power assigns more significance to electron affinity than to ionization potential. Hardness assesses resistance to the electron flow.

To make a comparison with other well-known antioxidant and antireductant substances, experimental values of I and A for F and Na atoms were used to obtain the corresponding $\omega+$ and $\omega-$ values. F represents a good electron acceptor, whereas Na represents a good electron donor. For any substance L, we define the electron acceptance index as:

$$\mathrm{Ra} = \frac{\omega_L^+}{\omega_F^+} \tag{3}$$

If Ra = 1, then $\omega_L^+ \cong \omega_F^+$ and L represents as effective an electron acceptor as F. If Ra > 1, then $\omega_L^+ > \omega_F^+$ and L represents a more effective electron acceptor than F. If Ra < 1, then $\omega_L^+ < \omega_F^+$ and L represents a less effective electron acceptor than F. In the same way, the electron donation index is defined as

$$\mathrm{Rd} = \frac{\omega_L^-}{\omega_{Na}^-} \tag{4}$$

If Rd = 1, then L is as effective an electron donor as Na. If Rd >1, then L is a less effective electron donor than Na. If Rd < 1, then L is a more effective electron donor than Na. If Ra and Rd are both known, then any substance L can be characterized in terms of its electron donor−acceptor capacity. These values allow us to place any substance L on the donor acceptor map (DAM), shown in Figure 2.

Results and Discussion

Ionization Potential and Electron Affinity. Table 1 presents the vertical ionization potentials (I) and vertical electron affinities (A), for all molecules studied. Two carotenoids (β-carotene (BC) and astaxanthin (ASTA)) and some vitamins previously described[4] are included for comparison. As can be seen, psittacofulvins and vitamins have higher I values than anthocyanins and carotenoids. The low I values represent the most easily oxidized substances and therefore the most efficient antiradicals, expressed in terms of their electron donating capacity. Generally, I values from Table 1 indicate that anthocyanins and carotenoids act as more effective antioxidants than psittacofulvins and vitamins, in terms of their electron donor mechanism.

Regarding electron affinity (A), the results in Table 1 show that carotenoids, psittacofulvins and anthocyanins have both large and positive values, whereas vitamins present either negative or very small positive values (vitamin A). Positive A values indicate that the anion is more stable than the neutral. We can derive from these results that naturally occurring pigments are more capable of accepting electrons than vitamins, and thus they represent the most efficient antiradicals (expressed in terms of their electron accepting capacity). Among natural pigments, the most effective electron acceptor is ASTA and the least effective acceptors are anthocyanins.

As to trap free radicals substances must either donate or accept electrons, we can say that carotenoids and anthocyanins represent better antiradicals than vitamins and psittacofulvins. Psittacofulvins have lower I values (meaning that they are better antioxidants), whereas vitamins represent the worst antiradicals, in terms of their electron accepting mechanism, because carotenoids, psittacofulvins, and anthocyanins are all more effective owing to the fact that they are able to accept electrons without losing energy (as they are antireductants).

Electrodonating ($\omega-$) and Electroaccepting Power ($\omega+$). Electrodonating and electroaccepting power is analyzed using $\omega-$ and $\omega+$, as expressed in eqs 1 and 2. Effective electron donors must present low values for electrodonating power ($\omega-$). In the case of electroaccepting power ($\omega+$), high values indicate effective electron acceptors. In general, the results presented in Table 1 indicate that high values for I likewise imply high values for $\omega-$, except in the case of vitamins whose $\omega-$ values are

TABLE 1: Vertical Ionization Energies (*I*), Vertical Electron Affinities (*A*), Electron Donation, and Acceptance Powers ($\omega-$ and $\omega+$) and Indexes (Rd and Ra) Obtained Using Equations 1−4[a]

molecule	*I* (eV)	*A* (eV)	$\omega-$ (donating power)	$\omega+$ (accepting power)	Ra	Rd
BC	**5.10**	**1.47**	**4.84**	**1.56**	**0.46**	**1.40**
ASTA	**5.70**	**2.42**	**7.27**	**3.21**	**0.94**	**2.10**
vitamin C	8.53	−0.39	4.46	0.38	0.11	1.29
vitamin E	6.70	−5.55	1.08	0.51	0.15	0.31
vitamin A	6.22	0.54	4.06	0.68	0.20	1.17
tetradecahexenal	**6.81**	**1.47**	**5.61**	**1.47**	**0.43**	**1.62**
hexadecaheptenal	**6.57**	**1.63**	**5.76**	**1.66**	**0.49**	**1.66**
octadecaoctenal	**6.37**	**1.76**	**5.91**	**1.84**	**0.54**	**1.71**
eicosanonenal	**6.21**	1.87	**6.05**	**2.01**	**0.59**	**1.75**
peonidin	5.45	0.93	4.13	0.94	0.28	1.19
cyanidin	5.49	0.92	4.14	0.94	0.27	1.20
delphinidin	5.51	0.99	4.25	1.00	0.29	1.23
pelargonidin	5.49	0.91	4.13	0.93	0.27	1.19
petunidin	5.38	0.97	4.15	0.98	0.29	1.20
malvidin	5.38	0.94	4.10	0.94	0.28	1.19
PEONIDIN+	**10.2**	**5.13**	**15.7**	**8.02**	**2.36**	**4.54**
CYANIDIN+	**10.5**	**5.22**	**15.9**	**8.08**	**2.38**	**4.61**
DELPHINIDIN+	**10.5**	**5.23**	**16.0**	**8.14**	**2.39**	**4.62**
PELARGONIDIN+	**10.5**	5.23	**15.9**	**7.92**	**2.33**	**4.59**
PETUNIDIN+	**10.2**	**5.15**	**15.8**	**8.17**	**2.40**	**4.58**
MALVIDIN+	**10.1**	**5.06**	**15.5**	**7.98**	**2.35**	**4.49**

[a] Reference values for the well known oxidant (Fluor) and the reductant (sodium) are also shown. Complete optimizations without symmetry constraints were undertaken at B3LYP/6-311G** level (for psittacofulvins) and BPW91/D95V level (for anthocyanins). Some psittacofulvins were also calculated at BPW91/D5DV level to assess the influence of the functional and the bases set. As was previously reported,[4] the relative values for *I* and *A* do not depend on the functional or on the basis set.

lower than those of other substances and contrast with their *I* values. As previously discussed,[4] this indicates that $\omega-$ is a better indicator of antioxidant power than *I*, as it was reported that vitamin E is one of the most important lipid-soluble antioxidants present in cell membranes.[40] The electrodonating power of anthocyanins is greater than that of psittacofulvins and carotenoids. The order of reactivity expressed in terms of facility for oxidation, referring to the $\omega-$ value is as follows:

vitamin E > vitamins A and C ≈ anthocyanins > BC > psittacofulvins > ASTA

Vitamin E represents the best antioxidant, whereas ASTA represents the worst. To determine whether color in animals is an indication of their antiradical status, it is important to analyze the second mechanism for electron transfer, that is, electron capture. For this purpose, it is possible to see in Table 1 that $\omega+$ correlates well with *A* and animal pigments are effective electron acceptors. The reactivity order, considering *A* and $\omega+$ values, is as follows:

ASTA > psittacofulvins > BC > anthocyanins > vitamins

ASTA represents the best antireductant, whereas vitamins represent the worst. Thus, both $\omega-$ and $\omega+$ results appear to indicate that among animals, pigmentation is an indication of antiradical status (either antioxidant or antireductant). As previously pointed out,[10−18] anthocyanins are considered valuable antioxidants in plant foods and have important antiradical properties. This evidence conforms to the values of *I* and $\omega-$ presented here. We should emphasize that vitamin E is a better antioxidant than these natural pigments. Moreover, psittacofulvins are not better antiradicals than ASTA but can scavenge free radicals more easily than BC, anthocyanins, and vitamins (in terms of the electron capture mechanism). It is also important to note that it would appear that anthocyanins and psittacofulvins

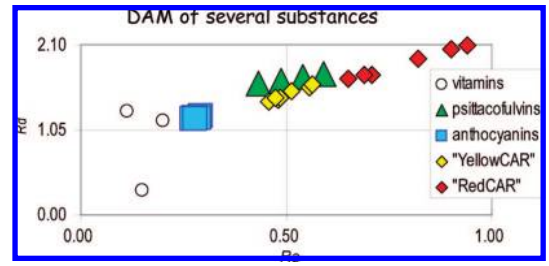

Figure 3. DAM for psittacofulvins and anthocyanins. Pssitacofulvins and anthocyanins located in the DAM. Results for vitamins, yellow, and red carotenoids (CAR) reported previously[4] are included for comparison. Dividing lines are only indicative, to clarify the image.

use different mechanisms for scavenging free radicals. Anthocyanins are effective electron donors, and psittacofulvins are effective electron acceptors. In this sense, the mechanism used by psittacofulvins for scavenging free radicals is similar to the mechanism suggested for carotenoids,[4] whereas the reaction of anthocyanins with free radicals is comparable to that manifested when vitamins scavenge free radicals.[4]

Donator Acceptor Map (DAM). Table 1 presents Ra and Rd for BC, ASTA, psittacofulvins, anthocyanins, and vitamins. Figure 3 shows the DAM for these substances. Carotenoids (Yellow CAR and Red CAR) as well as some vitamins previously reported are included for comparison. The DAM indicates that psittacofulvins are very similar to the Yellow CAR. They are located between a good antiradical zone (good antireductant section) and the worst antiradical region. Anthocyanins are also close to the worst antiradical zone but nearer to the antioxidant section than to the antireductant sector. When anthocyanins are compared with psittacofulvins, the former are revealed as better antioxidants than the latter; that is to say, psittacofulvins represent better antireductants than anthocyanins. Psittacofulvins may act as antireductants and thus also as antiradicals, in a way similar to that of Yellow CAR. As antiradicals, they are certainly less effective than Red CAR. On the other hand, anthocyanins are able to scavenge free

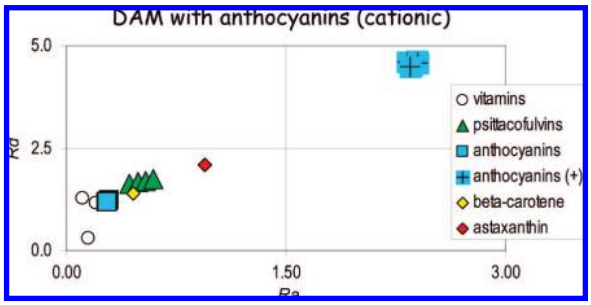

Figure 4. DAM for cationic anthocyanins. Psittacofulvins, anthocyanins and anthocyanins (+1) located in the DAM. Results for vitamins, beta-carotene, and astaxanthin reported previously[4] are included for comparison. Dividing lines are only indicative, to clarify the image.

radicals more efficiently than psittacofulvins, mainly by donating electrons, but the capacity of these substances for accepting electrons is very poor. It is not possible to assert from these results that psittacofulvins are better antiradicals than anthocyanins or vice versa, because the mechanisms employed to scavenge free radicals are different. Anthocyanins are better antioxidants, whereas psittacofulvins are better antireductants. It may be the case that under some conditions anthocyanins will be more effective against oxidative stress than psittacofulvins, but with an alternative free radical or in different circumstances, psittacofulvins may prove better antiradicals than anthocyanins. Moreover, for the purpose of scavenging free radicals, the chemical environment, where the molecules are present in a living organism, is also important, as well as the solubility of these substances in different solvents and the location or site of action of these antiradical molecules. The statement that substances are either antioxidant or antireductant is meaningless, unless we specify the medium to which we refer. What we can do is to compare one molecule with another, as was undertaken for anthocyanins and psittacofulvins. From the results presented here, it was possible to reach the conclusion that anthocyanins manifest a similar reaction mechanism for scavenging free radicals as vitamins, whereas psittacofulvins are comparable to carotenoids, as both, carotenoids and psittacofulvins represent very effective electron-acceptors.

DAM for Anthocyanins (Cations). There are many different stable configurations where anthocyanins are able to exist, with different pH values.[41,42] The pH dependence of the radical scavenging capacity of these molecules is very important, as it has been suggested that they may be positively charged in an acidic environment, and because the pH range of different body fluids vary widely, ranging from 1 in the stomach to 9 in the duodenum. Moreover, it was suggested that anthocyanins should be regarded as natural, stable carbocations.[25] If this is the case, the antiradical capacity of these positively charged molecules may be very important. To study the antiradical capacity of these positively charged molecules, it is necessary to calculate Ra and Rd values. For this purpose, the cation was optimized, and using this optimized geometry, the energy of the double cation and the neutral was obtained. Subsequently, I and A, $\omega+$ and $\omega-$, and Ra and Rd were calculated. The results for these cations are presented in Table 1. Figure 4 shows the DAM including these carbocations. As is evident, these positively charged molecules are very effective electron acceptors. It seems that they may represent better antiradicals than carotenoids, psittacofulvins, and anthocyanins, in terms of the electron acceptor mechanism.

Solvent Effects. I and A for some of the molecules were calculated considering polar (water (wt)) and non polar (benzene (bz)) solvent effects. The results are presented in Table 2. Evidently, they are largely consistent, that is, the relative order is the same using solvents and in gas phase. Solvent effects decrease I values and increase A values in comparison to those in the gas phase, but the general pattern is preserved. Similar results were previously reported for carotenoids,[4] vitamins,[4] delphinidin,[16] and pelargonidin.[16] Hence, the conclusions already reached, concerning I and A, are qualitatively the same when solvent effects are taken into account. However, solvent effects strongly influence Ra and Rd values, as can be seen in Figure 5 for all molecules studied to date (ref 4 and this work). Whereas in gas phase and benzene the results are similar, they are very different when we consider water. All of the molecules are much more effective electron acceptors in water than in either the gas phase or benzene. Thus, the molecules may also be better antiradicals in water than they are in either benzene or in the gas phase. To analyze the solvent effects on the anthocyanins (+), the DAM for these molecules is presented in Figure 6. As is evident, in this case the solvent effects are lesser than among the neutral molecules that are also described in this paper. The donator−acceptor properties of these cations are more or less the same in gas phase, water, or benzene.

Accepting Electrons: A Possible Mechanism for Preventing Oxidative Stress? The key condition for biological antiradical systems is that they should lessen, rather than exacerbate, the effects of oxidative stress, and they should not generate toxic byproduct as a result of their function. It is well-known that this is true for vitamins C and E, which are very important

TABLE 2: I and A Values (in eV), Considering Solvent Effects for Selected Molecules[a]

	I (gas)	A (gas)	I (wt)	A (wt)	I (bz)	A(bz)
tetradecahexenal	**6.81**	**1.47**	**5.14**	**2.83**	**5.90**	**2.22**
octadecaoctenal	**6.37**	**1.76**	**4.88**	**2.94**	**5.56**	**2.41**
peonidin	5.45	0.93	3.85	2.58	4.56	1.78
pelargonidin	5.49	0.91	3.83	2.55	4.56	1.77
petunidin	5.38	0.97	3.87	2.60	4.54	1.85
malvidin	5.38	0.94	3.87	2.61	4.55	1.80
CYANIDIN+	**10.51**	**5.22**	**6.01**	**3.84**	**8.08**	**4.57**
DELPHINIDIN+	**10.49**	**5.23**	**5.97**	**3.89**	**7.89**	**4.33**
PELARGONIDIN+	**10.74**	**5.23**	**6.10**	**3.83**	**8.24**	**4.56**

[a] Polar (water (wt)) and non-polar (benzene (bz)) solvent effects were included by using the polarisable continuum model. In this work, full geometry optimizations at B3LYP/6-311G** level for psittacofulvins and BPW91/D95V level for anthocyanins were carried out. Results for the gas phase and those using benzene and water are largely consistent. The relative order is the same using various solvents and in gas phase.

Figure 5. DAM with solvent effects. Psittacofulvins and anthocyanins located in the DAM, considering solvent effects. Polar (water (wt)) and nonpolar (benzene (bz)) were included by using the polarizable continuum model. Results for vitamins, yellow, and red carotenoids (CAR) reported previously[4] are included for comparison. Dividing lines are only indicative, to clarify the image.

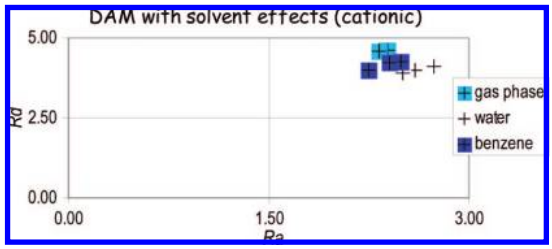

Figure 6. DAM with solvent effects for cationic anthocyanins. Anthocyanins (+1) located in the DAM considering solvent effects. Polar (water (wt)) and nonpolar (benzene (bz)) were included by using the polarizable continuum model. Dividing lines are only indicative, to clarify the image.

antioxidant substances. Our results conform to this discovery, as we concluded that vitamins represent very effective electron donors, as is also the case for anthocyanins. However, whereas solvent effects do not alter antioxidant properties (electron donor capacity) of vitamins, anthocyanins in water represent effective electron acceptors rather than effective electron donors. Hence, depending on the chemical environment, anthocyanins may be good antiradicals by means of two different mechanisms: antioxidation or antireduction.

The only experiment[9] that focuses on the antioxidant properties of psittacofulvins indicates that only octadecaoctenal is able to act as a potent inhibitor of the hydroxyl radical formation. The results of our experiments do not tally with this experimental observation. A possible explanation for this discrepancy is that the reaction mechanism is not related to electron transfer, or perhaps under other experimental conditions, the results would be very different. Furthermore, there are other free radicals that psittacofulvins may scavenge. Further experiments, taking into account the electron acceptor mechanism for scavenging free radicals, as well as more theoretical studies analyzing alternative reaction mechanisms for scavenging free radicals are imperative to define whether psittacofulvins represent good antiradicals.

We proposed in a previous work[4] that carotenoids might play an antiradical role accepting electrons from free radicals, that is, oxidizing free radicals. However, we did not realize that carotenoids were also able to remove electrons from other molecules and thus might also be toxic and not always beneficial for organisms. This result might explain why high doses of carotenoids accelerate cancer among heavy smokers.[43] It is well-known that at times, the toxicity of a given substance depends on the dose. In small amounts carotenoids can be beneficial, but in higher concentrations they can be toxic, as Young and Lowe[44] indicated. These authors found that carotenoids lose their effectiveness as radical scavengers at high concentrations, and they become toxic at even higher concentrations. An explanation of this phenomenon may be related to the oxidant (not antioxidant) properties of carotenoids. It may be that at higher concentrations, carotenoids oxidize free radicals as well as other molecules, producing toxic effects. Analyzing the other animal pigments that are described in this paper, it becomes clear that psittacofulvins and anthocyanins (in water) also represent effective electron acceptors. Following this logic, they may perhaps be toxic oxidants instead of important antiradicals. It is thus evident that further research is necessary to clarify these ideas.

In the literature concerning free-radical scavenging, the idea of scavenging free radicals by means of the electron-accepting mechanism is not very common because electron-accepting substances are oxidants (they accept electrons lost by some other molecule). It is difficult to accept that a substance that is an oxidant can employ its oxidation capacity in order to prevent further oxidation. The suggestion is that the capacity to accept electrons prevents oxidative stress, not only because of the capacity to scavenge free radicals, but also because free electrons are present that produce superoxide ($O_2^{-\bullet}$). This represents a very important reactive oxygen species that contributes to oxidative stress. The electron-acceptor molecules may capture these free electrons and thus prevent oxidative stress, even though strictly speaking, this reaction is not considered to be a mechanism for scavenging free radicals.

Taking the results reported here along with those previously published,[4,5] it is not possible to conclude whether carotenoids, psittacofulvins and anthocyanins are good antiradicals or not. The only firm conclusion is that these animal pigments (anthocyanins in water) represent effective electron acceptors. It is likely that this property has a big influence on oxidative stress, either diminishing or exacerbating it.

To understand the real value of carotenoids, anthocyanins and psittacofulvins as protective antiradicals, it is important to fully comprehend the chemistry of these molecules. The electron transfer mechanism provides important information which allows us to characterize these systems, but it is necessary to analyze the other reaction mechanisms, namely hydrogen atom transfer (HAT) and radical addition to these molecules, in order to amplify our understanding of the antiradical capacity of these animal pigments. Previous reports exist, describing the HAT mechanism, characteristic of carotenoids and anthocyanins,[5,16] but this does not apply to psittacofulvins. For this reason and in order to provide more information about the free radical scavenge machinery, work concerning these aspects continues.

Conclusions

The Donator Acceptor Map indicates that anthocyanins represent effective electron donors, whereas psittacofulvins represent effective electron acceptors. Anthocyanins and vitamins are antioxidants, whereas psittacofulvins and carotenoids are antireductants (oxidants).

Animal pigments (carotenoids, psittacofulvins and anthocyanins) are much more effective electron acceptors in water, than in either the gas phase or benzene. Solvent effects do not alter the electron donor capacity of vitamins, but anthocyanins become effective electron acceptors in water, as opposed to good electron donors. Depending on the chemical environment, anthocyanins may transfer electrons using two different mechanisms, either oxidation or reduction.

To prevent oxidative stress, substances must either donate or accept electrons. Molecules that scavenge free radicals by accepting electrons oxidize free radicals. However, they can also oxidize other molecules and hence, they may be toxic oxidants rather than important antiradicals. Possibly, this effect is related to the concentration, that is, at high concentration these substances turn out to be toxic. Taking these theoretical results, it is not possible to conclude whether these animal pigments represent good antiradicals or dangerous oxidants. However, it may definitely be concluded that carotenoids, psittacofulvins and anthocyanins (in water) are good electron acceptors. It is likely that this property has a big influence on oxidative stress, either diminishing or exacerbating it. Keeping in mind that there are free electrons that produce superoxide (a very important reactive oxygen species), the electron-acceptor molecules may capture these free electrons, preventing oxidative stress. Doubtlessly this is an area that requires further research but the results reported here may stimulate and guide further experiments.

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