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Antiradical Power of Carotenoids and Vitamin E: Testing the Hydrogen Atom Transfer Mechanism

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The antiradical capacities of 13 carotenoids (CAR) and vitamin E are explored, by assessing CAR–H bond dissociation energy. Density functional theory (DFT) calculations were performed, in order to evaluate the hydrogen atom transfer (HAT) antiradical mechanism. Results indicate that C4 or C4' is not always the reactive position when it is unsubstituted and also that CAR without H atoms in the 4 position may be as effective against free radicals as other CAR with H atoms in C4 and C4'. Lutein is the most effective antiradical for the purpose of hydrogen abstraction, whereas the least effective antiradical for this process is canthaxanthin, which is one of the reddest CAR. Vitamin E is not as effective as most of the yellow CAR but may be a better antiradical than canthaxanthin. In addition to the CAR–H bond dissociation energy, the number of reactive positions as we report in this paper represents another important aspect for consideration, when analyzing capacity for scavenging free radicals. Many additional aspects exist, which we do not consider here; thus we cannot attempt to reflect all the factors seen in vivo. However, our results provide comparative information on the relative ability of CAR to protect against free radicals, using the CAR–H bond dissociation energy, as one useful parameter. We hope that our theoretical results will contribute to the advancement of this complex research field.

Introduction

Many secondary sexual characteristics in birds and fishes are manifested in bright yellow and red coloring. It has been repeatedly demonstrated that females prefer males with larger and brighter ornaments. Where male coloration is varied, females tend to prefer red males to yellow ones.^{1–4} The pigments used to color scales, feathers, bills, or skin in red and yellow often consist of carotenoids (CAR).^{1–7} It has been proposed that these molecules as well as some vitamins are involved in a number of physiological processes, due to their antioxidant properties.^{1–31} CAR and vitamins are classed as antioxidants because these molecules scavenge free radicals, thus limiting cellular damage. Due to their capacity for scavenging free radicals, we prefer to call these substances “antiradicals”.

Three mechanisms are discussed in the literature,^{8–31} referring to the reactions of free radicals with CAR and vitamins: the electron transfer reaction, the hydrogen atom transfer (HAT) from the CAR, and radical addition to the CAR. Two other aspects of these mechanisms should also be taken into consideration: the relationship between structure and kinetics and the relationship between energetics and structure. In a previous work,¹³ we analyzed the energetic–structure relationship for the first reaction mechanism in detail, i.e. the electron transfer reaction of 13 CAR (presented in Figure 1). These molecules were identified in the feathers of the house finch (*Carpodacus mexicanus*);³ a species where the coloration of male house finches may vary from pale yellow to bright red. It was reported

that some of the CAR presented in Figure 1 confer a red hue ($\lambda_{\max} > 460$ nm) whereas others are yellow in color^{2,3,28} ($\lambda_{\max} < 460$ nm). Overall, in our previous work, we found that CAR can easily accept electrons during the charge transfer process. If the charge process of yellow and red CAR is compared to that of vitamins, it becomes evident that they are able to neutralize free radicals by accepting electrons, whereas vitamins are able to scavenge free radicals more efficiently than CAR, mainly by donating electrons. Contrastingly, vitamins appear to have a very poor capacity for accepting electrons. These results are useful for understanding the role of CAR, when acting as radical scavengers. In order to increase our knowledge about the antiradical capacity of CAR, it is important to analyze the other two reaction mechanisms which occur with free radicals. In this work, we study the relationship between energetics and structure, inherent in the hydrogen atom transfer (HAT) mechanism, in the case of the CAR presented in Figure 1.

The HAT mechanism has been studied previously by other authors.^{10,21,29–31} It has been suggested that: (i) hydrogen abstraction in CAR is from the 4 position (see Figure 2) of the cyclohexene ring and (ii) CAR without H atoms in this position are less effective against free radicals, when using this mechanism. Nevertheless, it was also reported that the ability to scavenge free radicals of CAN (with a substituted 4 position) is comparable to that of BC (which has an unsubstituted 4 position in the cyclohexene fragment), and thus, the suggestion has been that an alternative reactive mechanism must be responsible for the reactivity of CAN (either electron transfer or radical addition). Other studies exist, concerning the HAT mechanism, focusing on the antioxidant properties of several compounds.^{32–34} In the case of chalcones, Kozłowski et al.³⁴ reported that the O–H bond dissociation enthalpy parameter

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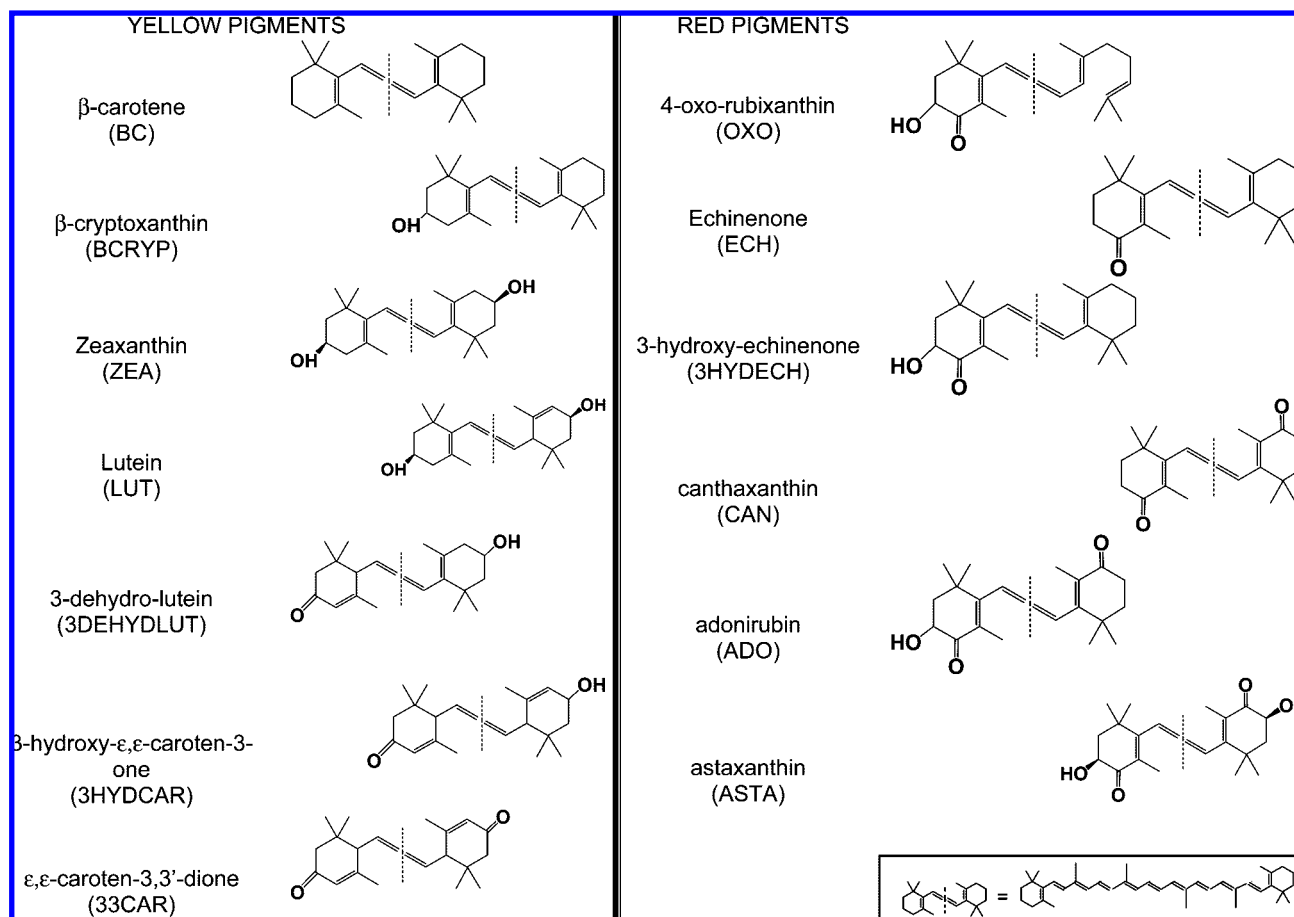


Figure 1. Carotenoid pigments: schematic representation of the molecular structure of 13 carotenoid pigments found in the feathers of the male house finch.³

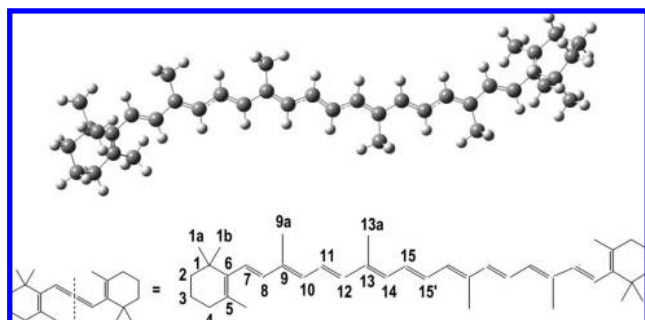


Figure 2. Scheme showing the numbers assigned to each reaction site. The optimized molecular structure of BC as an example is also shown.

seems to be the best indicator concerning the antiradical capacity of these molecules. They demonstrated with these results that the HAT mechanism is important for explaining capacity to scavenge free radicals.

In spite of the existence of previous studies about the HAT mechanism, there are no density functional investigations which analyze the capacity of CAR to carry out HAT and neither are there any concerning the relationship between H dissociation energy and the capacity of these molecules for scavenging free radicals. In order to understand the HAT mechanism inherent in CAR, it is necessary to study the H-atom abstraction for each important H atom in the molecule. The hydrogen abstraction capacity can be estimated by assessing the energy involved for the dissociation of the hydrogen atom from the CAR, thus the main goal of this work is to explore whether a correlation exists

between the dissociation energy (ΔE), involved in the removal of one hydrogen atom from the CAR and thus its capacity for scavenging free radicals. An assessment of the ΔE , relating to several hydrogen atoms in each CAR permits us to identify the hydrogen atom that can be dissociated with the lowest energetic cost, thus defining its reactive position. In this article, we report quantum chemical calculations for 13 CAR (presented in Figure 1). It was previously reported¹⁷ for CAR that several different conformers with respect to the relative orientations of the β -ionone rings are possible. However, since the energy difference between them is quite small (see ref 17), in this study we used all *s-cis* conformers, as they were reported as the most stable ones. The antiradical capacity of these molecules is analyzed in terms of ΔE . As will become apparent, a detailed analysis of the lowest hydrogen dissociation energy for each CAR suggests that the HAT mechanism is useful for explaining certain experimental results.

Computational Details

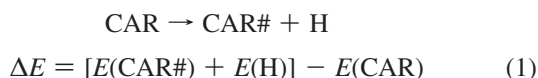
Density functional theory^{35–37} as implemented in Gaussian 03³⁸ was used for all calculations. Becke's 1988 functional, which includes the Slater exchange, along with corrections involving the gradient of the density³⁹ and Perdew and Wang's 1991 gradient-corrected correlation functional⁴⁰ were employed in the calculations of complete optimizations, without symmetry constraints. D5DV basis sets were also employed.^{41–43} Harmonic frequency analyses permitted us to verify optimized minima.

The methodology used in this work was validated, by comparing our results with those obtained using the B3LYP⁴⁴ and 6-311G* basis set.⁴⁵ These calculations correspond to the

gas phase. Solvent effects were included, using the polarizable continuum model (PCM),^{46,47} with water and benzene acting as the solvents for polar and nonpolar environments, respectively.

Optimization of geometry was applied to the radicals, beginning with the optimized structure of the parent molecule, after the H atom had been removed from all the positions at the cyclic end, from the CH₃ group and from the OH groups. In general, free radical abstraction of the hydrogen atom from the C4 position of a β -ring results in a radical with a resonance-stabilized carbon center, which is beneficial because of the delocalization of the unpaired electron over the polyene chain. The other CH₂ groups in the ring are less reactive because they are not allylic. The C=C–H bonds are very strong and do not permit H abstraction from positions along the polyene chain.

The dissociation energy (ΔE) of the H atom was calculated, as expressed in the following equation:



where CAR# denotes the CAR radical, missing one H atom. In order to validate our results, we determined ΔE for vitamin E (following the same procedure for the reaction, as that for CAR). The gas phase experimental dissociation energy for the hydrogen atom⁴⁸ is equal to 3.3 eV. Our theoretical value in the case of both methodologies (BPW91/D5DV and B3LYP/6-311G*) is 3.2 eV. Hence, our theoretical results are in good agreement with the experiment. For each CAR and CAR#, ΔE values were obtained at the BPW91/D5DV level of theory for all possible H abstractions, permitting the hydrogen atom associated with the lowest ΔE to be identified and therefore the reactive position. In order to assess the effect of the functional and the basis, we repeated the optimization of CAR and CAR#, considering the dehydrogenation of the hydrogen atom with the lowest ΔE and using B3LYP/6-311G*. In order to analyze the effects of solvents (water and benzene), single point energy calculations were performed at the BPW91/D5DV level of theory for selected molecules. These results are available in the Supporting Information.

The relative values for hydrogen dissociation energy do not depend on the functional, on the basis set, or on the solvent. As we are interested in comparing the values of ΔE for different CAR and with that for vitamin E, it is possible to apply these results with confidence.

Results and Discussion

Identification of the Reactive Position. In order to assess the antiradical capacity of CAR, we can consider the homolytic dissociation of the CAR–H bond (HAT mechanism for the reaction). This reaction can occur for each H atom in the CAR molecule and is governed by the dissociation energy (ΔE) of the H atom as expressed in eq 1. The lower the ΔE value, the easier the H abstraction and the greater potential role played by that particular CAR, as an antiradical. Before comparing the various CAR, it is necessary to identify the reactive position, that is the carbon atom associated with the lowest ΔE for each molecule. In Figure 2, we present a scheme showing the numbers assigned to each reaction site. Optimized molecular structure of BC as an example is also shown. In Figures 3 and 4, we present the ΔE values for every relevant H atom for the structures presented in Figure 1. Previous reports²⁶ indicate that hydrogen loss from the methyl group on the 1a, 1a', 1b, and 1b' positions of the cyclohexene ring is unlikely because this produces only an allyl radical that is not observed in the

experiments. Hydrogen loss from the CH and CH₃ groups of the polyene chain is unlikely since the product radical is not stabilized by resonance. In spite of these results and in order to verify these previously reported conclusions, we calculated the dehydrogenation energy of some molecules considering CH and CH₃ groups of the polyene chain. The bond dissociation energy of 9a and 13a positions is approximately 0.6 eV higher than the lowest bond dissociation energy reported in this paper. For this reason, we did not include the bond dissociation energy of these hydrogen atoms.

In Figures 3 and 4, the reactive position is indicated in bold. As is apparent, the ΔE value for the hydroxyl group is very high in comparison with other ΔE values. It is also possible to conclude that neither C4 nor C4' are always the reactive position, if they are unsubstituted. For LUT, 3DEHYDLUT, 3HYDCAR, and 33CAR, the reactive position is C6 or C6' and is related to an increment in electron delocalization, when the H atom is abstracted. In these molecules, the dissociation of the hydrogen atom increases the number of conjugated double bonds in the molecule and enhances the stability of CAR#. This explains the low ΔE value for this H atom, compared to the others. With the exception of these four CAR, the reactive position is unsubstituted C4 or C4'. For ADO and ASTA, the H atom is dissociated from the H–COH group at C3, whereas for CAN, a molecule with two C=O substituents at C4 and C4' and without H–COH chemical groups, the reactive position is a hydrogen of CH₃ at C5 or C5'. The ΔE value for CAN is the highest.

In the case of all CAR molecules, the lowest ΔE is obtained for any dehydrogenation, producing the most stable CAR# radicals due to the enhancement of the π -conjugated system. These results are not breathtaking since the reactivity of C4 and C4' was recognized before.^{20,21} It was also reported that the most stable hydrogen abstraction in CAR referred to the 4 position of the cyclohexene ring and thus CAR without H atoms in this position would be less effective against the radical, when using this mechanism. However, as can be seen in Figures 3 and 4, the hydrogen abstraction for some CAR relates to the C6, C6' position and not the C4, C4' position, in spite of the absence of a substituent in the C4 position. Moreover, ASTA with two substituents in the C4 and C4' has a similar ΔE value to BC, BCryp, and ZEA. Clearly, the influence of the substituent at the 4, 4' position modifies the reactivity of CAR, but for ASTA this is not the case, since its ΔE is the same as for other CAR with unsubstituted C4, C4' positions. This result for ASTA indicates that CAR without H atoms in the 4, 4' position may be as effective for eliminating free radicals as other CAR with H atoms in C4 and C4'.

Antiradical Power of CAR. In order to assess varying capacities for scavenging free radicals through the HAT reaction mechanism, it is important to compare the lowest ΔE values for each CAR. The results are presented in Table 1. A figure indicating the reactive position is indicated for each case. Out of this group of molecules LUT represents the best antiradical and CAN the worst; in terms of the HAT reaction mechanism.

Evidently, it is possible to divide CAR into five groups (see Table 1), depending on the reactive position. In the first one, the hydrogen abstraction derives from the carbon atom that participates in the bond between the β -ring and the polyene chain (C6, C6' position). These have the lowest ΔE and, therefore, represent the best antiradicals, in terms of the HAT reaction mechanism.

The second group consists of BC, BRYP, and ZEA. All have the same chromophore, i.e. nine conjugated double bonds of

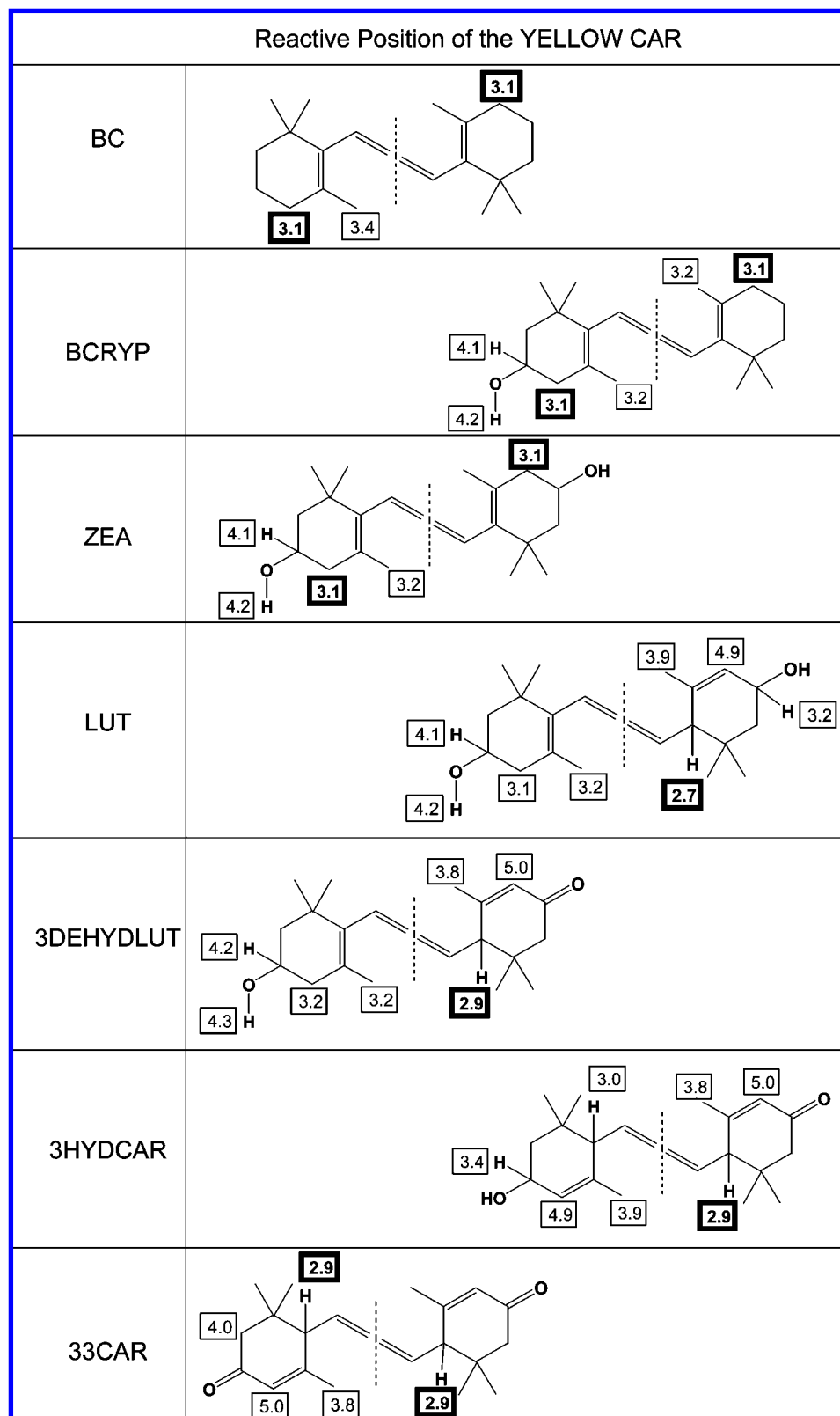


Figure 3. Reactive position of the yellow CAR: dissociation energy (ΔE in electronvolts) of every relevant H atom for the structures presented in Figure 1. Values represent the ΔE (in electronvolts) for the indicated H atom. The reactive position corresponds to the carbon atom associated with the lowest ΔE value for each molecule, and it is reported in bold.

the polyene chain, plus a contribution of two double bonds from the β -rings, even though these are not coplanar with the polyene chain. Consequently, they have similar absorption spectrum maxima. ΔE results indicate that the allylic C4, C4' position in the ring of these molecules constitutes a reactive position and

available evidence^{20,21} indicates that this position is susceptible to hydrogen abstraction on the part of free radicals.

The third group consists of ECHE, 3HYDECH, and OXO. These CAR are classified as red, since λ_{\max} is greater than 460 nm. They have substituent groups (C=O) at C4 positions, but

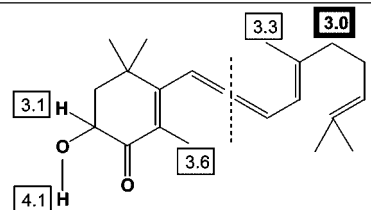
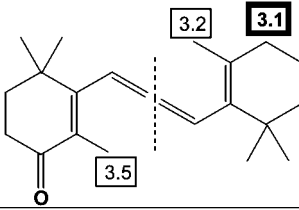
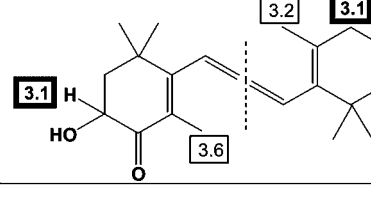
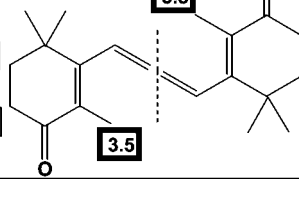
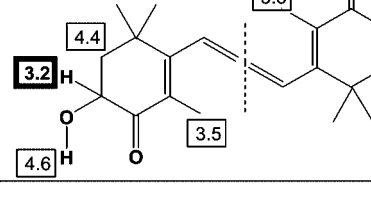
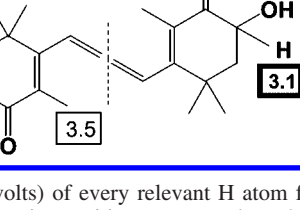
Reactive Position of the RED CAR	
OXO	
ECH	
3HYDECH	
CAN	
ADO	
ASTA	

Figure 4. Reactive position of the red CAR: dissociation energy (ΔE in electronvolts) of every relevant H atom for the structures presented in Figure 1. Values represent the ΔE (in electronvolts) for the indicated H atom. The reactive position corresponds to the carbon atom associated with the lowest ΔE value for each molecule, and it is reported in bold.

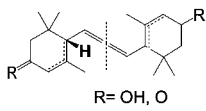
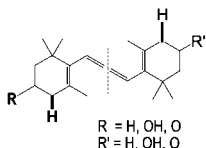
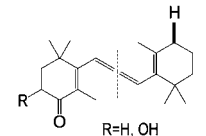
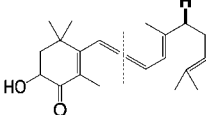
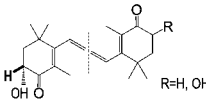
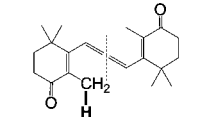
they also manifest a C4' position which is allylic to the chromophore, which lacks substituent groups and which readily undergoes dehydrogenation. The ΔE of these CAR show similar values to those of the second group and higher ones than those from the first group, in spite of their differences in color. Taking these results we can conclude that some yellow CAR represent better antiradicals, as some of them (those that belong to the first group) have lower ΔE than the red CAR.

The fourth group consists of ADO and ASTA, whereas CAN belongs to a fifth group (where the relevant H atom is bonded to a methyl group). These are the reddest CAR and are diketo compounds, with substituent groups at C4 and C4' positions. The ΔE of CAN is greater than the ΔE for other CAR, but this is not so in the case of ADO and ASTA. These are the reddest CAR but their ΔE is similar to the corresponding value for yellow CAR.

It is possible to compare the ΔE of CAR to the ΔE value for vitamin E, a well-known antioxidant substance. For vitamin E, the theoretical value for ΔE is equal to 3.2 eV. All CAR presented in Table 1, with the exception of ADO and CAN have lower ΔE values than vitamin E. This may indicate that CAR are better antiradicals than vitamin E, in terms of the HAT mechanism. However, other factors such as the solubility of CAR and vitamins and the position and orientation of the CAR in the membrane are relevant but are not considered in this analysis.

Theory versus Experiment. No direct link between our energetic values and the kinetic results previously reported is evident.^{20,21} However, it appears that we can find a correlation between ΔE and the number of reactive positions with experimental reaction rates, which will broaden knowledge concerning the reaction mechanism for scavenging free radicals. This will

TABLE 1: Lowest Dissociation Energy (ΔE in electronvolts) Values of Each CAR^a

	MOLECULE	ΔE (eV)		Reactive H atom (Indicated in bold)
		BPW91/D5DV	B3LYP/6-311G*	
Group 1	LUT	2.7	2.9	
	3DEHYDLUT	2.9	3.2	
	3HYDCAR	2.9	3.1	
	33CAR	2.9	3.1	
Group 2	BC	3.1	3.2	
	BCRYP	3.1	3.2	
	ZEA	3.1	3.2	
Group 3	ECH	3.1	3.2	
	3HYDECH	3.1	3.2	
	OXO	3.0	3.1	
Group 4	ADO	3.2	3.4	
	ASTA	3.1	3.2	
Group 5	CAN	3.5	3.5	

^a A figure indicating the reactive position is also included.

become clear in this section, as theoretical results are used to rationalize some of the available experimental information.^{20,21}

In Figures 3 and 4, it is possible to discern that CAR with two reactive positions exist (BC, BCRYP, ZEA, 33CAR, 3HYDECH, CAN, and ASTA), although others have only one reactive position. According to the number of reactive positions and the ΔE values, a similar reactive pattern is expected for BC, BCRYP, and ZEA, as these have the same ΔE and two equally reactive positions. This conforms with experimental results that report similar rates, concerning the reactivity of these three CAR with free radicals.^{20,21}

The number of reactive positions may also explain the reaction of CAR with peroxy radicals. LUT, the major CAR component in the human diet, reacts rapidly with peroxy radicals but its reactivity is rather lower than that of ZEA.²¹ LUT has lower ΔE than ZEA, but the reactive positions of the molecules are not the same. ZEA has two reactive positions, and LUT has only one. This is crucial in terms of reactivity, as there may be stereochemical impediments to accessing the free radical, which will thus affect the kinetics of the reaction. In this case, it is the number of reactive positions for the HAT mechanism which correlates with the fact that ZEA reacts faster than LUT with peroxy radicals.

It was also reported that, in spite of their longer chromophore, CAN and ASTA react with peroxy radicals much more slowly than BC. Besides this, it was shown that CAN and ASTA in the presence of an oxidation system (based on the Fenton reaction) react more slowly than BC.²¹ As the ΔE value for CAN is higher than the corresponding value for BC, a lower reactivity for the HAT reaction was to be expected. However, the ΔE of ASTA is the same as the ΔE of BC and both

molecules have two reactive positions. Therefore similar reactivity was expected. However, this is not the experimental result. In this case, the ΔE values related to the HAT mechanism and the number of reactive positions is uncorrelated with the experimental results.

It is important to remember that we are analyzing the relationship between energetics and structure, in order to explain antiradical capacity through the HAT reactive mechanism, whereas the available experimental results^{20,21} describe the relationship between structure and kinetics, assuming different reaction mechanisms. Kinetics and energetics do not necessarily give the same results. The reactions of CAR with free radicals are extremely rapid and the rate of the reaction may be dominated by several aspects in vivo that are very difficult to analyze, either experimentally or theoretically. One of them is the accessibility of the hydrogen atom that will become dissociated, as the possibility exists that small free radicals may access any part of the molecule, whereas big free radicals would probably only react with the most accessible hydrogen atoms. The chemical environment of the reactive position is also important, as CAR may be intercalated into the lipid bilayer or adsorbed by the surface of the membrane. This process is determined by the chemical characteristics of the molecule, which define whether or not the reactive positions are available for the HAT reaction.

In spite of these obstacles, we can employ ΔE values, referring to the reactive position in order to compare the reactivity of these molecules. This will provide valuable information for further studies. In order to understand the real value of CAR as protective antiradicals, it is important to fully comprehend the chemistry of these molecules. HAT mechanism and ΔE values provide important information which permits us to characterize these systems, but it is necessary to analyze the third reaction mechanism, namely radical addition to the CAR, in order to amplify our understanding of the antiradical capacity of CAR. For this reason, work concerning these aspects continues.

Conclusions

In order to analyze the antiradical capacity of CAR, we followed the homolytic dissociation of the CAR–H bond (HAT mechanism of the reaction). This reaction is governed by the ΔE of the H atom. The lower the ΔE value, the easier the H abstraction and the more important the role played by CAR as an antiradical. We identified the reactive position in all CAR, which indicates the carbon atom bounded to the H atom with the lowest Δ .

Our results indicate that C4 or C4' is not always the reactive position when it is unsubstituted and also that CAR without H atoms in the 4 position may be as effective against free radicals as other CAR with H atoms in C4 and C4'. A comparison between the ΔE of various CAR and vitamin E permits us to conclude that according to HAT, some yellow CAR are more effective radical scavengers than red ones. All CAR, except ADO and CAN have lower ΔE values than vitamin E, indicating that CAR are better antiradicals in terms of the HAT mechanism than vitamin E. The HAT reaction and ΔE values are important information that allows us to characterize these systems. ΔE values make it possible to qualitatively explain several experimental results related to the antiradical reactivity of these molecules. This information is relevant in studies examining the role of CAR as antioxidants.

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Supporting Information Available: Calculations of bond dissociation energies, considering solvent effects (water and benzene). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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