A Theoretical Study of the Reaction of *β*-Carotene with the Nitrogen Dioxide Radical in Solution

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A theoretical study of the reaction of *β*-carotene (BC) with the nitrogen dioxide radical (NO$_2$$^•$) in solution is carried out using the density functional theory (DFT) at the B3LYP/6-31G(d) level, to optimize the molecular geometries, and the polarizable continuum model (PCM), to account for solvent effects. The three most important reaction mechanisms—electron transfer from *β*-carotene to the radical, hydrogen abstraction by the radical, and radical addition to form an adduct—are studied in detail. Three solvents with different polarities—heptane, methanol, and water—are employed to investigate the effect of the environment on the reaction mechanisms. Our results show that electron transfer is thermodynamically favored only in the polar solvents, the abstraction reactions are spontaneous in the three solvents, although faster in the polar ones, and the addition reactions are all endergonic and, therefore, unlikely to occur in any of the solvents. In both the abstraction and addition mechanisms, the attack of the radical takes place preferentially at the *β*-ionone rings, in particular at positions H4 and C5, respectively. The higher stability of the reaction products in these cases is explained in terms of their molecular geometries and electronic structures.

I. Introduction

In recent years, epidemiological evidence has been reported that supports a protective effect of carotenoids in the development of chronic diseases, especially cardiovascular ones, and in cancer.[1−7] These diseases seem to have their origin in the oxidative damage that biological tissues suffer due to the action of free radicals,[8] and for this reason, it was suggested that antioxidants like *β*-carotene may play an important role in their prevention.[9−16] Against this evidence, however, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial[17] published in 1994 concluded, unexpectedly, that a supplement of *β*-carotene in the smokers diet not only does not decrease the incidence of lung cancer but raises it. This conclusion was later reinforced by other studies indicating that smokers should avoid a high intake of *β*-carotene in order to prevent this type of diseases.[18−24] All of this has led to a re-examination of the antioxidant activity of carotenoids,[25−39] with special focus on the interaction mechanisms of these species with the components of cigarette smoke, of which the nitrogen dioxide radical NO$_2$$^•$ is a major compound.

Among the different mechanisms existing to scavenge free radicals (R$^•$),[40] the most important for carotenoids (CAR) are electron transfer, hydrogen atom abstraction by the radical, and radical addition to the carotenoid to form an adduct.[26,27,31,33,35−37] These mechanisms can be written schematically as follows:

Electron transfer: $CAR + R^• \Rightarrow CAR^{•+} + R^-$ (I)

Hydrogen abstraction:
$$CAR + R^• \Rightarrow CAR(-Hx)^• + RH$$ (II)
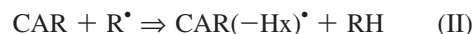
Radical addition:
$$CAR + R^• \Rightarrow [CAR(Cx)-R]^•$$ (III)

where Hx stands for the abstracted hydrogen atom and Cx for the carbon atom to which the radical adds to form the adduct. The relative importance of these mechanisms will depend both on the specific chemical properties of the reactants, the carotenoid and the radical, and on the polarity of the solvent in which the reaction takes place.

The reaction of *β*-carotene (BC) with NO$_2$$^•$ in particular has been studied experimentally in different environments,[28,29,32,39,41−48] with no definitive conclusions being reached about the influence of the solvent on the reaction mechanisms. Böhm et al.[41,42] have given experimental evidence of the formation of both carotenoid radical cations and adduct products in the reaction of NO$_2$ generated photochemically with *β*-carotene in water solution. In a pulse radiolysis study, Everett and co-workers[43,44] have shown, in turn, that nitrogen dioxide reacts exclusively by electron transfer with *β*-carotene in a tertbutyl alcohol/water mixture to produce the radical cation. This experimental finding was confirmed quite recently in another pulse radiolysis study by Zhao el al,[48] which clearly excludes the formation of carotenoid addition radicals

Kikugawa et al.,[45] on the other hand, have observed that *β*-carotene scavenges NO$_2$$^•$ in hexane very effectively, with no significant release of HNO$_2$ or NO. This could be indicative of

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the formation of a radical adduct, despite the fact that the reaction products were not characterized in this work. Khopde et al.[46,47] have studied the direct reaction of NO$_2^{\bullet}$ with β-carotene in acetonitrile and chloroform by absorption spectrometry, finding evidence which also supports the formation of addition products. Liebler and co-workers[28,29] have investigated the oxidation of β-carotene by cigarette smoke in gas phase and in toluene, identifying 4-nitro-β-carotene as a major reaction product. Moreover, the authors proposed a reaction mechanism for the formation of this product in which a molecule of β-carotene reacts first by electron transfer with a molecule of NO$_2$, or with peroxyl radicals, to generate the β-carotene cation radical, which, after deprotonation, reacts with a second molecule of NO$_2$ to yield the nitro product.[28] In another study, by Rahman et al.,[32] on the degradation of β-carotene by cigarette smoke in hexane at a temperature of 20 °C, the release of 4-nitro-β-carotene could not be confirmed. However, Lowe et al. [39] quite recently reported the formation of this nitro product in hexane when the reaction is conducted at the lower temperature of −20 °C, with the C4(4′) position of the terminal rings of β-carotene playing a key role in the reaction.

From the theoretical side, a number of studies have been recently reported on the reactions of carotenoids with free radicals within the framework of the density functional theory.[49−52] Specifically, Gao et al.,[49] using different density functionals and basis sets, have studied the structure and energetics of the β-carotene radical cation and different deprotonated neutral radicals, both in the gas phase and in a polar water environment. They conclude that deprotonation of the methyl group at the double bond of the terminal β-ionone ring of the radical cation gives the most stable neutral radical, due to the retention of the π conjugated system, with no significant effects being caused by the polar environment. Galano[50] has analyzed the relative antioxidant capability by electron transfer of a large series of carotenoids against various free radicals, including NO$_2^{\bullet}$, using the B3LYP hybrid density functional. In this study, the influence of the environment is accounted for by employing water and benzene as polar and nonpolar solvents. This work shows that the oxidation of β-carotene by NO$_2^{\bullet}$ is exergonic in the polar water solvent but becomes highly endergonic in nonpolar benzene, in agreement with the experimental observations. The antiradical capacity of β-carotene by electron donating was later confirmed by Martinez et al. [51] in a DFT study on the donator acceptor properties of carotenoids, melatonin, and vitamins, based on the calculation of ionization energies, electronic affinities, and other related quantities. In a subsequent work, Martinez et al.[52] have extended their theoretical study on the antiradical power of carotenoids to the hydrogen atom transfer mechanism by computing the CAR-H dissociation energies, reaching the conclusion that, for the specific case of β-carotene, the C4 or C4′ positions of the rings are the most reactive for hydrogen abstraction.

In this work, we carry out a theoretical study of the reaction of β-carotene with the NO$_2^{\bullet}$ radical in solution, using the DFT method at the B3LYP/6-31G(d) level of theory to determine the structure and energetics of the different species involved in the reaction, and the polarizable continuum model (PCM) to account for the effect of the solvent. The three viable reaction mechanisms—electron transfer, hydrogen abstraction, and radical addition—are considered, and all possible reaction pathways are investigated in detail. Water, methanol, and heptane are used as solvents to mimic different polar environments. The paper is organized as follows. In section II, we give the details of the computational methodology employed, section III is devoted

TABLE 1: Gibbs Free Energies (in kcal/mol) at 298.15 K for the Electron Transfer Reaction of β-Carotene with NO$_2^{\bullet}$ in Heptane, Methanol, and Water Solutions

solvent	$\Delta G_{BC \rightarrow BC^{+\bullet}}$	$\Delta G_{NO_2^{\bullet} \rightarrow NO_2^-}$	ΔG^0
heptane	114.83	−85.37	29.46
methanol	103.59	−118.57	−14.98
water	103.06	−119.75	−16.69

to the discussion of the results obtained for each reaction mechanism, and conclusions are given in section IV.

II. Computational Methodology

All of the geometry optimizations were carried out in the gas phase using the B3LYP functional[53−55] in combination with the 6-31G(d) basis set, since this level of theory has been shown to provide good results for the geometry and vibrational spectrum of β-carotene and its isomers.[56,57] The harmonic vibrational frequencies of the reaction complexes were calculated at the same level of theory, and used first to determine whether the optimized structures are true minima or transition states (TSs) and later to evaluate the contributions to the Gibbs free energy at 298.15 K. In order to verify that the transition states connect the reactants with the appropriate products, we calculated the intrinsic reaction coordinates (IRC) using the algorithm developed by Gonzalez and Schlegel.[58,59] The spin densities were in turn obtained using the standard Mulliken population analysis.

To account for the solvent effects, the polarizable continuum model (PCM) developed by Tomassi and co-workers[60,61] was used, with the energies being improved by performing single point calculations at the higher B3LYP/6-311++G(d,p) level of theory on the optimized structures. Heptane, methanol, and water were chosen as solvents to represent low, medium, and high polar environments, with the dielectric constants being 1.92, 32.63, and 78.39, respectively. We should note that the methodology employed in this work closely follows that used by Matxain et al.[62−64] to study the antioxidant properties of the B$_6$ vitamin. All calculations were performed with the Gaussian 03 program package.[65]

III. Results and Discussion

As previously mentioned, the NO$_2^{\bullet}$ radical can react with the molecule of β-carotene basically through three distinct mechanisms—electron transfer, hydrogen abstraction, and radical addition. We have studied the three mechanisms in the solvents proposed using the theoretical methodology described above. The results obtained for each mechanism are presented and discussed below.

A. Electron Transfer. The electron transfer reaction determines, in principle, the antioxidant capability of β-carotene.[41−44,48,50,51] In order to investigate the viability of this reaction with NO$_2^{\bullet}$ in solution, we have calculated the corresponding changes in the Gibbs free energy at 298.15 K for the three solvents. The ΔG^0 values obtained are given in Table 1, along with the individual contributions of the reactants. As observed, the electron transfer is thermodynamically favored in the two polar solvents, methanol and water, with negative values of $\Delta G^0 = -14.98$ and -16.69 kcal/mol, respectively, and is unfavored in the nonpolar solvent of heptane with $\Delta G^0 = 29.48$ kcal/mol. This agrees with the experimental observations by Bohm et al.[41,42] and by Everett et al.[43,44] that β-carotene reacts with NO$_2^{\bullet}$ through electron transfer in the polar solvents of water and a tertbutyl alcohol/water mixture, respectively. The ΔG^0 value calculated
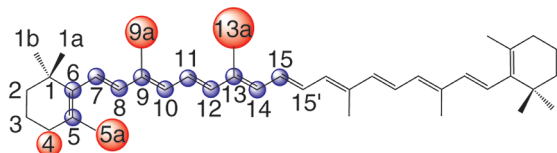
Figure 1. Molecular structure of β-carotene. The carbon atoms bonded to allylic hydrogens that can be abstracted by the radical are labeled in red, and the carbon atoms which can be attacked by the radical to form an adduct are labeled in blue.

in this work in water solution is also in good agreement with that of -19.28 kcal/mol computed by Galano[50] at a similar level of theory but using a continuum-structured solvent model which includes two molecules of water. The reason which explains why the electron transfer from β-carotene to $NO_2^\bullet$ is favored in the polar solvents lies fundamentally in the greater stabilization achieved by the radical when it converts into its anionic form in polar environments. According to our calculations (see Table 1), the NO_2^- anion is ~ 30 kcal/mol more stable in the polar solvents than in the nonpolar one, which eventually tips the balance in favor of negative, exergonic, values of ΔG^0.

Our calculations also confirm that the loss of the electron in β-carotene modifies the geometry of the molecule, in keeping with the results obtained by Gao et al.[49] in their DFT study of the β-carotene cation radical. One of the main changes concerns the spatial orientation of the β-ionone rings, which undergo an important twist toward the central polyene plane. The twist angle of the terminal rings reduces specifically from $47.2°$ in neutral β-carotene to $34.0°$ in its cationic form, with the latter value also being in close agreement with that calculated by Gao et al. The cation radical thus stabilizes by partial extension of the central polyene conjugated system into the double bonds of the β-ionone rings.

B. Hydrogen Abstraction. The hydrogen abstraction and radical addition reactions of β-carotene with $NO_2^\bullet$ follow the general pattern of reactions of alquenes with radicals, according to which the radical may either abstract an allylic hydrogen or add to one of the double bonds of the molecule to form an adduct.[66] In Figure 1, we show all the possible reactive sites of the molecule of β-carotene, labeling in red the carbon atoms bonded to allylic hydrogens that can be transferred to the $NO_2^\bullet$ radical and in blue the carbon atoms to which the radical may add. The hydrogen atoms belonging to the C1a and C1b methyl groups are not marked, since they are not in allylic positions with respect to the polyene chain. Moreover, we consider only the reactivity of one-half of the molecule, up to C15, since the other half is equivalent by symmetry.

Previous experimental studies of abstraction of an allylic hydrogen of β-carotene[28,29,39] by $NO_2^\bullet$ seem to indicate that this reaction constitutes a step prior to nitration of the C4 carbon atom in the β-ionone ring, a conclusion supported by the theoretical results provided recently by Martinez et al.[52] To get more insight into this reaction mechanism, we have systematically calculated the optimized geometries and energies of the reactant, transition state (TS), and product complexes for all possible abstractions of allylic hydrogens of β-carotene—H4, H5a, H9a, and H13a (see Figure 1)—and their corresponding Gibbs free energies in solution. The results are given in Table 2, which also contains the distances between the atoms involved in the abstractions, and the imaginary frequencies of the TSs, which are basically associated with the stretching C–Hx bonds. All of the abstraction reactions are exergonic, with the abstraction of the H4 hydrogen being the most thermodynamically favored in the three solvents. The abstraction reactions are more

likely to occur in the polar solvents, although the influence of the environment is, in this case, less accentuated than in the electron transfer reaction (see Table 1). The analysis of the energetics of the transition states also reveals that the abstraction of the H4 hydrogen is the one which has the lowest activation free energy, so this reaction is expected to be the most kinetically favored as well. For all of the hydrogen abstractions, the activation free energies are again substantially lower in the polar solvents, in a range of about $7-10$ kcal/mol, and these reactions are, accordingly, expected to be faster in polar environments.

The structure of the transition states is quite similar for all of the abstraction reactions, as demonstrated by the small differences existing in the C–Hx and O–Hx distances and the imaginary frequencies given in Table 2. There are, however, some changes in the structure of products that are worth noting. To see them clearly in Figure 2, we show the optimized geometries of the product complexes for the four possible abstraction reactions form both the normal and lateral views. As indicated in this figure, the C6C7 bond distances in the deprotonated BC(−H4) and BC(−H5b) complexes (Figure 2a and 2b) are, respectively, 1.371 and 1.367 Å, which are characteristic of double bonds, in contrast with the 1.475 Å, single-bond value of this distance in neutral β-carotene.[56] A change in the double bond pattern of these deprotonated β-carotene complexes therefore takes place that gives rise to an extra extension of the π conjugated system, thus explaining the higher stability of the products derived from abstractions of the ring hydrogens. In contrast, deprotonation of the H9a and H11a hydrogens located in the polyene chain results in a partial loss of the planarity of the chain, as observed in Figure 2c and 2d and evidenced by the values of the C7C8C9C10 and C11C12C13C14 dihedral angles also included in these figures, which accounts for the lower stability of the BC(−H9a) and BC(−H11a) abstraction products.

C. Radical Addition. In the addition mechanism, the $NO_2^\bullet$ radical may attack, in principle, any carbon atom of the double bonds of β-carotene, both in the central polyene chain and in the terminal β-ionone rings (see Figure 1). As with the abstraction mechanism, we have again systematically optimized the geometries of the reactant, transition state, and product complexes for all of the possible addition reactions in the gas phase, and, subsequently, calculated their corresponding Gibbs free energies in the three solvents. The results are given in Table 3, in which we specifically include the distances between the radical nitrogen atom and the carbon atom of β-carotene attacked by the radical, the imaginary frequencies of the transition states, basically associated with the Cx–N bond created during the adition reaction, and the activation and total Gibbs free energies in solution. We should note that all of the addition reactions proceed in one step, since no other minima associated with some possible intermediates of this reaction were found.

As shown in Table 3, the addition reactions are all endergonic in the three solvents. This is presumably due to the larger entropic factor of the radical additions, resulting from the fact that a unique product, the radical adduct, is released in this case, as opposed to the entropic factors of the electron transfer and hydrogen abstraction reactions, in which two different products are formed. The addition reactions are therefore expected to occur spontaneously only at low temperatures, thus ruling out in principle the possibility of this direct reaction mechanism occurring in biological environments where the temperature is around 298.15 K. From the specific values of the Gibbs free energies given in Table 3, we see also that the addition at the C5 position in the β-ionone ring is the least unfavored, although

TABLE 2: Optimized Geometry Data[a] and Gibbs Free Energies[b] (in kcal/mol) at 298.15 K for the Hydrogen Abstraction Reactions of β-Carotene with NO₂•

	optimized geometries						Gibbs free energies					
	reactants		transition states			products		$\Delta G^{\ddagger}$			ΔG^{0}		
Hx[c]	R_{C-Hx}	R_{O-Hx}	R_{C-Hx}	R_{O-Hx}	freq[d]	R_{C-Hx}	R_{O-Hx}	heptane	methanol	water	heptane	methanol	water
H4	1.10	2.80	1.22	1.46	1154.2i	2.27	0.98	15.56	5.95	5.30	−11.64	−16.37	−16.55
H5a	1.10	2.90	1.24	1.42	1202.7i	2.19	0.99	18.79	9.60	9.03	−7.44	−12.12	−12.31
H9a	1.10	2.77	1.27	1.38	1000.9i	2.13	0.99	17.77	10.80	10.36	−1.84	−6.44	−6.74
H13a	1.10	3.25	1.28	1.36	1159.6i	2.16	0.99	18.93	12.00	11.56	−0.04	−4.82	−4.80

[a] Distances between the carbon atom of β-carotene and the abstracted Hx hydrogen atom, R_{C-Hx}, and between the O atom of NO₂• and the Hx, R_{O-Hx}, in Å. [b] Energies, B3LYP/6-311++G(d,p); geometries and thermodynamic corrections, B3LYP/6-31G(d). [c] Hydrogen abstracted (see text). [d] Imaginary frequencies for the TSs, in cm⁻¹.
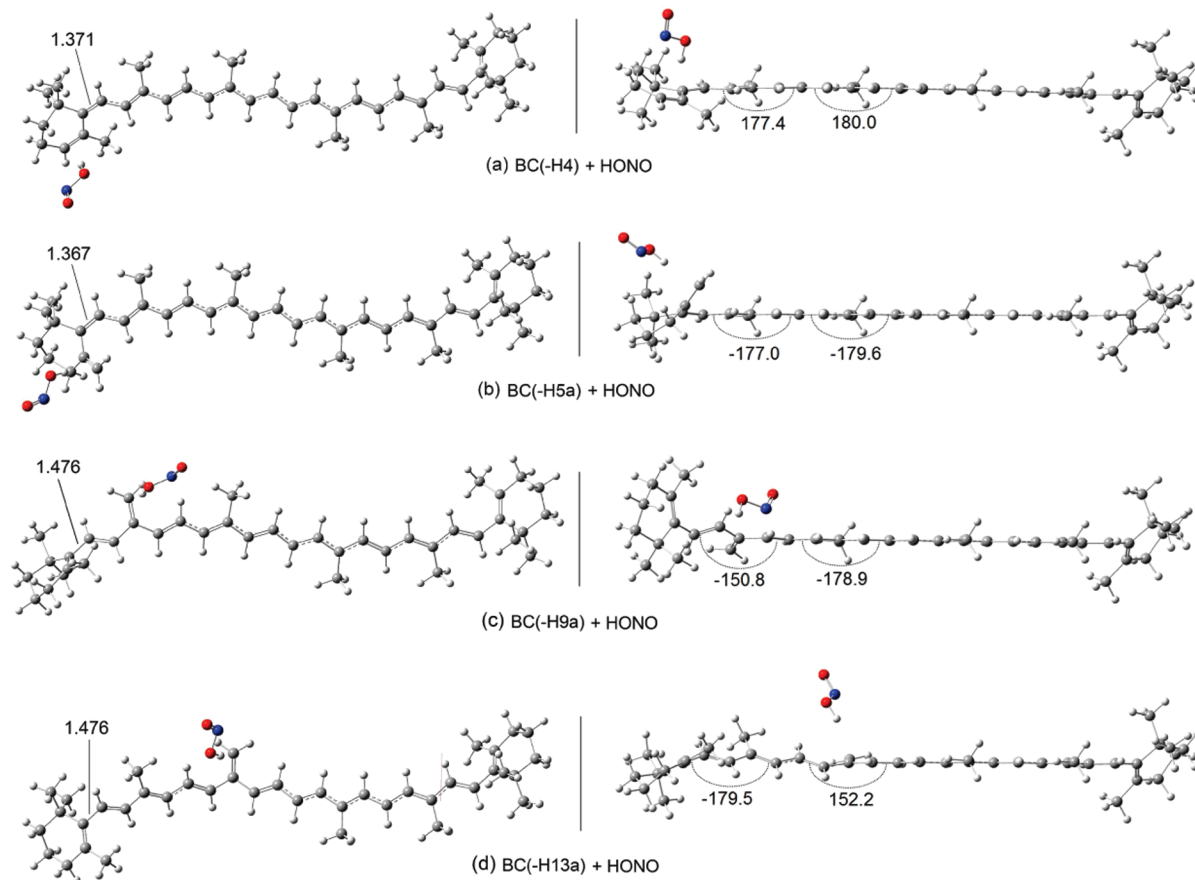

Figure 2. Optimized geometries of the four possible product complexes resulting from the hydrogen abstraction reactions of β-carotene with NO₂•. C6−C7 bond distances are given in angstroms in the normal views (left). C7C8C9C10 and C11C12C13C14 dihedral angles are given in degrees in the lateral views (right).

followed so closely in energy by the additions to the C9 and C11 positions (with differences of 2−3 kcal/mol depending on the solvent) that these three reactions can be considered equally unfavored within the accuracy limits of the DFT calculations. The addition reactions in the polar solvents, methanol and water, are less unfavored than those in the nonpolar solvent of heptane, with differences of about 2−3 kcal/mol for the Gibbs free energies, and some larger ones, of 3−5 kcal/mol, for the activation Gibbs free energies. This pattern is similar, although less pronounced, to that found in the hydrogen abstraction reactions. Nevertheless, the variations of the relative Gibbs free energies with the solvent are not large enough to change the stability order of the radical adducts.

It is also of interest to analyze the structural changes that take place in the molecule of β-carotene during the addition reactions. It is expected, first, that the addition of the NO₂• radical to any of the carbon atoms of the polyene chain (C 7 ≤

Cx ≤ C 15) will give rise to the breaking of the conjugated system, due to a partial loss of the planarity of the chain, and this is certainly what occurs, as is clearly observed in Figure 3, where the adduct resulting from the addition of the radical to the C15 carbon atom is shown. In contrast, the addition of the radical to the C5 and C6 carbon atoms in the β-ionone ring should not, in principle, have a noticeable effect on the central conjugated system, since the double bond of the ring is only partially conjugated with the polyene chain because of the ring twisting.[57] As a consequence, the attack of the radical at the double bond of the ring is expected to be favored against the attack at the double bonds of the polyene chain, and this is indeed the case for the addition reaction at the C5 position. Surprisingly, however, according to the values of the Gibbs free energies given in Table 3, the least stable radical adduct is precisely that resulting from the attack of the radical at the C6 carbon atom of the ring, with $\Delta G^{\circ} \approx 30$ kcal/mol. In order to

TABLE 3: Optimized Geometry Data[a] and Gibbs Free Energies[b] (in kcal/mol) at 298.15 K for the Radical Addition Reactions of β-Carotene with NO2·

| | optimized geometries | | | | Gibbs free energies | | | | | |
| | reactants | transition states | | products | $\Delta G^{\ddagger}$ | | | ΔG^0 | | |
Cx[c]	R_{Cx-N}	R_{Cx-N}	freq	R_{Cx-N}	heptane	methanol	water	heptane	methanol	water
C5	3.02	2.25	248.4i	1.56	12.30	9.33	9.16	3.13	1.75	1.74
C6	3.35	1.95	244.1i	1.61	31.06	29.33	29.27	30.04	28.96	28.90
C7	4.52	2.26	195.8i	1.54	10.70	7.11	6.77	7.45	6.08	5.87
C8	3.16	1.97	322.4i	1.58	19.96	17.79	17.63	17.99	16.65	16.57
C9	3.11	2.23	228.7i	1.58	9.50	6.29	6.02	5.43	4.16	4.17
C10	3.30	2.05	277.9i	1.56	14.25	11.37	11.11	10.99	9.06	8.85
C11	2.91	2.20	232.9i	1.56	10.19	6.81	6.47	4.63	3.32	3.24
C12	2.92	2.09	282.9i	1.56	13.38	10.15	9.89	9.83	8.11	7.91
C13	3.10	2.19	241.3i	1.56	10.44	6.83	6.52	8.81	7.65	7.55
C14	3.29	2.14	246.4i	1.56	13.37	10.02	9.67	8.29	6.55	6.44
C15	3.06	2.15	219.3i	1.56	10.68	7.45	7.16	7.02	5.54	5.36

[a] Distances between the N atom of NO2· and the Cx carbon atom of β-carotene, R_{Cx-N}, in Å. [b] Energies, B3LYP/6-311++G(d,p); geometries and thermodynamic corrections, B3LYP/6-31G(d,p). [c] β-Carotene carbon atom attacked by the NO2 radical (see Figure 1).
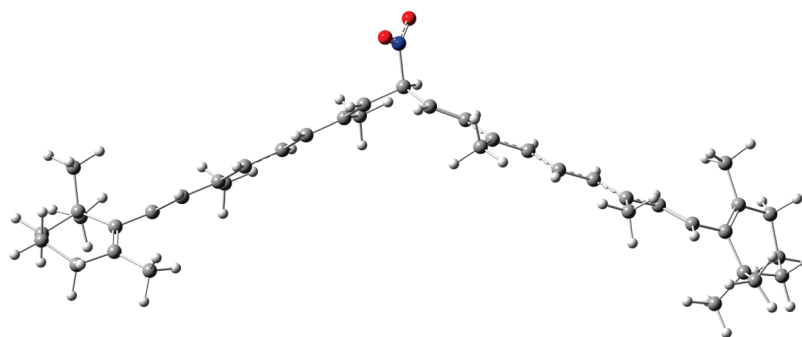
Figure 3. Optimized geometry of the radical adduct arising from the addition of NO2· to β-carotene at the C15 position.
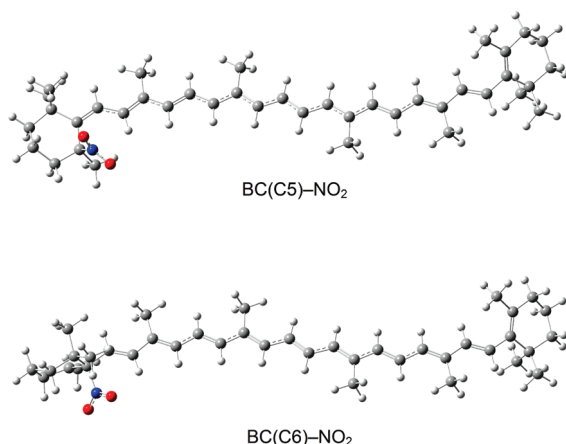
BC(C5)–NO2

BC(C6)–NO2

Figure 4. Optimized geometries of the most and the least stable radical adducts, BC(C5)−NO2 and BC(C6)−NO2, respectively, arising from the direct addition of NO2· to β-carotene.

explain this contradictory result, we show in Figure 4 the optimized geometries of the two adducts, BC(C5)−NO2 and BC(C6)−NO2. We see then that the addition of the radical to the C5═C6 double bond substantially modifies the orientation of the ring with respect to the polyene chain in both cases but with quite different consequences. In the BC(C5)−NO2 adduct, the bond formed with the radical shifts the 5a methyl group out of the polyene chain plain. The C5C6 bond then becomes a single bond and tries to settle in the plane of the polyene chain, as evidenced by the change of the C5C6C7C8 dihedral angle, whose value goes from 47.2° in the free β-carotene molecule to 6.2° in the BC(C5)−NO2 adduct. In the BC(C6)−NO2 adduct, however, the formation of the bond

between the radical and the C6 carbon atom generates a ring with a cyclohexane radical-type structure, which precludes the participation of the polyene chain in the delocalization of the unpaired electron.

In order to consider in more detail the role that the polyene chain plays in the stabilization of the BC(C5)−NO2 and BC(C6)−NO2 adducts, in Figure 5, we plot the carbon−carbon bond lengths and the atomic spin densities of the two adducts, excluding the methylic carbons which do not contribute to the stabilization of the adducts because their spin densities are negligible. It is clearly observed in this figure that the geometries and the electronic structures of the two adducts are completely different. In the BC(C5)−NO2 adduct (Figure 5a), the unpaired electron delocalizes along the polyene chain in such a way that none of the carbon atoms of the chain supports an atomic spin density higher than 0.29, which is the maximum on the C15′ carbon atom. The stability of the BC(C5)−NO2 adduct is, therefore, due to the fact that the entire polyene chain becomes involved in the delocalization of the unpaired electron. This delocalization gives rise, in turn, to some changes in the single and double bonds which are closer to the rings, in such a way that new double bonds C6═C7, C8═C9, and C10═C11, which were single bonds in β-carotene (see Figure 1), are formed in the radical adduct. Moreover, the conjugation degree in the central part of the chain becomes larger than at the ends of the chain. The central bond C15′−C14′ has, for instance, the same length, 1.40 Å, as the C14′−C13′ and C15−C15′ bonds, while the lengths of the C6═C7 (1.37 Å) and C7−C8 (1.44 Å) bonds located at the end of the polyene chain differ noticeably in 0.07 Å.

As far as the BC(C6)−NO2 adduct is concerned, Figure 5b shows that in this case there is no stabilization by resonance,
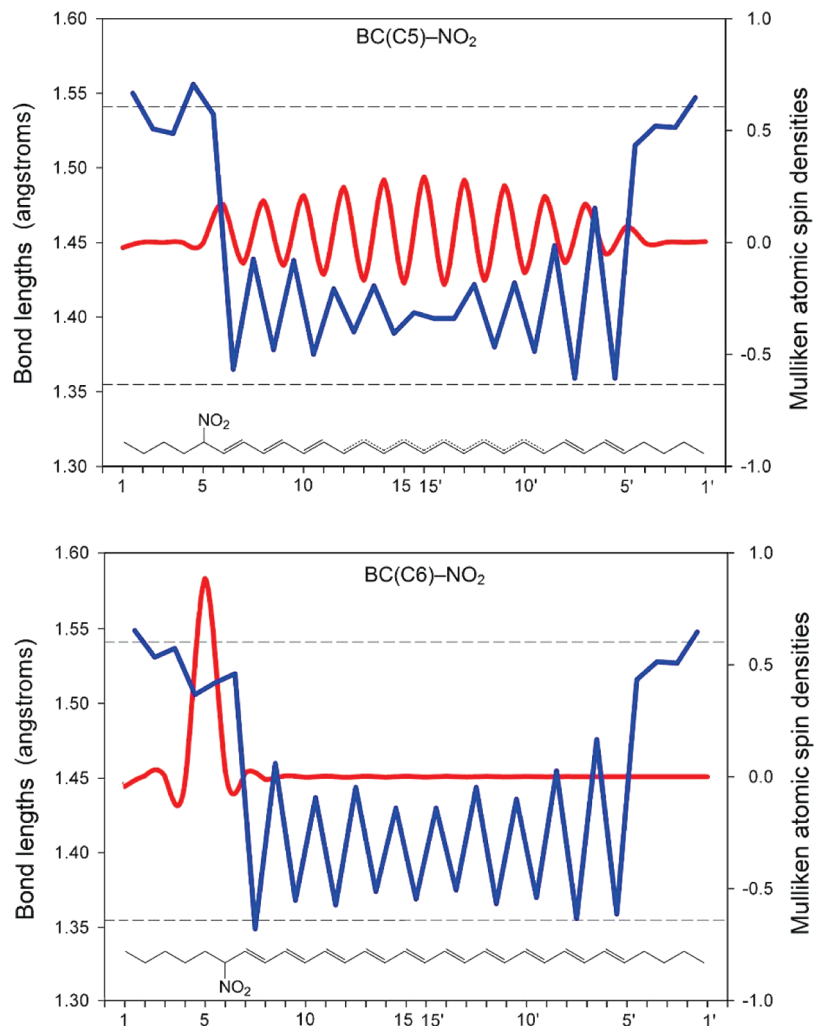
Figure 5. CC bond lengths (blue line) and atomic spin densities (red line) for the BC(C5)−NO$_2$ and BC(C6)−NO$_2$ radical adducts, including simplified schemes of the molecular structures of the adducts. The dashed lines correspond to typical values of single and double CC bond lengths, 1.540 and 1.355 Å, respectively.

TABLE 4: Gibbs Free Energies (in kcal/mol) at 298.15 K for the Most Favored Reactions of β-Carotene with NO$_2$$^\bullet$, in Heptane, Methanol, and Water Solutions

mechanism	products	ΔG^0		
		heptane	methanol	water
electron transfer	Car$^{\bullet+}$ + NO$_2$$^-$	29.46	−14.98	−16.69
hydrogen abstraction	BC(−H4)$^\bullet$ + HONO	−11.64	−16.37	−16.55
radical addition	[BC(C5)−NO$_2$]$^\bullet$	3.13	1.75	1.74

since the polyene chain does not participate in the delocalization of the unpaired electron, as evidenced by the fact that the spin densities on the carbon atoms of the central part of the chain all vanish. Consequently, the unpaired electron delocalizes almost exclusively on the C5 carbon atom, whose spin density is 0.89, and no significant differences in the polyene conjugation degree of the radical adduct are found with respect to the conjugation degree of the free β-carotene molecule.[57]

IV. Conclusions

Density functional theory has been used to study the reaction of β-carotene with the NO$_2$$^\bullet$ radical in solution. The results obtained are summarized in Table 4. According to these, electron transfer is thermodynamically favored at room temperature only in the polar solvents. Hydrogen abstractions are favored in the three environments, although more in the polar ones, and radical additions are all endergonic and therefore unlikely to occur in

any of the solvents. In addition, the abstraction of the H4 hydrogen of the β-ionone ring occurs preferentially in the three solvents, whereas the addition of the radical to the C5 carbon, also in the ring, gives the most stable radical adduct. The higher reactivity of these ring sites is due, in both cases, to an increase of the conjugation degree of the polyene chain, which results in turn in an extra stabilization of the products formed. The addition mechanism has, furthermore, the peculiarity that both the most and the least stable adducts, BC(C5)−NO$_2$ and BC(C6)−NO$_2$, respectively, arise from the addition of the radical to the β-ionone double bond. The stability of the BC(C5)−NO$_2$ radical adduct is due to the combination of two effects, the stabilization by resonance of the unpaired electron, which does not occur in the BC(C6)−NO$_2$ adduct, and the partial extension of the planarity of the conjugated polyene chain into the end groups by proper twisting of the β-ionone rings.

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In nonpolar solvents, like heptane, the reaction of β-carotene with $NO_2^\bullet$ is expected to occur spontaneously only through the hydrogen abstraction mechanism. This therefore supports, on theoretical grounds, the explanation that the experimental observation of 4-nitro-β-carotene as an oxidation product of degradation of β-carotene by cigarette smoke in nonpolar environments[28,29,39] is due to an indirect mechanism involving abstraction of the H4 hydrogen, which is the most favored in nonpolar solvents. Further work in this line on the oxidation mechanism of β-carotene by $NO_2^\bullet$ is in progress in our group.

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