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Density Functional Theory Study of the Stability and Vibrational Spectra of the *β*-Carotene Isomers

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A density functional theory analysis of the stability and vibrational spectra of the *β*-carotene isomers is carried out. The study includes the 7-, 9-, 11-, 13-, and 15-*monocis* isomers and the 7,13′-, 9,13-, 9,13′-, 9,15-, 11,11′-, and 13,15-*dicis* isomers. The optimized geometries needed to study the stability of the isomers are calculated at the B3LYP/6-31G(d) level of theory, and their energies are further recalculated at the higher B3LYP/6-311+G(2d,2p) level. In addition, the Wiberg bond orders and the natural bond orbital charges of the isomers are computed study the effect of the torsion of the *β*-ionone rings on the conjugation degree of the polyene chain. The infrared and Raman spectra of the *β*-carotene isomers are then calculated at the B3LYP/6-31G(d) level, scaling the calculated frequencies with an overall factor to account for the anharmonicity effects. The calculated frequencies are shown to compare quite well with the experiment, and the normal modes of the key bands are theoretically interpreted.

I. Introduction

Carotenoids are the most abundant group of natural pigments, and they play an important role in protection from oxidation damage.[1−8] Among the more than 600 carotenoids identified so far, 50 have pro-vitamin A activity due, in part, to the presence of at least one unsubstituted *β*-ionone ring, which is a prerequisite for this important biological property.[9] Since *β*-carotene has two *β*-ionone rings, this carotenoid is considered to be one of the most important vitamin A precursors.

Although carotenoids are usually present in nature in their most stable forms, with all the double bonds in the polyene chain having an *all-trans* configuration, there are numerous processes that may lead to *cis* isomerization, such as radiation of solutions or heating in food processing.[10−15] As far as *β*-carotene is concerned, experimental evidence has been given for the existence of the 7-, 9-, 11-, 13-, and 15-*monocis* isomers and different *dicis* isomers, including the 7,13′-, 9,13-, 9,13′-, 9,15-, 11,11′-, and 13,15-*dicis* ones.[11,16−22] It has also been suggested that all these stereoisomers display different antioxidant capacities.[9,18,23,24]

From the theoretical point of view, DFT methods have recently been employed to study the properties of the most stable, and naturally abundant, *all-trans*,*s-cis* isomer of *β*-carotene.[22,25−30] In particular, we have recently shown[29] that the B3LYP hybrid functional[31,32] in combination with the 6-31G(d) basis set reproduces the equilibrium geometry and the IR and Raman vibrational spectra of this isomer quite well. Also, Schlüker et al.[22] have reported a computational study on the structural, energetic, and spectroscopic vibrational characteristics of the *all-trans*,*s-cis* and *all-trans*,*s-trans* conformers at the BPW91/6-31G(d) level, and Liu et al.[30] have recently analyzed the effect of the *β*-ionone ring rotation on the structures and vibrational spectra of these isomers at the same BPW91/6-31G(d) level of theory.

As far as the *cis*-*β*-carotene isomers are concerned, Doering et al.[11] have determined the ground-state energies of the *monocis* isomers using molecular mechanics (MM2), and Hu et al.[21] have provided optimized geometries of the *monocis* and the 11,11′-*dicis* isomers, again from MM2 calculations. At a higher level of theory, Qiu et al.[33] have reported very recently a DFT study of the structure and energetics of the *monocis* and *dicis* isomers of *β*-carotene at the B3LYP/6-31G(d) level, and Guo et al.[34] have used the same method to study the *all-trans* to *monocis* isomerization of *β*-carotene at both the ground singlet (S_0) and triplet (T_1) states.

In this investigation we carry out a theoretical DFT study of the stability and vibrational spectra of the isomers of *β*-carotene, including the 7-, 9-, 11-, 13-, and 15-*monocis* and the 7,13′-, 9,13-, 9,13′-, 9,15-, 11,11′-, and 13,15-*dicis* isomers, for all of which experimental spectroscopic information is available.[17,18,20,21] The ab initio calculations of the optimized geometries and infrared (IR) and Raman spectroscopies are performed at the B3LYP/6-31G(d) level of theory, and a recalculation of the relative energies of the isomers at the higher B3LYP/6-311+G(2d,2p) level, based on their previous optimized geometries, is also made. In addition, the Wiberg bond orders and the natural bond orbital (NBO) charges of the isomers are computed to analyze the effect of the torsion of the *β*-ionone rings on the conjugation degree of the polyene chain. The calculated vibrational frequencies, scaled to account for the anharmonicity effects, are shown to compare quite well with the frequencies measured spectroscopically. The main IR and Raman bands of the *β*-carotene isomers are finally interpreted on purely ab initio grounds.

II. Computational Details

To study the stability of the *β*-carotene isomers and to determine their IR and Raman spectra theoretically, it is

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necessary to have available the optimized geometries of the isomers. In accordance with our previous work on β-carotene,[29] we have calculated such geometries at the B3LYP/6-31G(d) level of theory. These calculations also include the optimization of the structures of the three *all-trans,s-cis*, *all-trans,s-trans,s′-cis*, and *all-trans,s-trans* conformers (hereafter referred to as *s-cis*, *s-trans,s′-cis*, and *s-trans*) at the same B3LYP/6-31G(d) level. In addition, we have recalculated the energies of the β-carotene isomers at the higher B3LYP/6-311+G(2d,2p) level on the basis of the previously optimized geometries to better check their relative stability, and we have determined the Wiberg bond order indices by means of the NBO analysis to study the effect of the torsion of the β-ionone rings on the stability of the isomers. For this last purpose, we have also considered the intermediate conformations that connect the three *all-trans-*β-carotene isomers by computing the potential energy surface (PES) for rotation of the β-ionone ring (see below). Due to the high number of geometries needed to generate this PES, these calculations were performed using the lower 3-21G basis set, which has been shown to be accurate enough for this type of conformational studies in carotenoids.[35]

The nature of the stationary points of the isomers is characterized by vibrational calculations at the B3LYP/6-31G(d) level, which yield no imaginary frequencies for the different structures, thus indicating whether the optimized structures obtained for the isomers correspond to local minima. These vibrational calculations are used in turn to obtain the zero-point vibrational energies (ZPEs) and ultimately to generate the theoretical IR and Raman spectra. The harmonic frequencies extracted directly from the theoretical calculations are scaled to account for the anharmonicity effects. In particular, the 0.9614 scale factor proposed by Scott and Radom[36] for the B3LYP/6-31G(d) method is used for all the isomers, since this factor has been successfully employed in our previous work[29] to improve the frequencies of β-carotene.

All the calculations presented in this work were carried out using the Gaussian 03 program package,[37] and the population analysis was made using the 3.1 version of the NBO program implemented in this package.[38−40]

III. Results and Discussion

A. Stability of the Isomers. Since the β-ionone rings may adopt different orientations with respect to the central polyene chain, we have first analyzed in some detail the effects of the rotations of the rings on the relative stability of the three *all-trans* isomers of β-carotene. The chemical structures of these three *s-cis*, *s-trans,s′-cis*, and *s-trans* isomers are shown in Figure 1.

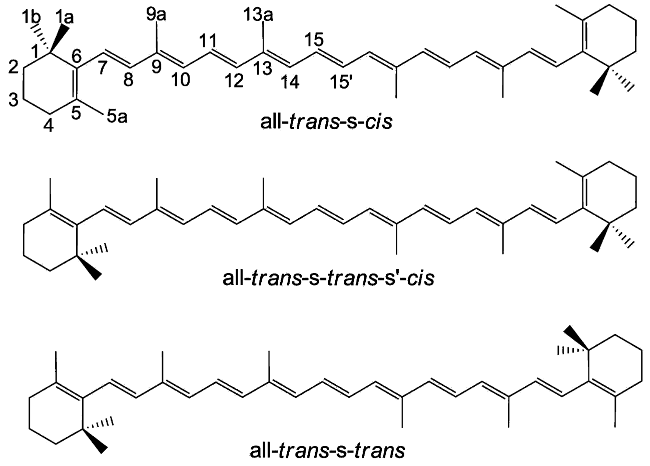

Figure 1. Molecular structures and atom numbering of the *all-trans* isomers of β-carotene.

In Table 1 we give the values of the dihedral angles C5=C6−C7=C8 (*D*) and C5′=C6′−C7′=C8′ (*D*′) of the three *all-trans* conformational isomers, together with their relative energies and the distances and Wiberg indices for the C6−C7 and C6′−C7′ bonds connecting the β-ionone ring to the polyene chain. First we note that the values of the dihedral angles for the *s-cis* (47.2°) and the *s-trans* (169.1°) isomers agree well with the angles computed by Schlüker et al.[22] at the BPW91/6-31G(d) level (42.9° and 171.9°, respectively) and that the relative energy of the *s-trans* isomer with respect to the most stable *s-cis* isomer, 2.75 kcal mol^{-1}, is somewhat higher than that provided by Schlüker et al. of 2.10 kcal mol^{-1}. In agreement with the results provided by these authors,[22] we see then that although the torsion of the rings with respect to the plane of the polyene chain results in a loss of conjugation in the *s-cis* isomer with respect to the *s-trans,s′-cis* and *s-trans* isomers, as evidenced by the increase of the C6−C7 and C6′−C7′ bond distances and the decrease of the corresponding Wiberg indices, the *s-cis* isomer continues to be the most stable one. In Figure 2 we show the potential energy surface calculated for the *all-trans* conformations of β-carotene as a function of the dihedral angles *D* and *D*′. The relative energies of this PES are calculated at the B3LYP/3-21G level, as commented above, with the dihedrals simultaneously held fixed at values varying in steps of 10° between 0° and −180° (*D*) and between 0° and +180° (*D*′) and all other geometrical parameters relaxed during the optimizations. As observed in this figure, the global minimum clearly appears at the dihedrals $D \simeq -50°$ and $D' \simeq +50°$, which correspond to the *s-cis* isomer, and any other orientation of the rings is therefore less stable. This result provides additional evidence of the higher stability of the *s-cis-*β-carotene isomer as previously explained by Schlüker et al.[22] by an energetically favored β-ionone ring conformation which compensates for the shorter conjugation length of this isomer compared with that of the *all-trans* isomer. The optimized geometries of the three *all-trans* isomers of β-carotene are displayed in Figure 3.

To study in more depth the effect of the conjugation of the polyene chain on the geometry and the electronic structure of the *s-cis-*β-carotene isomer, in Figure 4 we plot the bond distances and the NBO charges for the atoms situated in the chain (see the simplified model of β-carotene in the lower part of the figure). The variations of the bond distances shown in this figure clearly account for the presence of a conjugated system in the central part of the molecule, as evidenced by the alternation of the corresponding single and double bonds and by the decrease of the central C−C distances with respect to those of the terminal ring bonds lying outside the conjugated system. The distances marked with an asterisk correspond to the single C6−C7 and C6′−C7′ bonds, and they are larger than those of the C−C bonds in the chain, thus confirming the partial loss of conjugation previously stated. We see also in Figure 4 that the higher values of the NBO charges correspond to the carbon atoms bonded to the methyl groups. The effective electronic charge for these carbon atoms decreases to the point of becoming slightly positive for the C5 and C5′ atoms. The four carbon atoms situated at each end of the simplified model of β-carotene are atoms C1, C2, C3, and C4 and C1′, C2′, C3′, and C4′ of the rings. These atoms do not form part of the conjugate system and do not participate therefore in the charge delocalization. Accordingly, the bond distances between them

TABLE 1: Relative Energies (kcal mol^{-1}), Dihedral Angles (*D* and *D*′, deg), Bond Lengths (Å), and Wiberg Indices for the C6−C7 and C6′−C7′ Bonds for the *all-trans* Isomers of β-Carotene Calculated at the B3LYP/6-31G(d) Level of Theory

		dihedral angles		bond lengths		Wiberg indices	
isomer	Δ*E*[a]	*D*	*D*′	C6−C7	C6′−C7′	C6−C7	C6′−C7′
s-cis-β-carotene	0.00	−47.2	47.2	1.475	1.475	1.0813	1.0813
s-trans,*s*′*-cis*-β-carotene	1.42	−169.7	46.2	1.466	1.475	1.1372	1.0820
s-trans-β-carotene	2.75	−169.1	169.1	1.466	1.466	1.1368	1.1368

[a] Relative energies calculated at the B3LYP/6-311+G(2d,2p) level on B3LYP/6-31G(d) geometries.

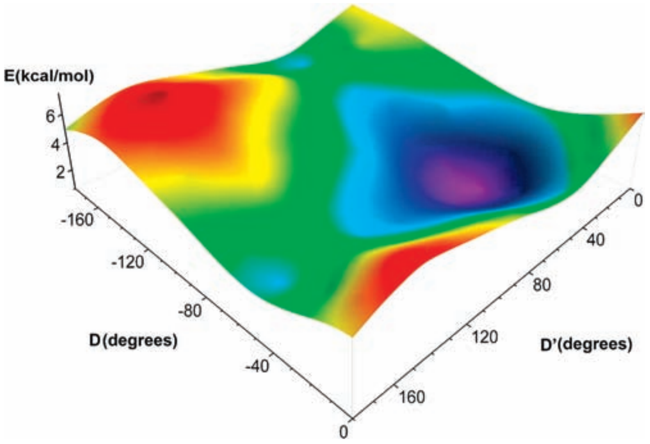

Figure 2. Potential energy surface for simultaneous rotation of the two β-ionone rings in β-carotene. Energies are given relative to the global minimum, which corresponds to the *s-cis* isomer.

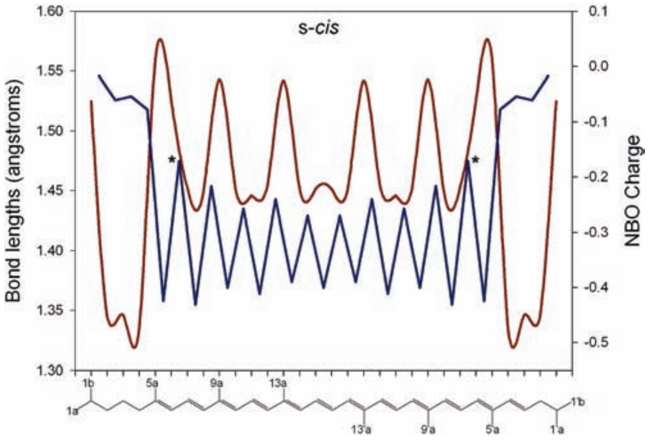

Figure 4. Bond lengths (blue line) and NBO charges (red line) calculated for the *s-cis*-β-carotene isomer based on the geometry optimized at the B3LYP/6-31G(d) level. The asterisk denotes the C6−C7 (left) and C6′−C7′ (right) bonds.

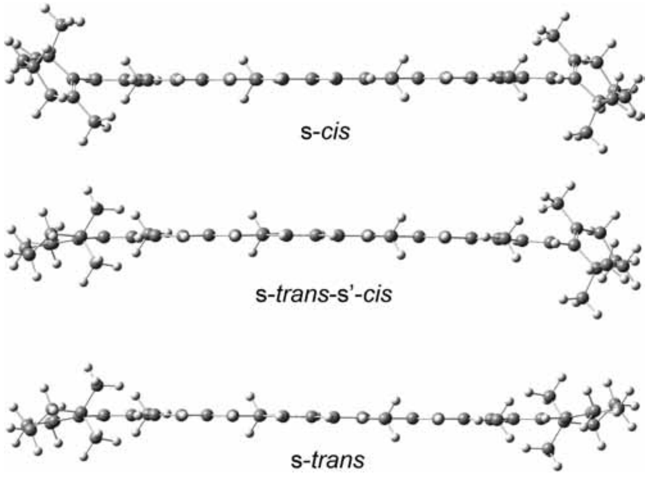

Figure 3. Optimized geometries of the *all-trans* isomers of β-carotene.

are larger than the distances between the central carbon atoms, and their charges are more negative, except for the C1 and C1′ atoms, which are bonded to methyl groups.

After characterizing the molecular structure of the *all-trans*-β-carotene isomers and determining the most stable spatial orientation of the β-ionone rings in them, we have proceeded to optimize the geometries of the *monocis* and *dicis* isomers of β-carotene at the B3LYP/6-31G(d) level of theory as exposed in section II. The molecular structures of the isomers are shown in Figure 5.

In Table 2 we give the symmetries of the *monocis* and *dicis* isomers, their relative energies, including the ZPE correction, the values of the dihedral angles *D* and *D*′, and the C6−C7 and C6′−C7′ bond lengths and Wiberg indices. As observed, the dihedral angles *D* and *D*′ take practically the same values for all the isomers ($D \approx -47°$ and $D' \approx +47°$), except for the 7-*cis* and 7,13′-*dicis* isomers, for which the dihedral angle *D* corresponding to the β-ionone ring closer to the *cis* C7═C8 double bond is larger ($D \approx -67°$). According to the structures of these two isomers shown in Figure 5, a strong steric hindrance is expected to occur between the 5a- and 9a-methyl groups, which results in a larger repulsion and an extra increase of the dihedral angle *D*. This in turn brings about a decrease of the conjugation degree as evidenced by the longer lengths (1.486 and 1.485 Å) and the lower Wiberg indices (1.0419 and 1.0442) of the C6−C7 bonds of these isomers with respect to those of the most stable *all-trans*,*s-cis* isomer (1.475 Å and 1.0813). A consequence of these changes in the structure of the 7-*cis* and 7,13′-*dicis* isomers is their destabilization, as demonstrated by their calculated relative energies of 5.41 and 6.51 kcal mol^{-1}, respectively.

A pronounced difference is also noticed in Table 2 between the energy of the *all-trans* isomer and those of the 11-*cis* and 11,11′-*dicis* isomers, with relative energies of 5.46 and 10.66 kcal mol^{-1}, respectively. This destabilization is due to the steric repulsion between the 13a-methyl group and the olefinic H10 hydrogen for the 11-*cis* isomer and to this repulsion and the equivalent one between the 13′a-methyl group with the H10′ hydrogen for the 11,11′-*dicis* isomer (see Figure 5). As a consequence, there is an increase of the bond angles C10−C11−C12 (130.7°) and C11═C12−C13 (133.1°) with respect to the corresponding angles of the *all-trans*,*s-cis* isomer, which are 122.9° and 126.7°, respectively.

The electronic energies for the different isomers included in Table 2, calculated at the higher B3LYP/6-311+G(2d,2p) level, confirm that the *all-trans*,*s-cis*-β-carotene isomer is the most stable, in accordance with previous theoretical results reported by Doering[11] and Hu[21] from molecular mechanics MM2 calculations and by Qiu et al.[33] from calculations at the B3LYP/6-31G(d) level. The predicted stability of *monocis*- and *dicis*-β-carotene isomers, as extracted from the relative energies included in Table 2, follows the order: *all-trans* > 13-*cis* ≃ 9-*cis*

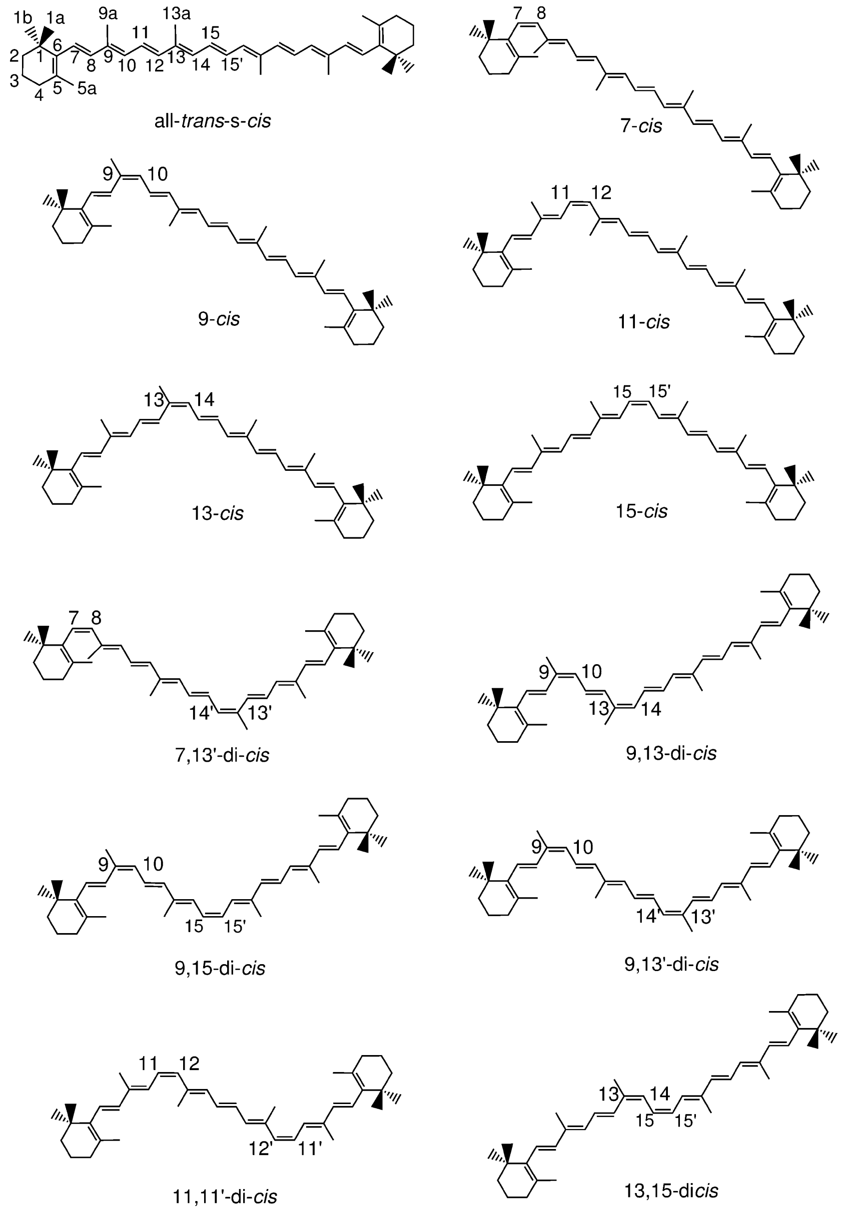

Figure 5. Molecular structures and atom numbering of the *cis* isomers of β-carotene.

TABLE 2: Total Electronic Energy (E, au), Zero-Point Vibrational Energy (ZPE, au), Relative Energy (ΔE, kcal mol^{-1}), Relative Energy Corrected for ZPE (ΔE_{corr}, kcal mol^{-1}), Dihedral Angles (D and D', deg), Bond Lengths (Å), and Wiberg Indices for the C6−C7 and C6′−C7′ Bonds for β-Carotene Isomers Calculated at the B3LYP/6-31G(d) Level of Theory

						dihedral angles		bond lengths		Wiberg indices	
isomer	symmetry	E	ZPE[a]	ΔE[b]	ΔE_{corr}	D	D'	C6−C7	C6′−C7′	C6−C7	C6′−C7′
all-trans,s-cis	C_i	−1558.3697	0.8584	0.00	0.00	−47.2	47.2	1.475	1.475	1.0813	1.0813
7-*cis*	C_1	−1558.3615	0.8588	5.14	5.41	−67.6	48.0	1.486	1.476	1.0419	1.0778
9-*cis*	C_1	−1558.3683	0.8588	0.90	1.19	−45.9	46.0	1.475	1.475	1.0819	1.0822
11-*cis*	C_1	−1558.3619	0.8593	4.90	5.46	−47.4	47.1	1.475	1.476	1.0797	1.0797
13-*cis*	C_1	−1558.3681	0.8587	1.01	1.22	−46.8	47.5	1.475	1.476	1.0804	1.0789
15-*cis*	C_s	−1558.3661	0.8590	2.28	2.69	−46.9	46.9	1.475	1.475	1.0802	1.0802
7,13′-*dicis*	C_1	−1558.3598	0.8588	6.22	6.51	−66.0	46.9	1.485	1.475	1.0442	1.0803
9,13-*dicis*	C_1	−1558.3666	0.8586	1.95	2.11	−48.0	45.6	1.476	1.475	1.0772	1.0828
9,15-*dicis*	C_1	−1558.3647	0.8589	3.17	3.53	−47.8	48.3	1.476	1.476	1.0780	1.0772
9,13′-*dicis*	C_1	−1558.3667	0.8587	1.89	2.08	−47.7	47.4	1.476	1.476	1.0780	1.0789
11,11′-*dicis*	C_i	−1558.3542	0.8599	9.73	10.66	−46.6	46.6	1.475	1.475	1.0813	1.0813
13,15-*dicis*	C_1	−1558.3644	0.8589	3.35	3.67	−47.0	47.5	1.476	1.476	1.0798	1.0787

[a] ZPE corrections are obtained at the B3LYP/6-31G(d) level. [b] Differences between potential energy minima calculated at the B3LYP/6-311+G(2d,2p) level on B3LYP/6-31G(d) geometries.

> 9,13′-*dicis* ≃ 9,13-*dicis* > 15-*cis* > 9,15-*dicis* > 13,15-*dicis* > 7-*cis* ≃ 11-*cis* > 7,13′-*dicis* > 11,11′-*dicis*, which is basically the same given by Qiu et al.[33] from the B3LYP/6-31G(d) calculations. For the *monocis* isomers, this sequence disagrees,

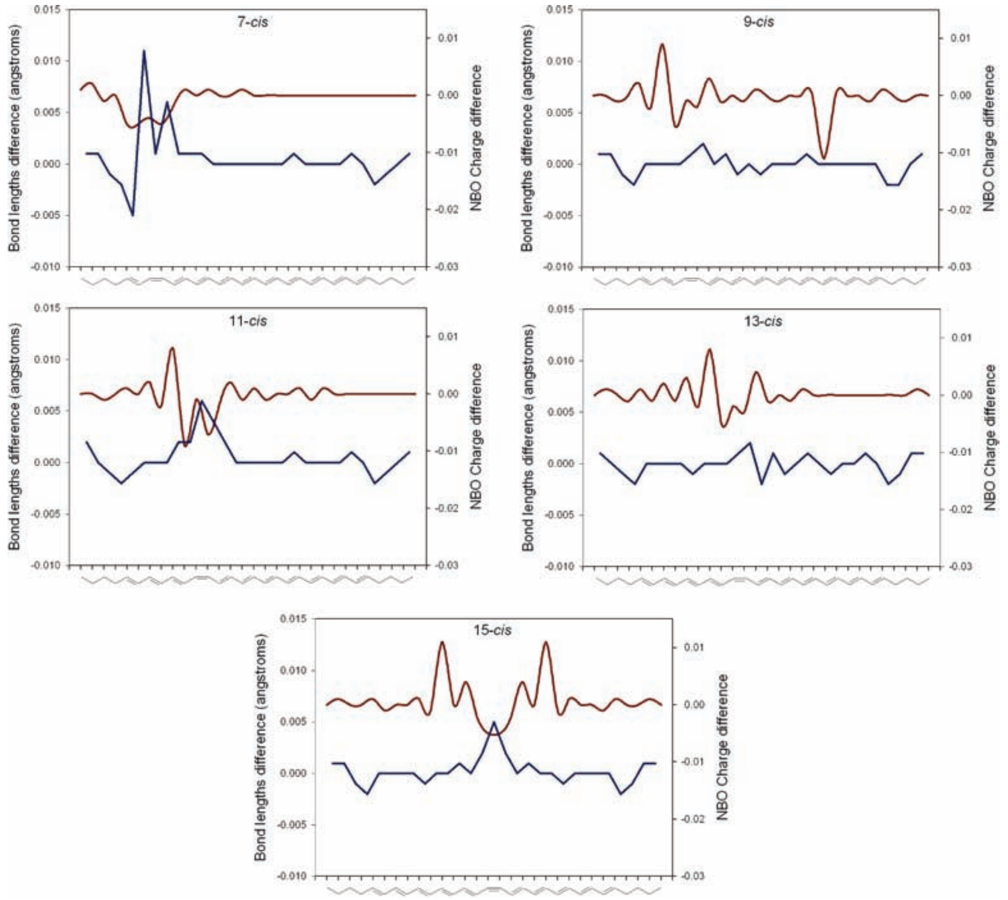

Figure 6. Differences between the bond lengths (blue line) and the NBO charges (red line) of the *monocis*-β-carotene isomers and those for the *all-trans*,*s-cis* isomer based on the optimized geometries calculated at the B3LYP/6-31G(d) level.

however, with that provided by Doering and by Hu from MM2 calculations. This is presumably due to the lower accuracy of such calculations with respect to the DFT ones. We should note at any rate that the stability order of the *monocis* isomers obtained from the DFT calculations coincides with the natural abundance of these isomers, according to which the *all-trans*,*s-cis* isomer is the most abundant, the 13-*cis* and 9-*cis* isomers are often observed, the 15-*cis* isomer is sometimes observed, and the 7-*cis* and 11-*cis* isomers are rarely observed.[23]

To see how the *cis* isomerization affects the stability of the β-carotene molecule, we show in Figures 6 and 7 the differences between the bond distances and NBO charges calculated for each isomer and those corresponding to the most stable *all-trans*,*s-cis* isomer. Although these differences are, in general, not very large, they account for certain modifications of the properties of the isomers around the *cis* bond. We notice first that the less stable 7-, 11-, and 15-*cis* and 7,13′-, 9,15-, 13,15-, and 11,11′-*dicis* isomers are precisely those for which the deviations in the distances and charges with respect to the values of the *all-trans*,*s-cis* isomer are larger. For the 7-*cis* and 7,13′-*dicis* isomers, these differences clearly show the effects of the extra torsion of the β-ionone ring closer to the *cis* C7═C8 bond discussed above. Concretely, there is a shortening of the double C5═C6 and C7═C8 bonds and a lengthening of the single C6−C7 bond for these isomers, accompanied by an increase of the negative charges of these carbon atoms, all of which confirm the loss of conjugation of the C5═C6 bond with the rest of the polyene chain caused by the increase of the β-ionone ring rotation angle. These results are consistent with the work by Schlücker et al.[22] and support the importance of the ring torsions to the stability of β-carotene.

Figures 6 and 7 also reveal that the degree of destabilization of the *dicis*-β-carotene isomers may be interpreted as the addition of the individual destabilization degrees of the corresponding *monocis* isomers. This additivity is in fact simply checked by noticing that the relative energies of the *dicis* isomers included in Table 2 approximately satisfy the expressions

$$\Delta E_{\mathrm{corr}}(7,13'\text{-}dicis) \simeq \Delta E_{\mathrm{corr}}(7\text{-}cis) + \Delta E_{\mathrm{corr}}(13\text{-}cis)$$

$$\Delta E_{\mathrm{corr}}(9,13\text{-}dicis) \simeq \Delta E_{\mathrm{corr}}(9\text{-}cis) + \Delta E_{\mathrm{corr}}(13\text{-}cis)$$

$$\Delta E_{\mathrm{corr}}(9,13'\text{-}dicis) \simeq \Delta E_{\mathrm{corr}}(9\text{-}cis) + \Delta E_{\mathrm{corr}}(13\text{-}cis)$$

$$\Delta E_{\mathrm{corr}}(9,15\text{-}dicis) \simeq \Delta E_{\mathrm{corr}}(9\text{-}cis) + \Delta E_{\mathrm{corr}}(15\text{-}cis)$$

$$\Delta E_{\mathrm{corr}}(11,11'\text{-}dicis) \simeq \Delta E_{\mathrm{corr}}(11\text{-}cis) + \Delta E_{\mathrm{corr}}(11\text{-}cis)$$

$$\Delta E_{\mathrm{corr}}(13,15\text{-}dicis) \simeq \Delta E_{\mathrm{corr}}(13\text{-}cis) + \Delta E_{\mathrm{corr}}(15\text{-}cis)$$

To conclude, the results obtained in this work confirm that the *all-trans*,*s-cis*-β-carotene isomer is effectively the most stable and that extra torsions of the β-ionone rings and/or double bond isomerizations of the polyene chain destabilize the molecule by distorting its geometry, therefore bringing about an alteration of the charge distribution. Moreover, the effects induced by the *dicis* isomerization may be interpreted as the addition of the individual effects of the corresponding *monocis* isomers separately.

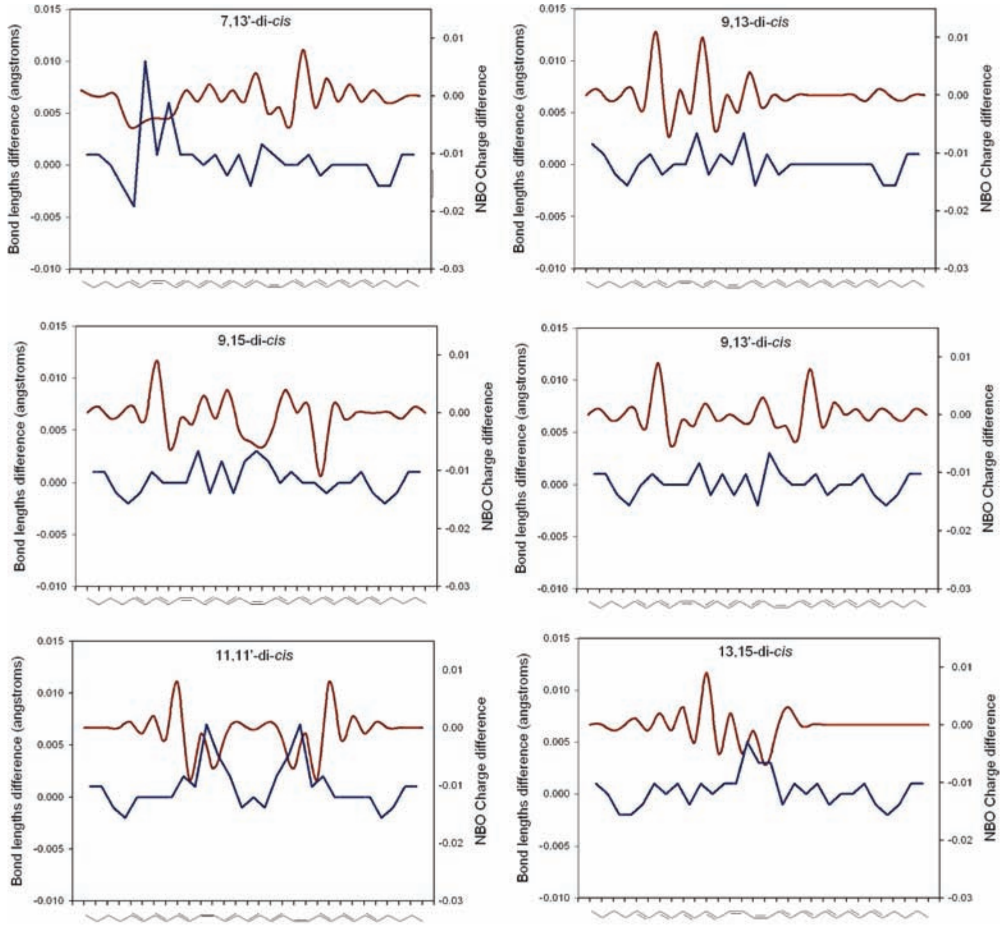

Figure 7. Differences between the bond lengths (blue line) and the NBO charges (red line) of the *dicis*-β-carotene isomers and those for the *all-trans*,*s-cis* isomer based on the optimized geometries calculated at the B3LYP/6-31G(d) level.

TABLE 3: Theoretical and Experimental Wavenumbers (cm^{-1}) for Bands of the IR Spectra of the *monocis*-β-Carotene Isomers[a]

	ν(C=C)		δ(CH$_3$)$_a$		δ(=C−H)$_{ip}$ + δ(CH$_3$)$_s$		δ(CH$_3$)$_{rocking}$		δ(=C−H)$_{oop}$		δ(UC)$_{oop}$	
isomer	theory	experiment[b]	theory	experiment[b]	theory	experiment[b]	theory	experiment[b]	theory	experiment[b]	theory	experiment[b]
s-cis	1567	1560	1452	1442	1373	1368	995	1003	975	966		
					1358	1359			957	950		
									828	828		
7-*cis*	1562	1565	1454	1445	1375	1373	998	1002	976	964	733	741
					1360	1359			831	830		
9-*cis*	1563	1563	1455	1445	1377	1373	997	1005	975	962		
					1359	1359			826	825		
11-*cis*	1565		1461		1373		996		975		785	
					1352				881			
13-*cis*	1569	1559	1457	1445	1379	1375	995	1004	974	964		
					1357	1359			825	824		
15-*cis*	1566	1560	1452	1437	1398	1381	997	1005	971	968	786	775
					1361	1359			960	952		

[a] The theoretical wavenumbers are calculated at the B3LYP/6-31G(d) level and scaled with an overall scaling factor to account for anharmonicity (see the text). Vibrations: ν, stretch; δ, deformation; ip, in-plane; oop, out-of-plane. Symmetry: s, symmetric; a, antisymmetric. [b] From ref 20.

B. Vibrational Spectra. Let us consider now the vibrational spectra of the β-carotene isomers calculated at the B3LYP/6-31G(d) level. As far as the IR spectra are concerned, we have concentrated on the *monocis* isomers, for which the experimental IR spectra are available.[19,20] The IR spectra are, in general, difficult to interpret thoroughly due to the accumulation of bands with comparable intensity observed in them.[20] It is possible, however, to extract some key bands from the calculated IR spectra of the β-carotene isomers and compare them with the experimental bands.[22,26] In Table 3 we give the scaled wavenumbers for the normal modes characteristic of β-carotene which appear in specific regions of the IR spectrum, regardless of the presence of isomerized double bonds. These signals correspond essentially to normal modes associated with the polyene chain. As observed there is a good agreement between the calculated and the observed infrared wavenumbers of the *monocis* isomers included in this table. Moreover, our calculations allow us to identify the key unmethylated *cis* (UC) band,[41,42] which appears only in the isomers with no methyl groups on the isomerized double bond, that is, in the 7-*cis*, 11-*cis*, and 15-*cis* isomers

TABLE 4: Theoretical and Experimental Wavenumbers (cm^{-1}) for Bands of the Raman Spectra of β-Carotene Isomers[a]

	$\nu(\mathrm{C{=}C})$		$\delta(\mathrm{UC})_{\mathrm{ip}}$		$\nu(\mathrm{C{-}C})$		$\delta(\mathrm{CH_3})_{\mathrm{rocking}}$	
isomer	theory	experiment	theory	experiment	theory	experiment	theory	experiment
s-cis	$1520\ (9.1 \times 10^5)$	$1529s^b$			$1157\ (2.6 \times 10^5)$	$1159s^b$	$994\ (9.8 \times 10^4)$	$1006s^b$
7-*cis*	$1521\ (7.0 \times 10^5)$	$1530s^b$			$1176^*\ (4.8 \times 10^4)$	$1184m^b$	$996\ (3.0 \times 10^4)$	$1003m^b$
					$1162\ (1.5 \times 10^5)$	$1158s^b$		
9-*cis*	$1521\ (6.5 \times 10^5)$	$1534s^b$			$1155\ (1.7 \times 10^5)$	$1160s^b$	$997\ (4.7 \times 10^4)$	$1007m^b$
11-*cis*	$1519\ (4.9 \times 10^5)$	$1529s^c$			$1207^*\ (4.5 \times 10^4)$	$1205m^c$	$996\ (4.1 \times 10^4)$	$1007m^c$
					$1187^*\ (1.9 \times 10^5)$	$1192m^c$		
					$1178\ (3.9 \times 10^4)$	$1177m^c$		
					$1155\ (1.7 \times 10^5)$	$1158s^c$		
13-*cis*	$1533\ (3.5 \times 10^5)$	$1542s^b$			$1190^*\ (6.4 \times 10^4)$	$1194m^b$	$1008\ (1.9 \times 10^4)$	$1009m^b$
					$1175^*\ (4.2 \times 10^4)$	$1176m^b$		
					$1154\ (3.6 \times 10^4)$	$1161m^b$		
					$1127\ (8.5 \times 10^4)$	$1138m^b$		
15-*cis*	$1527\ (3.5 \times 10^5)$	$1539s^b$	$1245\ (8.4 \times 10^4)$	$1245m^b$	$1191^*\ (8.9 \times 10^4)$	$1197m^b$	$996\ (2.9 \times 10^4)$	$1006m^b$
	$1514\ (1.0 \times 10^5)$	$1531s^b$			$1152\ (8.1 \times 10^4)$	$1160s^b$		
7,13′-*dicis*	$1534\ (3.7 \times 10^5)$				$1185^*\ (1.2 \times 10^4)$		$1007\ (1.1 \times 10^4)$	
					$1139\ (4.8 \times 10^4)$			
					$1128\ (6.5 \times 10^4)$			
9,13-*dicis*	$1536\ (4.4 \times 10^5)$	$1543s^b$			$1191^*\ (5.8 \times 10^4)$	$1195m^b$	$996\ (2.2 \times 10^4)$	$1007m^b$
					$1174^*\ (5.2 \times 10^4)$	$1174m^b$		
					$1144\ (4.6 \times 10^4)$	$1150m^b$		
					$1128\ (6.4 \times 10^4)$	$1135s^b$		
9,15-*dicis*	$1528\ (5.1 \times 10^5)$	$1543s^b$	$1246\ (5.1 \times 10^4)$	$1243m^b$	$1191^*\ (6.7 \times 10^4)$	$1198m^b$	$996\ (2.7 \times 10^4)$	$1004m^b$
					$1147\ (5.8 \times 10^4)$	$1154m^b$		
					$1129\ (3.2 \times 10^4)$	$1138m^b$		
9,13′-*dicis*	$1537\ (4.0 \times 10^5)$	$1548s^b$			$1194^*\ (9.6 \times 10^4)$	$1200m^b$	$998\ (1.1 \times 10^4)$	$1008m^b$
					$1153\ (2.8 \times 10^4)$	$1159m^b$		
					$1130\ (1.3 \times 10^5)$	$1140s^b$		
11,11′-*dicis*	$1518\ (6.0 \times 10^5)$	$1529s^c$	$1266\ (1.5 \times 10^5)$	$1268m^c$	$1193\ (3.5 \times 10^5)$	$1197s^c$	$989\ (4.7 \times 10^4)$	$998m^c$
					$1161\ (1.1 \times 10^5)$	$1155m^c$		
13,15-*dicis*	$1533\ (7.9 \times 10^5)$	$1548s^b$	$1253\ (8.7 \times 10^4)$	$1254m^b$	$1230^*\ (6.7 \times 10^4)$	$1232m^b$	$994\ (2.7 \times 10^4)$	$1006m^b$
					$1192^*\ (1.1 \times 10^5)$	$1198m^b$		
					$1167\ (3.3 \times 10^4)$	$1161m^b$		

[a] The theoretical wavenumbers are calculated at the B3LYP/6-31G(d) level and scaled with an overall scaling factor to account for anharmonicity (see the text). The Raman scattering activities (Å^4/amu) are given in parentheses. Vibrations: ν, stretch; δ, deformation. The intensities s and m correspond to 100−50% and 50−25% absorption. The asterisk denotes stretching−bending coupling: ν (C−C) + $\delta(\mathrm{{=}C{-}H})_{\mathrm{ip}}$. [b] From ref 18. [c] From ref 21.

(see Figure 5). This IR band is essentially due to the out-of-plane deformation of the hydrogens located on the *cis* bond, so it is labeled as $\delta(\mathrm{UC})_{\mathrm{oop}}$.

The Raman spectra of the β-carotene isomers are less dense and therefore easier to analyze than the IR spectra since they present fewer bands with higher intensities. Moreover, the Raman spectra of the different isomers show important differences depending on the double bond configuration of the polyene chain. In Table 4 we give the vibrational wavenumbers calculated for the main Raman signals in the so-called “fingerprint” region, which is located between 1000 and 1600 cm^{-1}, together with the corresponding observed wavenumbers[18,20,21] when available. Note that only experimental signals with strong and medium intensity are included in this table.

Again in Table 4 we see that all the vibrational wavenumbers calculated for the different β-carotene isomers agree reasonably well with their experimental values. Going into detail, we note first that the brightest vibrational band for each isomer appears around 1520 cm^{-1} and is assigned to the stretching C═C normal mode.[18] The normal-mode analysis shows that this mode is mainly located in the double bonds of the central part of the polyene chain, with practically no contribution from the double bonds of the terminal rings. Next in Table 4 we have the stretching C−C bands, which are located around 1160 cm^{-1}, and their profiles depend on the double bond *cis* isomerization of the polyene chain. In particular, for the *all-trans*,*s-cis*-, 7-*cis*-, 9-*cis*-, 11-*cis*-, 15-*cis*-, 9,13-*dicis*-, and 11,11′-*dicis*-β-carotene isomers, a strong intensity signal stands out in the range of 1200−1130 cm^{-1}, whereas for the rest of the isomers only signals with medium intensity appear in that region. In this last case, the band with highest wavenumber corresponds to a C−C stretching mode coupled to the in-plane C−H bends ($\delta(\mathrm{{=}C{-}H})_{\mathrm{ip}}$). Besides, we have in Table 4 the vibrational bands observed in the lowest region of the spectra at ∼1000 cm^{-1}, which are assigned to the rocking normal mode of the methyl groups ($\delta(\mathrm{CH_3})_{\mathrm{rock}}$).

In the Raman spectra of the β-carotene isomers a key band in the range of 1240−1270 cm^{-1} also appears, which corresponds to a UC normal mode.[20] The presence of this band may be used, therefore, to identify the 7-*cis*, 11-*cis*, and 15-*cis* isomers or any *dicis* isomer with no methyl groups on, at least, one of the isomerized double bonds, i.e., in our case, the 7,13′-, 9,15-, 11,11′-, and 13,15-*dicis* isomers (see Figure 5). In contrast to what happens in the IR spectra, the normal-mode analysis shows that this UC band is due to the in-plane deformation of the hydrogen in the *cis* double bond, so it is a $\delta(\mathrm{UC})_{\mathrm{ip}}$ band. Our theoretical calculations show that these $\delta(\mathrm{UC})_{\mathrm{ip}}$ bands are weak for the 7-*cis*, 11-*cis*, and 7,13-*dicis* isomers, in agreement with the experimental evidence,[18,21] which is why they are not included in Table 4. We should note that this is the first time that this $\delta(\mathrm{UC})_{\mathrm{ip}}$ band is interpreted on the basis of DFT calculations, confirming the assignment given by Koyama and co-workers.[20]

The theoretical spectroscopic results obtained for the β-carotene isomers also show that the deviations from planarity of the terminal β-ionone rings do not basically modify their Raman spectra. As previously discussed, the rotation of the rings alters the stability of the β-carotene isomers by decreasing the conjugation degree of the C5═C6 and C5′═C6′ double bonds with the rest of the polyene chain. However, the normal-mode analysis carried out in this work reveals that these double bonds do not contribute to the main ν(C═C) band of the IR and Raman spectra, which are therefore unaffected by the ring torsion.

IV. Conclusions

In this work we have carried out a DFT conformational study of isomers of β-carotene at the B3LYP/6-31G(d) level of theory including the *all-trans* isomers, all the *monocis* isomers, and a number of the *dicis* isomers. The relative energies computed for all these isomers confirm that the *all-trans*,*s-cis* isomer is the most stable, as previously reported by other authors.[11,22,33,34] The optimized geometries show that the terminal β-ionone rings form an angle with the polyene chain plane of about 47° for all the isomers, except for the 7-*cis* and 7,13′-*dicis* ones, for which the rotation angle is larger, about 65°. Both the geometric parameters and the bond indices calculated indicate that this distortion of the molecular conformation is due to the combination of two contrary effects, the stabilization of the molecule induced by the conjugation of the double bonds on one hand and the destabilization of the molecule caused by the steric repulsion of the methyl groups in the rings with the hydrogen atoms in the polyene chain on the other. For the 11-*cis* and 11,11′-*dicis* isomers there is an additional contribution to the destabilization, due to the steric repulsion between the 13a-methyl group and the olefinic H10 hydrogen, which results in an additional increase of the bending angles in the neighborhood of the *cis* bonds.

The effect of the double bond *cis* isomerization on the stability of the molecule has been studied by comparing the bond lengths and the NBO charges of the different isomers with those of the most stable *all-trans*,*s-cis* isomer. Our results show that there is a modification in the geometry and the electronic charge distribution in the vicinity of the isomerized double bond which is directly related to the degree of destabilization of the *cis* isomers. The stability order of the *monocis* isomers, as extracted from the calculated relative energies, is also shown to agree with the natural abundance of the isomers.

In addition, we have calculated the IR and Raman spectra of the β-carotene isomers, also at the B3LYP/6-31G(d) level of theory. The wavenumbers thus obtained are in good accordance with the experimental data. Despite the difficulties in interpreting the denser IR spectra, we have been able to identify theoretically the presence of the $\delta(\mathrm{UC})_{\mathrm{oop}}$ band which characterizes unambiguously the C7═C8, C11═C12, and C15═C15′ *cis* isomerizations. Regarding the Raman spectra, we have shown first that the stretching C═C bands are quite insensitive to *cis* isomerization and that the double bonds of the β-ionone rings basically do not contribute to this normal mode, although they affect the stability of the isomers. In contrast, the calculated Raman spectra of the β-carotene isomers show strong differences in the 1300−1100 cm^{-1} region, where the bands corresponding to the stretching ν(C−C) normal modes (coupled in some cases to the bending $\delta(=\mathrm{C-H})_{\mathrm{ip}}$) and to $\delta(\mathrm{UC})_{\mathrm{ip}}$ modes are predominantly located.

Since at present there are no standards for all the molecular conformations of β-carotene available,[23] the theoretical results presented in this work on the stability and the vibrational spectra of the β-carotene isomers are expected to be of help in the experimental analysis of admixtures of these isomers. These results could be useful as well in the identification of the β-carotene isomers in photosystems in which the double bond configurations have not yet been clearly elucidated. On the other hand, the good agreement shown between the calculated and the observed frequencies of the vibrational spectra give additional support to the use of the B3LYP/6-31G(d) method for studying these isomers theoretically. Finally we should emphasize that, as long as the study of the electronic excited states of carotenoids is currently a field of great interest,[43−50] the optimized geometries calculated in this work may be used as a good starting point to further characterize the molecular and electronic properties of the *cis* isomers of β-carotene theoretically.

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References and Notes

(1) Stahl, W.; Sies, H. *Mol. Aspects Med.* **2003**, *24*, 345–351.
(2) El-Agamey, A.; Lowe, G.; McGarvey, D.; Mortensen, A.; Phillip, D.; Truscott, T.; Young, A. *Arch. Biochem. Biophys.* **2004**, *430*, 37–48.
(3) Krinsky, N.; Johnson, E. *Mol. Aspects Med.* **2005**, *26*, 459–516.
(4) Krinsky, N. *Nutrition* **2001**, *17*, 815–817.
(5) Omoni, A.; Aluko, R. *Trends Food Technol.* **2005**, *16*, 344–350.
(6) Nishino, H. *J. Cell. Biochem.* **1995**, *22*, 231–235.
(7) Mayne, S. *FASEB J.* **1996**, *10*, 690–701.
(8) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. *J. Agric. Food. Chem* **2005**, *53*, 6649–6657.
(9) Schieber, A.; Carle, R. *Trends Food Sci. Technol.* **2005**, *16*, 416–422.
(10) Kuki, M.; Koyama, Y.; Nagae, H. *J. Phys. Chem.* **1991**, *95*, 7171–7180.
(11) Doering, W.; Sotiriou-Leventis, C.; Roth, W. *J. Am. Chem. Soc.* **1995**, *117*, 2747–2757.
(12) Gao, G.; Wei, C. C.; Jeevarajan, A. S.; Kispert, L. D. *J. Phys. Chem.* **1996**, *100*, 5362–5363.
(13) Konovalov, V. V.; Kispert, L. D. *J. Chem. Soc., Perkin Trans. 2* **1999**, *1999*, 901–909.
(14) Marx, M.; Stuparic, M.; Schieber, A.; Carle, R. *Food Chem.* **2003**, *83*, 609–617.
(15) Gao, Y.; Kispert, L. D.; Konovalova, T. A.; Lawrence, J. N. *J. Phys. Chem. B* **2004**, *108*, 9456–9462.
(16) Tsukida, K.; Saiki, K.; Takii, T.; Koyama, Y. *J. Chromatogr.* **1982**, *245*, 359–364.
(17) Koyama, Y.; Kito, M.; Takii, T.; Tsukida, K. S. K.; Yamashita, J. *Biochim. Biophys. Acta* **1982**, *5*, 109–118.
(18) Koyama, Y.; Takii, T.; Saiki, K.; Tsukida, K. *Photobiochem. Photobiophys.* **1983**, *5*, 139–150.
(19) Saito, S.; Tasumi, M. *J. Raman Spectrosc.* **1983**, *14*, 310–321.
(20) Koyama, Y.; Takatsuka, I.; Nakata, M.; Tasumi, M. *J. Raman Spectrosc.* **1988**, *19*, 37–49.
(21) Hu, Y.; Hashimoto, H.; Moine, G.; Hengartner, U.; Koyama, Y. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2699–2710.
(22) Schlücker, S.; Szeghalmi, A.; Schmitt, M.; Poop, J.; Kiefer, W. *J. Raman Spectrosc.* **2003**, *34*, 413–419.
(23) Mortensen, A. *Food. Res. Int.* **2005**, *38*, 847–853.
(24) Jaime, L.; Mendiola, J.; Ibáñez, E.; Martín-Álvarez, P.; Cifuentes, A.; Reglero, G.; Señoráns, F. *J. Agric. Food. Chem.* **2007**, *55*, 10585–10590.
(25) Himo, F. *J. Phys. Chem. A* **2001**, *105*, 7933–7937.
(26) Berezin, K.; Nechaev, V. *J. Appl. Spectrosc.* **2005**, *72*, 164–171.
(27) Gao, Y.; Focsan, A.; Kispert, L.; Dixon, D. *J. Phys. Chem. B* **2006**, *110*, 24750–24756.
(28) Galano, A. *J. Phys. Chem. B* **2007**, *111*, 12898–12908.
(29) Requena, A.; Ceron-Carrasco, J.; Bastida, A.; Zúñiga, J.; Miguel, B. *J. Phys. Chem. A* **2008**, *112*, 4815–4825.
(30) Liu, W.; Wang, Z.; Zheng, Z.; Li, A.; Su, W. *J. Phys. Chem. A* **2008**, *112*, 10580–10585.
(31) Lee, C.; Yang, W.; Parr, R. *Phys. Rev. B* **1988**, *37*, 785–789.
(32) Becke, A. *J. Chem. Phys.* **1993**, *98*, 5648–5652.

(33) Qiu, D.; Chen, Z.; Li, H. *J. Mol. Struct.: THEOCHEM* **2008**, *865*, 44–48.

(34) Guo, W.; Tu, C.; Hu, C. *J. Phys. Chem. B* **2008**, *112*, 12158–12167.

(35) Durbeej, B.; Eriksson, L. A. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4190–4198.

(36) Scott, A.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502–16513.

(37) Frisch, M. J. ; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N. ; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision D.02; Gaussian, Inc.: Wallingford, CT, 2004.

(38) Foster, J.; Weinhold, F. *J. Am. Chem. Soc.* **1980**, *102*, 7211–7218.

(39) Reed, A.; Weinstock, R.; Weinhold, F. *J. Chem. Phys.* **1985**, *83*, 735–746.

(40) Carpenter, J.; Weinhold, F. *J. Mol. Struct.: THEOCHEM* **1988**, *169*, 41–62.

(41) Mukai, Y.; Koyama, Y.; Ito, M.; Tsukida, K. *J. Raman Spectrosc.* **1986**, *17*, 387–396.

(42) Koyama, Y.; Mukai, Y.; Umemura, J.; Ito, M.; Tsukida, K. *J. Raman Spectrosc.* **1984**, *15*, 300–307.

(43) Onaka, K.; Fujii, R.; Nagae, H.; Kuki, M.; Koyama, Y.; Watanabe, Y. *Chem. Phys. Lett.* **1999**, *315*, 75–81.

(44) Larsen, D.; Papagiannakis, E.; van Stokkum, I.; Vengris, M.; Kennis, J.; van Grondelle, R. *Chem. Phys. Lett.* **2003**, *381*, 733–742.

(45) Polívka, T.; Sundström, V. *Chem. Rev.* **2004**, *104*, 2021–2071.

(46) Dreuw, A. *J. Phys. Chem. A* **2006**, *110*, 4592–4599.

(47) Niedzwiedzki, D.; Sullivan, J.; Pol, T.; Birge, R.; Frank, H. *J. Phys. Chem. B* **2006**, *110*, 22872–22885.

(48) Marian, C.; Gilka, N. *J. Chem. Theory Comput.* **2008**, *4*, 1501–1515.

(49) Christensen, R.; Grace, M.; Galinato, I.; Chu, E.; Howard, J.; Broene, R.; Frank, H. *J. Phys. Chem. A* **2008**, *112*, 12629–12636.

(50) Bode, S.; Quentmeier, C.; Liao, P.; Barros, T.; Walla, P. *Chem. Phys. Lett.* **2008**, *450*, 379–385.

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