

## Effects of Molecular Symmetry on the Electronic Transitions in Carotenoids

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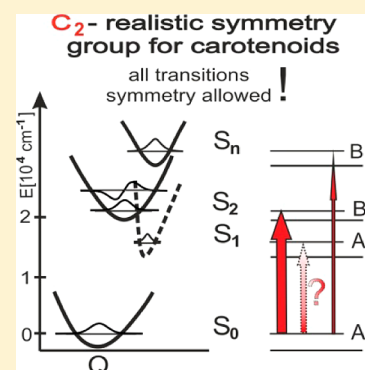
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### S Supporting Information

**ABSTRACT:** The aim of this work is the verification of symmetry effects on the electronic absorption spectra of carotenoids. The symmetry breaking in *cis*- $\beta$ -carotenes and in carotenoids with nonlinear  $\pi$ -electron system is of virtually no effect on the dark transitions in these pigments, in spite of the loss of the inversion center and evident changes in their electronic structure. In the *cis* isomers, the  $S_2$  state couples with the higher excited states and the extent of this coupling depends on the position of the *cis* bend. A confrontation of symmetry properties of carotenoids with their electronic absorption and IR and Raman spectra shows that they belong to the  $C_1$  or  $C_2$  but not the  $C_{2h}$  symmetry group, as commonly assumed. In these realistic symmetries all the electronic transitions are symmetry-allowed and the absence of some transitions, such as the dark  $S_0 \rightarrow S_1$  transition, must have another physical origin. Most likely it is a severe deformation of the carotenoid molecule in the  $S_1$  state, unachievable directly from the ground state, which means that the Franck–Condon factors for a vertical  $S_0 \rightarrow S_1$  transition are negligible because the final state is massively displaced along the vibrational coordinates. The implications of our findings have an impact on the understanding of the photophysics and functioning of carotenoids.



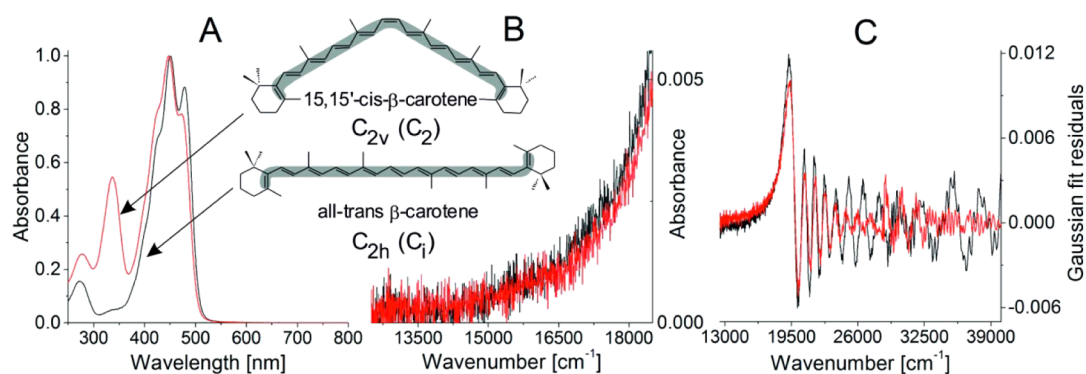
Carotenoids (Crts) are a large group of natural pigments that play numerous roles in biological systems. These range from light harvesting, photoprotection, and stabilization of the photosynthetic complexes to being physical and chemical quenchers of singlet oxygen, antioxidants, and precursors of visual pigments in animals.<sup>1–6</sup> Such a large variety of processes that engage Crts stem from the properties of their extended  $\pi$ -electron system and their understanding is crucial to the elucidation of the functioning of these pigments. However, their basic, relatively simple, polyene framework may be somewhat deceptive because the electronic structure of this system of alternating C–C and C=C bonds turns out to be surprisingly complicated. This complexity is well reflected in the peculiar photophysical properties of Crts, which have long since posed a challenge both to experimental and theoretical approaches. Yet, several quite basic issues, such as the presence or absence of some electronic levels and their energetic order in Crts, remain unresolved. One of the most puzzling is the fact that the electronic transition from the  $S_0$  state to the  $S_1$  state is never directly observed in Crts, in contrast to the very intense transition to the  $S_2$  state. The optically inactive  $S_1$  state in the isolated pigment can be populated either from the higher excited states, in two-photon processes<sup>7–10</sup> and by photosensitization.<sup>11</sup>  $S_1$  participates in excitation energy transfer to bacteriochlorophyll within bacterial LH2 and LH1 antennae<sup>9,12,13</sup> and plays a role in the regulation of photosynthetic

light harvesting in the major plant antenna LHCII,<sup>14</sup> being excitonically coupled to chlorophylls.<sup>15</sup> Another intriguing question is the structure–function relationship in the geometric isomers of Crts.<sup>16</sup> Thus, in photosynthetic light harvesting all-trans isomers are exclusively involved, whereas in the photoprotection of reaction centers, mainly the 15 *cis* isomers are involved.<sup>16–18</sup> The reason for this distinction is a matter for debate<sup>19</sup> and there are indications that light-induced *cis*–trans isomerization of the LHCII-bound Crts may occur in situ.<sup>20</sup>

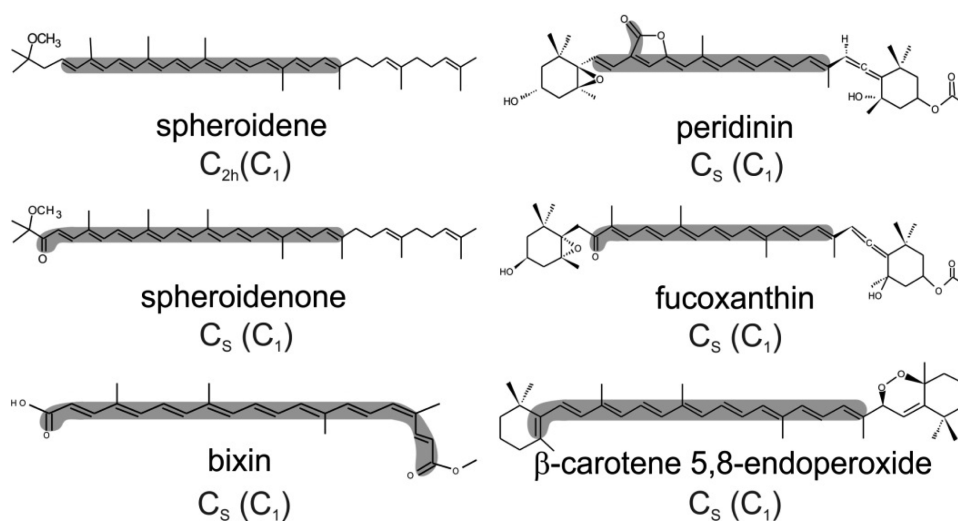
Considerations of molecular symmetry based on group theory have largely contributed to the understanding of electronic structures and photophysical features of important biological chromophores. The exceptional strength of the symmetry-based approach stems from the fact that a simple assignment of a molecule to a specific symmetry group precisely defines the symmetry properties of its wave functions, from which all observables can be derived and interpreted. The electronic absorption/emission and vibrational spectroscopies are such important areas where considerations of molecular symmetry are crucial. For instance, the changes in the symmetry of the macrocyclic  $\pi$ -electron system that occur

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**Figure 1.** Structures of all-trans and 15,15'-cis- $\beta$ -carotene and their electronic absorption spectra recorded in *n*-hexane (A), the overlapped tails of the absorption profiles, shown in the linear scale (B), and the overlapped residuals of the Gaussian deconvolution of the entire absorption spectra (C). The shape of the conjugated  $\pi$ -electron system in each carotenoid is marked in gray. The red lines refer to 15,15'-cis- $\beta$ -carotene. The methods of isolation of the pigments are described in the [Supporting Information](#).



**Figure 2.** Structures of carotenoids investigated in the present study, with the assignments of their  $\pi$ -electron systems and entire molecules (in parentheses) to symmetry point groups indicated. The shape of the conjugated  $\pi$ -electron system in each carotenoid is marked in gray.

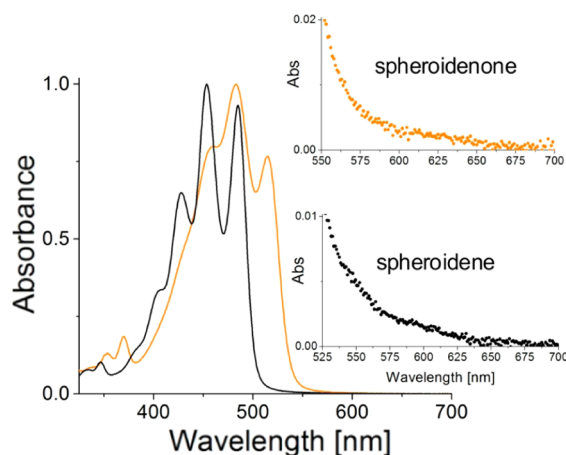
when going from porphyrin to chlorophyll and bacteriochlorophyll contribute to the removal of the degeneracy of electronic states, manifested in considerable shifts in their electronic levels.<sup>21</sup> Furthermore, the symmetry of the ligand field in the central binding pockets of these macrocyclic systems determines the character of the bonding and interactions with centrally bound metal ions.<sup>22,23</sup> Symmetry considerations are also important in the case of Crts. The selection rules for linear polyenes in the  $C_{2h}$  group state that the  $S_0(A_g) \rightarrow S_1(A_g)$  transition is strongly symmetry-forbidden because these two states have the same parity  $g$ , due to the presence of an inversion center.<sup>24,25</sup> This symmetry-based reasoning is carried over to Crts, close relatives of linear polyenes, and widely applied in the interpretation of the photophysical features of these pigments.<sup>26,27</sup> In addition, in several models (e.g., Hückel, PPP or Hubbard, which assume a zero-differential overlap (ZDO) between the atomic  $p_z$  orbitals), an artificial symmetry, the so-called Pariser alternancy symmetry, was introduced.<sup>28</sup> However, it was used only to simplify computations on polyenes and has no relevance to real molecular symmetries.<sup>29</sup>

It seems natural to ask what happens if the symmetry of a polyene is significantly broken. Structures of many naturally occurring Crts are asymmetric due to the side groups which are electronically coupled with the main  $\pi$ -electron system and

their molecules possess no inversion center. This approach has been applied by Frank et al., but intriguingly enough, they have found no effect of asymmetrically positioned side groups in Crts on the activity of the  $S_1$  state.<sup>30</sup> Another way to break the symmetry is the isomerization resulting in a *cis* bend, due to which the inversion center in Crts is evidently lost, thus reducing the symmetry group and changing the assignment to irreducible representations (see Table S1 in the [Suppl. Material](#)). In  $C_{2h}$ , the most often used for Crts in the all-trans conformation, these irreducible representations are  $A_g$  and  $B_w$  and when a reduction to  $C_v$ ,  $C_s$ , or  $C_2$  occurs, they become  $A_g/A_w$ ,  $A'/A''$ , and  $A/B$ , respectively. In another point group relevant to Crts,  $C_{2v}$ , the irreducible representations are  $A_1, B_1 / A_2, B_2$ , and when the symmetry is lowered to  $C_s$  or  $C_2$ , they become  $A'/A''$  and  $A/B$ , respectively. Thus, the  $\pi$  molecular orbitals in all-trans  $\beta$ -Car ( $C_{2h}$ ) belong to irreducible representations  $a_u$  and  $b_g$  but those of 15,15'-cis- $\beta$ -Car ( $C_{2v}$ ) have  $a_2$  and  $b_1$  symmetry. The other *cis* isomers possess only the plane of symmetry and hence they all belong to the  $C_s$  point group. In the present work we undertake to systematically examine how such a symmetry breaking does affect the electronic absorption spectra of Crts. To this end, we have prepared a series of Crts of asymmetric or severely distorted  $\pi$ -electron system, aiming to identify changes in their absorption spectra associated with the symmetry loss.

Both the theoretical analysis and the spectral properties show that all electronic transitions in nonsymmetrical Crts are symmetry-allowed, and the absence of some transitions, such as the dark  $S_0 \rightarrow S_1$  transition, must have another physical origin. We propose a simple mechanism by which this transition remains inactive.

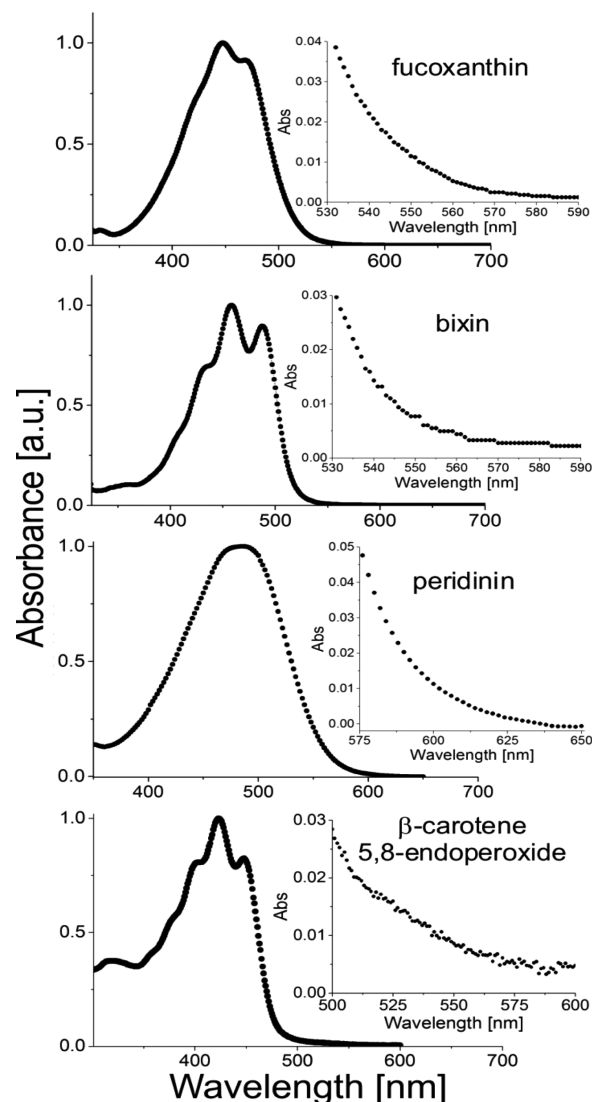
**Absorption Spectra.** The structures of the nonsymmetrical Crts investigated in the present work are shown in Figures 1 and 2. In order to reveal how in detail molecular symmetry affects the electronic transitions in Crts, the absorption spectra of all-trans and 15,15'-cis- $\beta$ -Car, were compared (Figure 1). In the range between 350 and 550 nm, their spectra are very similar. In particular, the shape of lower energy tails of the main absorption bands is identical, as it can be well seen in the 1000-fold enlarged region above 550 nm (Figure 1B). This identity is rather surprising in light of the fact that a new feature appears in the spectrum of the cis isomer below 350 nm, known as the "cis" peak, which indicates considerable changes in CrT electronic structure due to the cis bend (see below). In fact, a weak band in the same spectral region can also be seen in the absorption spectra of all-trans  $\beta$ -Car (Figure 1) and other all-trans Crts (Figure 3). Apparently, even small distortions at the



**Figure 3.** Absorption spectra of spheroidene and spheroidene recorded in *n*-hexane. In the insets, the enlarged fragments of the lower energy shoulder of the main absorption bands are shown. The methods of isolation of the pigments are described in the Supporting Information.

molecule periphery affect the electronic structure of the pigment. In spite of that, the symmetry-forbidden transition to the  $S_1$  level is not at all affected by this symmetry and linearity breaking. To clarify this issue, a thorough spectral deconvolution of the spectra of the two isomers of  $\beta$ -Car into Gaussian components has been carried out, which confirms that this state remains "dark", as evidenced by virtual identity of the fit residual (Figure 1C). This rigorous analysis shows that in both cases no additional bands are present on the lower energy side of the main transition, whose intensity would be greater than  $10^{-4}$ . It seems, however, that such weak transitions can be detected in Crts using a more sensitive/suitable methodology, for example, broadband 2D electronic spectroscopy.<sup>13</sup> This shows that the loss of the inversion center caused by the cis bend is of no effect on the activity of this forbidden transition. This lack of any effect of a severe symmetry breaking on the activity of the dark transition in  $\beta$ -Car prompted us to isolate and analyze the electronic spectra of other Crts whose

molecules have symmetries lower than  $C_{2h}$ , that is, in which the transition to the  $S_1$  state is formally allowed. To this end, neurosporene (Neu), lycopene (Lyc), spheroidene (Sph), spheroidenone (Spn), bixin, fucoxanthin, peridinin, 5,8-endoperoxide and 9-cis and 13-cis isomers of  $\beta$ -Car were obtained, and their spectra recorded. The absorption profiles and the lower energy tails of nearly linear Sph and Spn are compared in Figure 3 and those of more severely distorted pigments (fucoxanthin, bixin, peridinin, and 5,8-endoperoxide of  $\beta$ -Car) in Figure 4. The tails of the spectra of Spn and Sph



**Figure 4.** Absorption spectra of fucoxanthin, bixin, peridinin and 5,8-endoperoxide of  $\beta$ -carotene recorded in *n*-hexane. The raw data for peridinin was kindly provided by Dr Tomáš Polívka. In the insets the enlarged fragments of the lower energy shoulder of the main absorption bands are shown. 5,8-Endoperoxide of  $\beta$ -carotene was prepared as described previously.<sup>4,31</sup> The isolation of other pigments is described in the Supporting Information.

are practically identical (see inserts in Figure 3). Similarly, no low energy bands can be found in the spectra of the other nonsymmetric or distorted pigments (see inserts in Figure 4). This is in line with the earlier results of measurements carried out at cryogenic temperatures for peridinin and fucoxanthin.<sup>30</sup>

**Table 1.** Positions of the Maxima and Spacing, in  $\text{cm}^{-1}$ , of Vibronic Bands in the Electronic Absorption Spectra of Carotenoids, Estimated Directly from the Experimental Data, in Parentheses, and via Deconvolution of the Spectra into Gaussian Components, and the Resulting Shift Parameters  $B$  Defined as the Displacement, Relative to the Mode Frequency, of the PES Minima from the Equilibrium Position for the  $S_0$  State

	vibronic band maxima and intensities				band spacing			shift parameter
	0 ← 0	0 ← 1	0 ← 2	0 ← 3	$\Delta_1$	$\Delta_2$	$\Delta_3$	$B$
neurosporene	21326 (21322) 0.97	22770 (22779) 0.99	24134 (24096) 0.56	25323	1444 (1457)	1364 (1317)	1189	1.43
spheroidene	20646 (20619) 0.91	22050 (22051) 0.99	23390 (23364) 0.60	24594	1404 (1432)	1340 (1313)	1204	1.48
lycopene	19882 (19900) 0.89	21188 (21231) 0.95	22539 (22523) 0.63	23744	1306 (1331)	1351 (1292)	1205	1.46
spheroidenone	19372 0.68	20611 0.92	21867 0.65	23036 0.35	1239	1256	1169	1.64
$\beta$ -carotene	20812 0.82	22137 0.85	23561 0.66	25025 0.16	1325	1424	1464	1.44
9- <i>cis</i>	21071 0.81	22386 0.84	23790 0.67	25363 0.24	1315	1404	1573	1.44
13- <i>cis</i>	21067 0.81	22390 0.85	23798 0.67	25255 0.22	1323	1408	1457	1.45
15- <i>cis</i>	20932 0.65	22252 0.87	23647 0.67	25052 0.32	1320	1395	1405	1.64

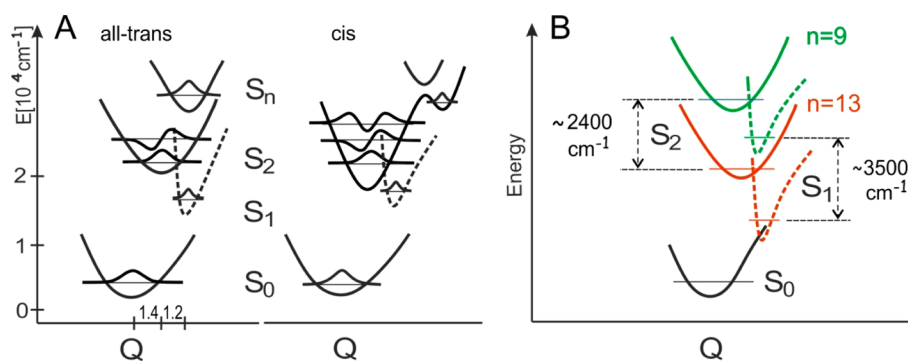
In order to further assess the structural effects on the electronic absorption spectra of Crts, the deconvolution has been applied to the spectra of Neu, Lyc, Sph, Spn, and  $\beta$ -Car and its 9-, 13-, and 15-*cis* isomers. The best fits to the experimental curves were obtained using purely Gaussian components and usually the coefficient of determination of the fits ( $r^2$ ) reached 0.9999. The results of this analysis are summarized in Table 1, which also shows how these parameters compare with the ones that could be determined directly from the spectra. The deconvolution enabled the exact estimation of the intensities and separations ( $\Delta_1 - \Delta_3$ ) between the vibrational components of the transition bands. In each spectrum, the  $\Delta$  values vary, indicating a significant anharmonicity of the potential energy surfaces (PES) of the  $S_2$  level, even in low oscillatory states of Crt molecules. On the other hand, from the ratios between the intensities of the  $0 \leftarrow 1$  and  $0 \leftarrow 0$  components, the PES shift parameters ( $B$ ) can be calculated. The  $B$  values, changing from 1.45 to 1.64 in the case of Spn and 15,15'-*cis*- $\beta$ -Car, show that structural changes significantly influence the position of the PES minimum of the  $S_2$  state.

*Symmetry versus Electronic and Vibrational Properties of Carotenoids.* For rigid molecules frozen in flat conformations, the  $\pi$  molecular orbitals in all-trans  $\beta$ -Car ( $C_{2h}$ ) belong to irreducible representations  $a_u$  and  $b_g$  and those of 15,15'-*cis*- $\beta$ -Car ( $C_{2v}$ ) to  $a_2$  and  $b_1$ . [If the end rings are not twisted, the all-trans  $\beta$ -Car molecule belongs to the  $C_{2h}$  group, the one to which simpler linear polyenes are also assigned, whereas the 15-*cis* isomer must be assigned the  $C_{2v}$  group (see Figure S1 and Table S2 in the Supporting Information). The (flat) molecules of the other geometric isomers, with the *cis* bend in the 7, 9, 11, or 13 position, possess only the plane of symmetry and hence they all belong to the  $C_s$  point group. Any twist of the end rings further lowers the molecular symmetry as the symmetry plane and the inversion center are lost, which changes the assignments to irreducible representations. In  $C_{2h}$  they are  $A_g$  and  $B_u$  and when a reduction to  $C_i$ ,  $C_s$ , or  $C_2$  occurs, they become  $A_g/A_u$ ,  $A'/A''$ , and  $A/B$ , respectively. By analogy, the irreducible representations of  $C_{2v}$  are  $A_1/B_2$ , and when the symmetry is lowered to  $C_s$  or  $C_2$ , they become  $A'/A''$  and  $A/B$ , respectively.] This enables one, first, to determine the symmetry of the corresponding molecular terms (= their wave functions) and, second, to indicate which electronic transitions are symmetry-forbidden. In  $C_s$  and  $C_{2v}$  all of the electronic transitions are allowed, whereas in  $C_{2h}$  the ones to the  $A_g$  state are strictly forbidden and to the  $B_u$  states allowed

(and indeed very intensive in Crts). The lowering of symmetry to  $C_i$  does not affect the transition to the  $A_g$  state (it remains forbidden), whereas other transitions, now to the  $A_u$  states, are symmetry-allowed. Further, in  $C_2$ , the lowest energy transition (now to the  $A$  state) becomes allowed and is polarized perpendicularly to the plane of the polyene chain. Thus, in all-trans  $\beta$ -Car with realistic symmetry the lowest energy transition is either forbidden ( $C_i$ ) or becomes allowed ( $C_2$ ), whereas in the *cis*-isomers all the transitions are symmetry-allowed.

Considering now the vibrational properties of Crts, the IR and Raman spectra of molecules which have an inversion center ( $C_{2h}$  and  $C_i$ ) should, at least roughly, complement each other, whereas in the  $C_{2v}$  and  $C_2$  groups (no inversion center), the normal vibrations should be active in both types of measurements. However, the Raman spectra of Crts show no complementarity whatsoever to their IR spectra.<sup>32–35</sup> Moreover, the IR spectra of all-trans  $\beta$ -Car and its *cis* isomers reveal a surprisingly high degree of similarity.<sup>33</sup> Practically, the only difference lies in an additional band situated near  $\nu = 780 \text{ cm}^{-1}$ , attributed to the deformational vibration of  $=C-H$  bond.<sup>34</sup> It appears then that the assignment of all-trans  $\beta$ -Car and 15,15'-*cis*- $\beta$ -Car to the  $C_{2h}$  and  $C_{2v}$  symmetry groups, respectively, finds no justification in the vibrational analysis, and the symmetry of all-trans  $\beta$ -Car must be lower. Also the crystallographic and theoretical studies indicate the  $C_i$  or  $C_2$  and clearly not  $C_{2h}/C_{2v}$  symmetry for this molecule.<sup>36–38</sup> Recent measurements and theoretical simulation of Crt geometry and Raman spectra fully confirm these conclusions.<sup>39–41</sup>

In the above analysis, full planarity of molecules has been assumed, but in reality, many other factors that lower the molecular symmetry of linear Crts have to be considered, such as (i) nonsymmetrically positioned side groups (see examples in Figures 2 and 3); (ii) the  $sp^3$  hybridization bars C atoms from being coplanar; (iii) in most instances, the endings are twisted; both the experiment and computations show a bend of about  $48^\circ$  in  $\beta$ -Car;<sup>39,42,43</sup> and (iv) interactions with solvent or chiral environment, for example, protein matrix, break the symmetry.<sup>44–46</sup> In particular, in light harvesting complexes the twist of the Crt skeleton seems important for the energy transfer.<sup>47,48</sup> Moreover, the present analysis and many examples of nonlinear Crts show that this symmetry-allowed  $S_0-S_1$  transition remains totally inactive.<sup>16,19,49–51</sup> The same concerns the Crts locked in the *cis* geometry.<sup>52,53</sup> Obviously, the electronic structure is changed in these molecules, as evidenced by the appearance of the *cis* band in their absorption spectra



**Figure 5.** Schematic presentation of the relationships between the potential energy curves (A) in all-trans  $\beta$ -Carotene (left) and its geometric (“cis”) isomers (right), and (B) of the  $S_0$ ,  $S_1$ , and  $S_2$  states in linear (all-trans) carotenoids. On the Q axis, the values of shift parameter B for the  $S_1$  and  $S_2$  states are indicated. The energetic separation (in  $\text{cm}^{-1}$ ) between the  $S_1$  and  $S_2$  states of carotenoids in organic solvent are taken from refs 69 and 12, respectively.

(see above). Actually, to the best of our knowledge, the  $S_0 \rightarrow S_1$  transition is virtually never observed in nonsymmetrical Crts.<sup>54</sup> Therefore, the often applied assignment of these molecules to the  $C_{2h}/C_{2v}$  group has to be used very cautiously and perhaps only when “pure” electronic transitions, limited to the polyene skeleton, are concerned. Only under these restrictions may the general conclusions derived for polyenes be carried over to Crts.<sup>24,25</sup> This does not apply, however, to the cis isomers because in these molecules the change in symmetry evidently affects the entire  $\pi$ -electron system but in spite of that the transition to the  $S_1$  level remains “dark”.

*Why Is the  $S_0 \rightarrow S_1$  Transition Not Active in Carotenoids?* The above analysis shows that in the case of Crts the molecular symmetry has to be considered and not just the symmetry of the  $\pi$ -electron system. This becomes clear in light of a strong coupling of electronic and nuclear motions (vibronic coupling) in these molecules, which implies that the symmetries of Crt electronic and vibrational wave functions cannot be taken separately. The vibronic coupling between  $S_0$  and the excited states, well verified by the Raman and time-resolved absorption spectroscopies,<sup>55–60</sup> does remove the restrictions on the electronic transitions imposed by symmetry. The vibronic coupling can easily be the reason for breaking of symmetry selection rules, even in highly symmetric small molecules. Benzene is perhaps the best example of a such a molecule, in which the intensities of symmetry-forbidden electronic transitions are comparable to the allowed ones. Furthermore, even the doubly forbidden transition, by both spin and symmetry, is clearly observed in its absorption spectrum.<sup>61</sup> However, in spite of vibronic coupling in Crts, the  $S_0 \rightarrow S_1$  transition is inactive and the question of the lack of its activity needs to be addressed if one wishes to better understand photophysics of these pigments. To this end, if the  $C_2$  group is realistically assumed for most cases,<sup>41</sup> the symmetries of Crt electronic states swap from  $A_g$  and  $B_u$  (in  $C_{2h}$ ) to A and B, respectively, and normal modes have a symmetry (symmetric) or b symmetry (antisymmetric), and all electronic transitions are allowed. [In the (unrealistic)  $C_{2h}$  group, some vibronic transitions are allowed. In this point group, the pure electronic function belongs to  $A_g$  and  $B_u$  irreducible representations and the vibrational function transforms as the symmetry of normal modes, which defines the ones that couple the ground ( $A_g$ ) and excited ( $A_g$ ) electronic states. The dipole moment operator transforms as  $a_u(z)$  and  $b_u(x; y)$ , and hence the direct product  $A_g a_g \begin{pmatrix} a_u \\ b_u \end{pmatrix} A_g a_g = \begin{pmatrix} a_u \\ b_u \end{pmatrix}$  shows which transitions are forbidden

when the vibrational function is full-symmetric. A transition becomes allowed when the  $A_g$  state couples to excitation of a vibration in which either the  $A_g a_u \begin{pmatrix} a_u \\ b_u \end{pmatrix} A_g a_g = \begin{pmatrix} a_g \\ b_g \end{pmatrix}$  or the  $A_g b_u \begin{pmatrix} a_u \\ b_u \end{pmatrix} A_g a_g = \begin{pmatrix} b_g \\ a_g \end{pmatrix}$  direct product contains full-symmetric representation. This implies that the orbitally forbidden  $A_g \rightarrow A_g$  transition in polyenes and Crts is in fact vibronically allowed.] The transitions to  $S_2$  and  $S_n$  (B symmetry) are polarized in the plane (X–Y) of the conjugated  $\pi$ -electron system and induce antisymmetric modes, while the  $S_0 \rightarrow S_1$  transition (A symmetry) can be polarized only vertically to this plane and only induces symmetric modes that necessarily involve collective off-plane (Z direction) movements of atoms. However, any deformation of Crt molecule in the Z direction will be strongly opposed by the  $\pi$ -electron system, due to the correlation effects. Moreover, such modes must violate the  $\pi$ – $\sigma$  separation in this molecule, leading to a change in hybridization of C atoms in the polyene skeleton from  $sp^2$  to  $sp^3$ . All these factors are expected to increase the force constants of these off-plane modes.

The occurrence of C atoms with pyramidal configuration in the  $S_1$  state of polyenes and Crts, which lowers their symmetry to  $C_1$ , becomes more and more evident.<sup>48,62</sup> A significant change in molecular geometry in the  $S_1$  state gives rise to a C=C stretching mode at  $\sim 1800 \text{ cm}^{-1}$  in time-resolved Raman spectra of all-trans  $\beta$ -Car and its derivatives.<sup>55,63</sup> Other indications of a large deviation from equilibrium geometry in the  $S_1$  state are strong temperature and solvent viscosity effects on the fluorescence quantum yield<sup>19</sup> and the ease of isomerization in this excited state.<sup>64</sup> Most likely, this deformation is the reason why this formally allowed transition remains extremely weak or nonexistent. In this case, at the instant of excitation, the Franck–Condon principle in Crts is not obeyed, because their ground and excited states differ significantly in geometries and symmetries. In other words, the molecular deformation required for the  $S_0 \rightarrow S_1$  transition to take place seems so drastic that  $S_1$  cannot be populated from the ground state, which implies peculiar relationship between the PES of these states, as shown schematically in Figure 5. Apparently, the deformation in the  $S_1$  state renders its PES much narrower than the PES of the  $S_0$  and  $S_2$  states, in line with the partial equalization of the lengths of the C–C and C=C bonds in the  $S_1$  state,<sup>65,66</sup> which reflects an increase in force constants of molecular vibrations in this state due to a stronger

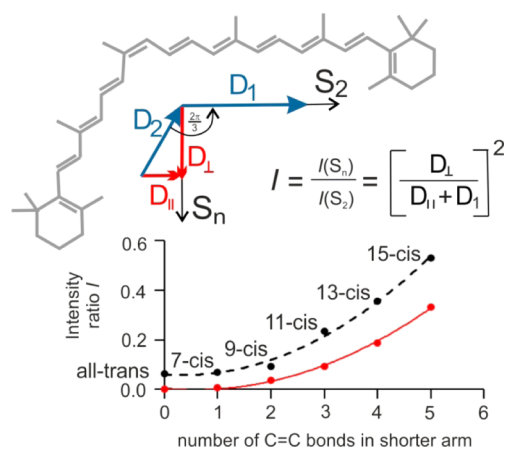
conjugation between carbon atoms. Crt in the  $S_1$  state is highly unrelaxed, as reflected in the occurrence of a very fast initial component in its decay,<sup>67</sup> and the lifetime of this state is much shorter than typical lifetimes of molecules in the  $S_1$  state. On the other hand, the  $S_1$  lifetime shortens from around 30 ps to almost 1 ps with the increasing conjugation length, both in free as well as LH-bound Crts,<sup>12,68,69</sup> which can be explained by increasing interaction between the  $S_0$  and  $S_1$  states, resulting in a better overlap of the respective vibrational wave functions, as the  $S_1$  and  $S_2$  state energies decrease,<sup>3,68</sup> shown schematically in Figure 5B. The analysis of the vibrational components in the steady state (see above) and transient absorption spectra of several all-trans Crts (recorded by Polívka et al.<sup>8,70</sup>) provides more information about the PESs. Thus, the  $B$  values show that the  $S_2$  minimum is shifted by 1.4 with respect to  $S_0$  (Table 1) and that the  $S_1$  minimum lies 1.2 further off, as indicated in Figure 5, in agreement with the trends deduced from the all-trans  $\beta$ -Car fluorescence profile.<sup>71</sup>

The population of  $S_1$  from  $S_2$  via internal conversion in one-photon processes is facilitated because the two respective PESs cross each other. In the two-photon processes, the  $S_0 \rightarrow S_1$  transition is promoted via virtual states,<sup>27,72,73</sup> and there is not enough time for the nuclear movements to take place during this event, that is, an intersection of the respective PESs is not required. The depopulation of the  $S_1$  state will occur then via overlapping oscillatory states. Furthermore, such transitions are not restricted to single symmetry and are equally allowed in many symmetries,<sup>74</sup> especially when one-photon processes are allowed.

We propose that the reason for inactivity of the  $S_0 \rightarrow S_1$  transition is not the symmetry but it is due to a severe molecular deformation in the final state, which cannot be achieved by one-photon excitation of the ground state. As discussed above, there are several physical effects which collectively strongly prevent the direct population of the  $S_1$  state from the  $S_0$  state.

**Origin of the "Cis" Band ( $S_0 \rightarrow S_n$  Transition).** The change in molecular geometry of Crts as introduced with the cis conformation affects the electronic structure of the pigment, which is reflected in the absorption spectra by the appearance of a high energy transition, as discussed above. The intensity of this new absorption band depends on the position of the cis bend, and the closer to the center of the molecule, the more intense it is.<sup>16,50</sup> It also appears to be inversely correlated with the intensity of the  $S_0 \rightarrow S_2$  transition, as if it borrows its intensity from the latter. Seemingly, this transition gains in activity because the  $S_2$  and  $S_n$  states begin to interact with each other as the minimum of the  $S_n$  PES shifts with respect to the  $S_2$  PES (Figure 5). A clear correlation between these intensities is shown in theoretical study of the excited states of  $\beta$ -Car isomers.<sup>75</sup> The  $S_2$ - $S_n$  interaction, in turn, depends on the degree of linearity of Crt molecule and becomes the strongest in the 15-cis isomer. A hint of such a dependence can be found even in the absorption spectra of all-trans  $\beta$ -Car (Figure 1A), spheroidenone (Figure 3), and xanthophylls<sup>76</sup> as a weak band located near 340 nm, which can be attributed to the terminal cis bends. On the other hand, the energies of the states do not change because the number of the conjugated  $\pi$ -electrons remains constant along the series of the isomers.

The above considerations allow us to propose a simple model of the influence of molecular geometry on the active electronic transitions in Crts. As shown in Figure 6, the cis bend divides the molecule into two fragments and the total



**Figure 6.** Experimentally determined (black) and predicted (red) ratio  $I$  between the intensities of the cis-peak ( $S_n$ ) and the  $S_2$  band in a series of cis isomers of  $\beta$ -carotene plotted against the number of conjugated C=C bonds in the chromophore shorter arm defined by the position of the cis bend.

transition moment  $D$  responsible for the transitions to  $S_2$  and  $S_n$  can be treated as a sum of two components  $D_1$  and  $D_2$ , oriented along the two respective arms of the C-C backbone. The  $D_2$  component, which is oriented along the shorter arm and which vanishes in the linear molecule, can be decomposed into two components  $D_\perp$  and  $D_\parallel$ . The cis transition ( $S_0 \rightarrow S_n$ ) is associated with the  $D_\perp$  component, whereas the transition to the state  $S_2$  with the  $D_1 + D_\parallel$  sum. These components of  $D$  give rise to the electronic transitions of total intensity proportional to  $D^2 = (|D_\perp|^2 + |D_\parallel + D_1|^2)$ , where  $D_\perp = \sqrt{3}/2 D_2$  and  $D_\parallel = 1/2 D_2$ . It follows then that the ratio of the intensities of these two transitions,  $I(S_n)/I(S_2)$ , is equal to  $[D_\perp/(D_\parallel + D_1)]^2$ . The maximum value of  $I(S_n)/I(S_2)$ , 1/3, is reached in the 15-cis isomer, when  $D_1 = D_2$ , and the minimum, zero, in all-trans  $\beta$ -Car, when  $D_2 = 0$ . The values of this ratio as calculated for a series of the  $\beta$ -Car isomers compare quite well with the experimentally estimated ones (Figure 6). In both cases, similar quadratic relationships are seen, however, the origin of the experimental curve (all-trans  $\beta$ -Car) is nonzero, most likely due to the contribution from the terminal cis bends to the absorption near 340 nm (see above). Second, the two curves are not parallel to each other, probably due to the changes in bond order along the chain.<sup>4,38,65,77</sup> In the present approach the lengths of the C-C and C=C bonds are assumed to be constant.

The present model predicts a strong effect of the molecular geometry on the cis transition, in agreement with the electroabsorption measurements,<sup>78</sup> and is consistent with the decrease in polarizability of Crt molecules upon cis-isomerization.<sup>79</sup> Moreover, it explains the dependence of the  $S_2$  transition intensity (i.e., intensity lending to  $S_n$ ) on the distance between the C-6 and C-6' atoms found in the computational study.<sup>75</sup>

The aim of the present work was to verify whether and how symmetry of  $\pi$ -electron system in Crts affects their electronic properties, in particular their absorption spectra. This has been approached by analysis of the effects related to the cis bend and other severe distortions of Crt molecular geometry. The change of molecular geometry of a Crt due to the cis bend results in a stronger interaction between the  $S_2$  and  $S_n$  states. The intensities of transitions to these two states are inversely correlated to each other and their ratio varies with the position

of the cis bend in the chain, as the effective transition dipole moments depend on the length of the molecular fragments defined by the bend position.

Because even in maximally distorted Crts, the so-called dark states could not be detected, our conclusion is that these states are inactive not due to the symmetry restrictions and  $C_{2h}$  and  $C_{2v}$  are improper symmetry groups for Crt molecules. In the case of all-trans  $\beta$ -Car and other Crts of similar symmetry (in solution or protein-bound), all the symmetry and spectroscopic considerations indicate  $C_2$  as the proper symmetry group. This implies that there are no symmetry-forbidden electronic transitions in Crts, contrary to prevailing opinion. However, it is hard to imagine that the absence of the  $S_0$ – $S_1$  transition may have different physical origins in linear and nonlinear Crts. The absence (“darkness”) of the  $S_0 \rightarrow S_1$  transition must then have another physical origin, common to all Crts and more powerful than the symmetry “forbiddenness”. It seems that there are several effects that collectively strongly prevent the direct population of the  $S_1$  state from the  $S_0$  state. One of them is a considerable electronic–vibrational coupling in these molecules due to which the Born–Oppenheimer approximation is not fulfilled. In effect, the Franck–Condon factors are small for the vertical transition from the equilibrium ground state ensemble to the  $S_1$  surface, which thus cannot be achieved in one-photon excitation. Besides, the  $S_1$  PES may have multiple minima.<sup>48</sup> In molecules such as carotenoids, in which electronic-oscillatory coupling is evident, it is not the fragmentary (system of conjugated  $\pi$  bonds) but the entire molecular symmetry that has to be considered. The application of symmetry notification for the electronic levels in Crts is of no use, unless realistic symmetries are known. Otherwise, it not only leads to an erroneous interpretation of their spectroscopic properties and photophysical features but may be somewhat deceiving. The above conclusions are based on a relatively simple approach, and a more sophisticated experimental and computational effort is needed to better understand these colorful compounds.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jpclett.6b00637](https://doi.org/10.1021/acs.jpclett.6b00637).

A detailed description of methods for pigment isolation and purification, the electronic absorption measurements and spectral deconvolution. Symmetry elements in the all-trans  $\beta$ -Carotene and 15,15'-cis- $\beta$ -Carotene molecules and conformations and point groups of cis isomers of  $\beta$ -carotene. State symmetries in the point groups relevant to the structure of carotenoids. (PDF)

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### Notes

The authors declare no competing financial interest.

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