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The T_1 resonance Raman spectra of biochemically relevant chromophores A theoretical investigation

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Abstract

The vibrational structure of the T_1 resonance Raman spectra of hexatriene, β-ionylidene acetaldehyde (C15 aldehyde) and β-carotene are analyzed with the help of semiempirical and ab initio quantum chemical calculations. The T_1 equilibrium structures and vibrational force fields of the mono-*cis*- and *trans*-isomeric species are obtained and employed to model the spectra under the assumption of a dominant Franck–Condon mechanism. The simulations are used to identify the species that contribute to the observed spectra and to map the shape of the T_1 potential energy surface. © 1997 Elsevier Science S.A.

Keywords: Resonance Raman spectroscopy; Triplet state; Retinal homologues; β-Carotene

1. Introduction

The recent advances in pulsed laser technology have triggered the development of time-resolved spectroscopical methods devised to study the time-evolution of short-lived molecular species like molecules in electronic excited states or radicals. In this sense, time resolved absorption and Raman spectroscopies contribute not only to the knowledge of the static molecular properties but also to the dynamics of the short-lived species.

Perhaps one of the more extensively studied dynamical processes is the *cis–trans* photoisomerization of conjugated molecules. In particular, the isomerization in the lowest triplet state of polyenes and polyene derivatives has received considerable attention, and several time-resolved absorption and resonance Raman (RR) studies have been reported [1–3] also in view of the importance of the lowest triplet state in reactions occurring in biochemical systems [4].

Compared to singlet states, the lifetime of the lowest triplet state of polyenes is considerably longer. This implies that, for sufficiently low energy barriers, the photoexcited species (conformers) that correspond to minima on the triplet potential energy surface can equilibrate. If equilibration is fast enough, the vibrational structure of the resonance Raman spectrum, whose measurement is time-delayed with respect to the triplet state pumping, is a source of indirect information on the shape and minima of the triplet potential energy surface, via the contribution of the equilibrated species.

The extraction of these details from the spectra is, however, considerably difficult because the observed vibrational structure is due to vibrations of the triplet excited state species. The associated frequencies can be substantially different from those of the ground state, and a one to one correspondence not often exists. Furthermore, the contributions from several intermediates can severely complicate the analysis of the vibrational structure.

The identification of the species that contribute to the activity observed in the spectra requires the independent determination of barriers heights, molecular structures and vibrational force fields which can be obtained with the help of quantum chemical calculations followed by modelling of the RR vibronic activity.

In this contribution we discuss the theoretical simulations of the T_1 RR spectra of 1,3,5-hexatriene, a model polyene; of β-ionylidene acetaldehyde (C15-aldehyde), an homologue of retinal and of β-carotene. The goal is twofold: to identify the species that contribute to the observed spectra and to discuss the dynamics that occurs in the time interval between the triplet build-up and the measurement of the RR spectra.

2. Computational methods

The excitation wavelength employed [3,5–8] to measure the T_1 RR spectra of the molecules studied in this work, is in

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resonance with a triplet electronic excited state T_n whose $T_1 \rightarrow T_n$ transition dipole moment is strongly allowed. As a consequence, the Franck–Condon (FC) mechanism (A term in Albrecht's notation [9,10]) is the main source of intensity and the vibrational structure of the T_1 RR spectra discussed in the following is dominated by the activity of totally symmetric (TS) modes.

In order to simulate the spectra we adopted the harmonic approximation and obtained, for each ith totally symmetric mode, the displacement parameters B_i relative to the T_1–T_n transition. The latter are defined as

$$B_i = \left(\frac{\omega_i}{\hbar}\right)^{1/2} Q_{i(T_1)}^{T_n,T_1} \tag{1}$$

Here, $Q_{i(T_1)}^{T_n,T_1}$ is the projection of the geometry change between the two states, expressed in Cartesian coordinates, onto the T_1-state normal coordinate $Q_i^{(T_1)}$, that is

$$Q_{i(T_1)}^{T_n,T_1} = [x_{T_n} - x_{T_1}]\mathbf{M}^{1/2}L_i^{T_1} \tag{2}$$

where $\mathbf{x}_K$ is the $3N$ dimensional vector of the equilibrium Cartesian coordinates in the Kth state, $\mathbf{M}$ is the $3N \times 3N$ diagonal matrix of the atomic masses and $L_i^{(T_1)}$ is the $3N$ vector describing the normal coordinate $Q_i^{(T_1)}$, in terms of mass-weighted Cartesian coordinates.

Under the assumption of resonance with the 0–0 band of the $T_1 \rightarrow T_n$ transition,the activity of each totally symmetric mode is related to the displacement parameter through the γ_i parameter

$$I_i \propto \gamma_i = \frac{1}{2}B_i^2 \tag{3}$$

It is clear from the above relations that the molecular parameters required to simulate the RR spectra are the equilibrium structures of the two states involved in the transition, T_1 and T_n, and the vibrational force field in T_1.

For the shortest system studied, hexatriene, we determined equilibrium geometries and force fields by employing the ab initio multi-configuration-self-consistent-field (MCSCF) procedure in the complete-active-space (CAS) formulation which is included in the Gaussian92/DFT suite of programs [11], in combination with the 6-31G basis set. The active space chosen for the calculations included the six π orbitals and electrons. The equilibrium structures, energies, and vibrational force fields of the T_1 intermediates were also studied by employing density functional theory (DFT) in conjunction with the Becke exchange functional [12] and the Lee, Yang and Parr correlation functional [13] (BLYP). For the larger systems we employed the DFT–BLYP method combined with the use of an updated version [15,16] of the semiempirical Quantum Consistent Force Field for π electrons (QCFF/PI) hamiltonian [14]. Excitation energies and $T_1 \rightarrow T_n$ transition dipole moments were computed with the latter hamiltonian in conjunction with a Configuration Interaction (CI) which included all single excitations generated from the selected π orbital space. The full π orbital space was used for hexatriene (3×3) and C15 aldehyde (4×4), whilst the six highest occupied and six lowest empty orbitals were selected for β-carotene.

As anticipated, the excitation wavelength employed in the RR experiments is in resonance with a state T_n whose $T_1 \rightarrow T_n$ transition dipole moment is strong. Beside the evidence from the experimental $T_1 \rightarrow T_n$ absorption spectra, QCFF/PI calculations of excitation energies and transition dipole moments showed that, for all the molecules under study, there is only one intense state in a wide energy region around the excitation energy employed experimentally. Thus, the identification of the resonant state was straightforward, and in the following we adopt the generic label T_n to indicate it, for every molecule and isomer discussed below. In the case of hexatriene, the equilibrium geometry of the T_n state was obtained at the CASSCF level. The state was identified by inspecting its wavefunction composition, and its geometry was optimized by selecting the corresponding root. No state averaging was required, since the state is well separated from lower lying excited states of the same symmetry.

3. Results and discussion

3.1. 1,3,5-hexatriene

Hexatriene (see Fig. 1) is the shortest polyene with both internal and external CC double bonds and as such it is a convenient model to understand the triplet state properties of longer polyenes. The RR spectra of both *trans*- and *cis*-1,3,5-hexatriene (HT) were extensively studied [3] and were found to be identical. This fact was initially interpreted as evidence of a common, perpendicularly twisted intermediate responsible for the RR spectrum [3]. Later, on the basis of semiempirical QCFF/PI calculations the contribution of the perpendicular form to the spectrum was ruled out because of off resonance conditions of the $T_1 \rightarrow T_n$ transition [15], and the observed vibrational structure was assigned to the planar species.

More recently we have shown that ab initio calculations at the CASSCF level predict the *trans*-form to be more stable than the *cis*-form by about 2 kcal mol^{-1} [17], in agreement with the indirect estimate deduced from experimental data [18]. A very low barrier separates the two isomers and fast equilibration occurs during the lifetime of the triplet state (ca.

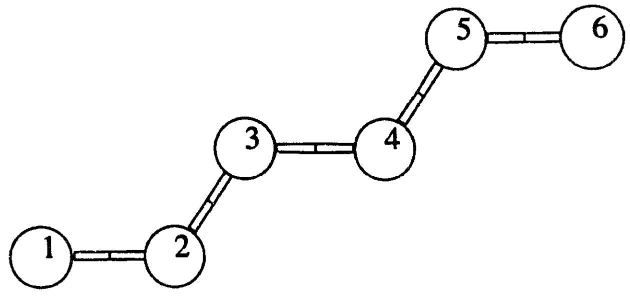

Fig. 1. Atom numbering of 1,3,5-hexatriene.

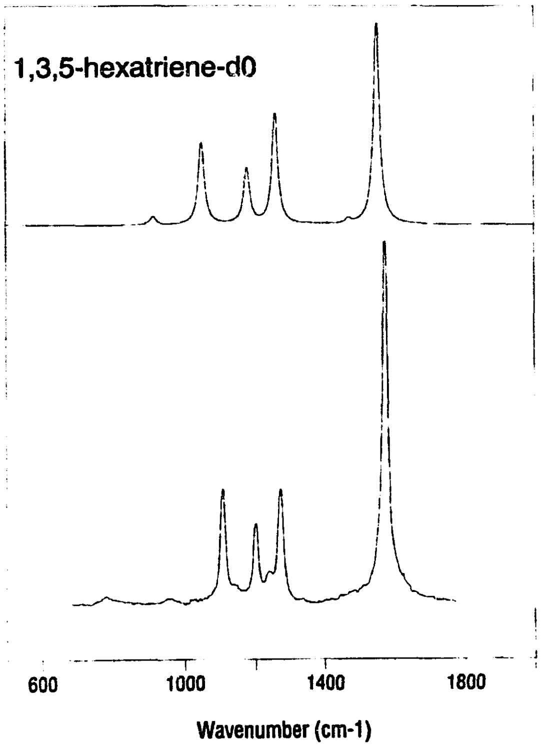

Fig. 2. Experimental (bottom) and simulated (top) resonance Raman spectra of 1,3,5-hexatriene.

100 ns). The RR spectra, recorded ca. 60 ns after pumping, can only detect the equilibrated mixture. Accordingly, the vibrational structure of the RR spectrum of both *cis*- and *trans*-HT is due to the *trans*-form which dominates the equilibrated population. The comparison between the observed and simulated T_1 RR spectrum of HT is depicted in Fig. 2.

The agreement between the spectrum simulated on the basis of CASSCF calculations [18] and the observed spectra is excellent, and indicates that ab initio CASSCF results are very reliable. Interestingly, a very similar energy difference between the planar *trans*- and *cis*-forms is predicted on the basis of DFT–BLYP/6-31G* and DFT–BLYP/6-311G** calculations: 2.58 and 2.65 kcal mol^{-1}, respectively.

Thus, for HT, the knowledge of the triplet potential energy surface along the isomerization coordinate is presently complete, and accounts for all the available experimental data.

The central CC double bond is considerably elongated in T_1 (1.470 or 1.465 Å according to CASSCF and DFT-BLYP calculations, respectively). At least two intermediates exist in T_1, one corresponding to the *trans*-form, the other corresponding to a structure perpendicularly twisted around the central double bond, and the barrier for interconversion between the two forms is very flat (ca. 0.5 kcal mol^{-1}).

3.2. *β-ionylidene acetaldehyde (C15-aldehyde)*

β-ionylidene acetaldehyde is a shorter homologue of retinal and the elucidation of the properties of its lowest triplet state is the first step toward the comprehension of the triplet photoisomerization of retinal. The latter process contributes considerably to the photo-chemistry of this important chromophore because of the high quantum yield of intersystem crossing. In this sense the photophysical properties of C15 aldehyde are very similar to retinal since the shortest homologue has a comparable quantum yield of intersystem crossing (0.42–0.45 [19]). Beside the *trans*-isomer, C15 aldehyde exits in two mono-*cis*-isomeric forms, the 7 *cis*- and 9 *cis*-form which are depicted in Fig. 3.

The triplet–triplet absorption spectrum shows a maximum extinction coefficient at 385 nm [19] and the lifetime was determined to be ca. 100 ns, that is, very similar to the triplet lifetime of hexatriene [3]. The T_1 resonance Raman spectra were obtained from each ground state mono-*cis*- and *trans*-isomer [5], by pumping the triplet state via direct photoexcitation and subsequently (5 ns of delay from the pump pulse) probe it with a pulse at 385 nm, in resonance with the strongest $T_1 \rightarrow T_n$ transition in the energy region [19,5].

The resulting resonance Raman spectra (see Fig. 4) are very similar since the vibronic activity appears in almost identical frequency regions, but with slightly different intensities, when starting from the 7 *cis*-, 9 *cis*- or *trans*-isomers. The quality of the spectra allows to identify only a restricted number of bands which show the strongest intensity. These are observed at ca. 1614, 1556, 1398, 1262 and 1220 cm^{-1}, respectively. The authors concluded that the spectra are sufficiently different to be attributed to different isomers and

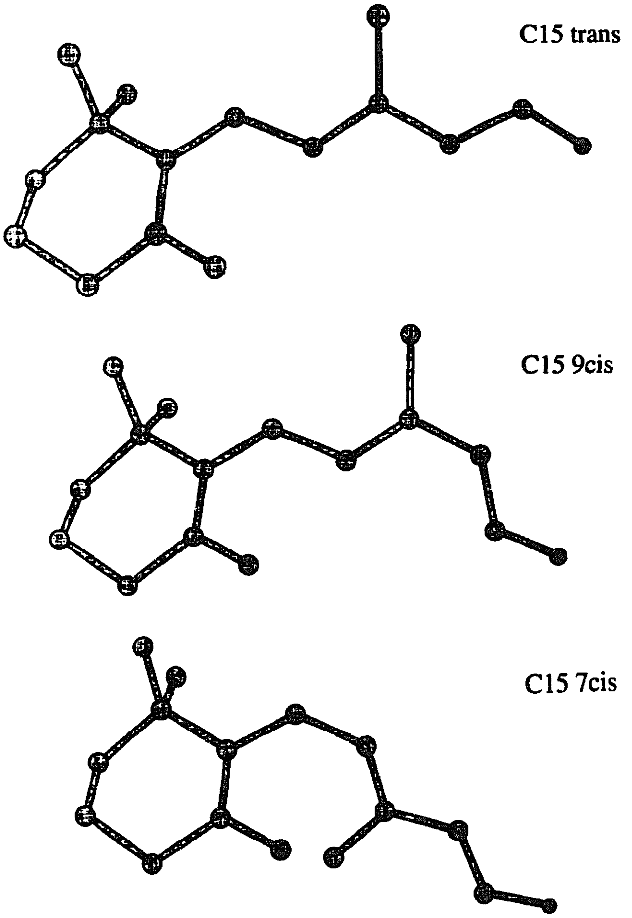

Fig. 3. Bond numbering for the three isomeric forms of *β*-ionylidene acetaldehyde (C15 aldehyde) investigated in this work.

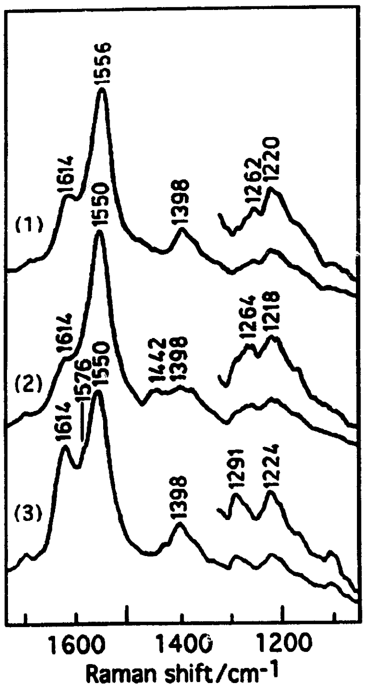

Fig. 4. Resonance Raman spectra of β-ionylidene acetaldehyde (C15 aldehyde) from Ref. [5]. The triplet species was produced from (1) all-*trans*, (2) 7 *cis*- and (3) 9 *cis*-isomers.

argued that the observation of different spectra indicates a very similar stability of the three isomeric forms [5] in T_1.

Indeed, beside the stability of intermediates in T_1, we have pointed out above that the dynamics on the triplet state, that is, whether or not isomerization and equilibration can take place, must also be taken into account to interpret the observed RR spectra.

Specifically, if we consider the experimental conditions in which the spectra were obtained, (the delay from the pump and probe pulses was 5 ns) we have to establish what is the fate of the initially pumped triplet state after 5 ns.

In the absence of time-resolved absorption studies on C15 aldehyde, we can make some assumption on the basis of similar studies available for retinal. It is known that intersystem crossing occurs within 20 ps from excitation, and that the time required for build-up of the triplet state is about 30–40 ps [1]. Thus, after few tens of picoseconds the triplet state of retinal has reached its maximum population. Given the similar intersystem crossing properties of C15 aldehyde, it is reasonable to assume that the same time scale will apply also to the shorter homologue. Thus, by adopting a simple kinetic model to estimate the interconversion rate between the *trans*- and *cis*-isomers it is concluded that the T_1 RR spectra observed in Ref. [5] can be attributed to different and non-equilibrated species only if barriers higher than 6–7 kcal mol^{-1} exist on the triplet surface.

The above considerations point to a triplet potential surface considerably different from that of HT, and prompted us to undertake a quantum chemical study of the C15 aldehyde in T_1 with two objectives: first, to simulate the resonance Raman spectra for each isomeric form and compare them with experimental data, second, to estimate the relative energies of the intermediates in T_1. To this end we have performed semiempirical QCFF/PI calculations to obtain equilibrium geometries and vibrational force fields in T_1 and in the T_n state in resonance with the exciting wavelength. We have evaluated the displacement parameters for the TS vibrations and simulated the vibronic structure of the RR spectra. In addition, we have performed ab initio calculations with the DFT–BLYP method and the 3-21G basis set, in order to get reliable estimates of the relative energies of the intermediates in T_1.

A survey of the computational results is presented in Fig. 5 where the bond lengths computed for the S_0, T_1 and T_n equilibrium structures of *trans*-C15 aldehyde are compared. The bond numbering adopted in Fig. 5 corresponds to that presented in Fig. 3. Similar pictures are obtained for the two mono-*cis*-isomers. Of the several geometrical parameters that characterize the equilibrium structures we have selected the bond lengths of the conjugated framework because these represent the molecular moiety more affected by electronic excitation and, specifically, the associated changes are responsible for most of the vibronic activity observed in the RR spectra.

Inspection of Fig. 5 shows that semiempirical and density functional calculations give very similar results for the ground state and lowest triplet state structures. The only notable difference pertains to the bond lengths of the first two conjugated CC bonds in the T_1 state. It appears that DFT–BLYP calculations extend the bond order inversion with respect to the ground state, over a larger number of CC bonds compared to QCFF/PI results.

From Fig. 5, it is clear that both methods predict a structural change upon excitation to the lowest triplet state, which affects the entire CC conjugated framework. The similarity of QCFF/PI and DFT–BLYP results indicates that the former

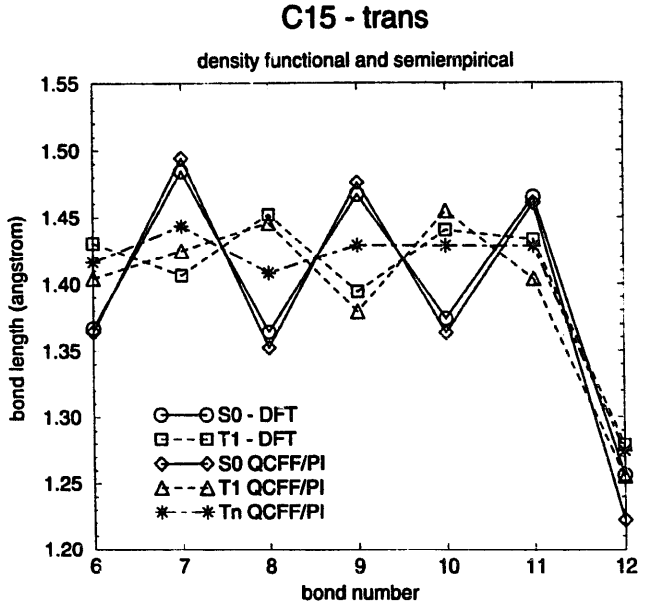

Fig. 5. Bond lengths of the conjugated framework of C15, as computed by QCFF/PI and DFT–BLYP/3-21G methods.

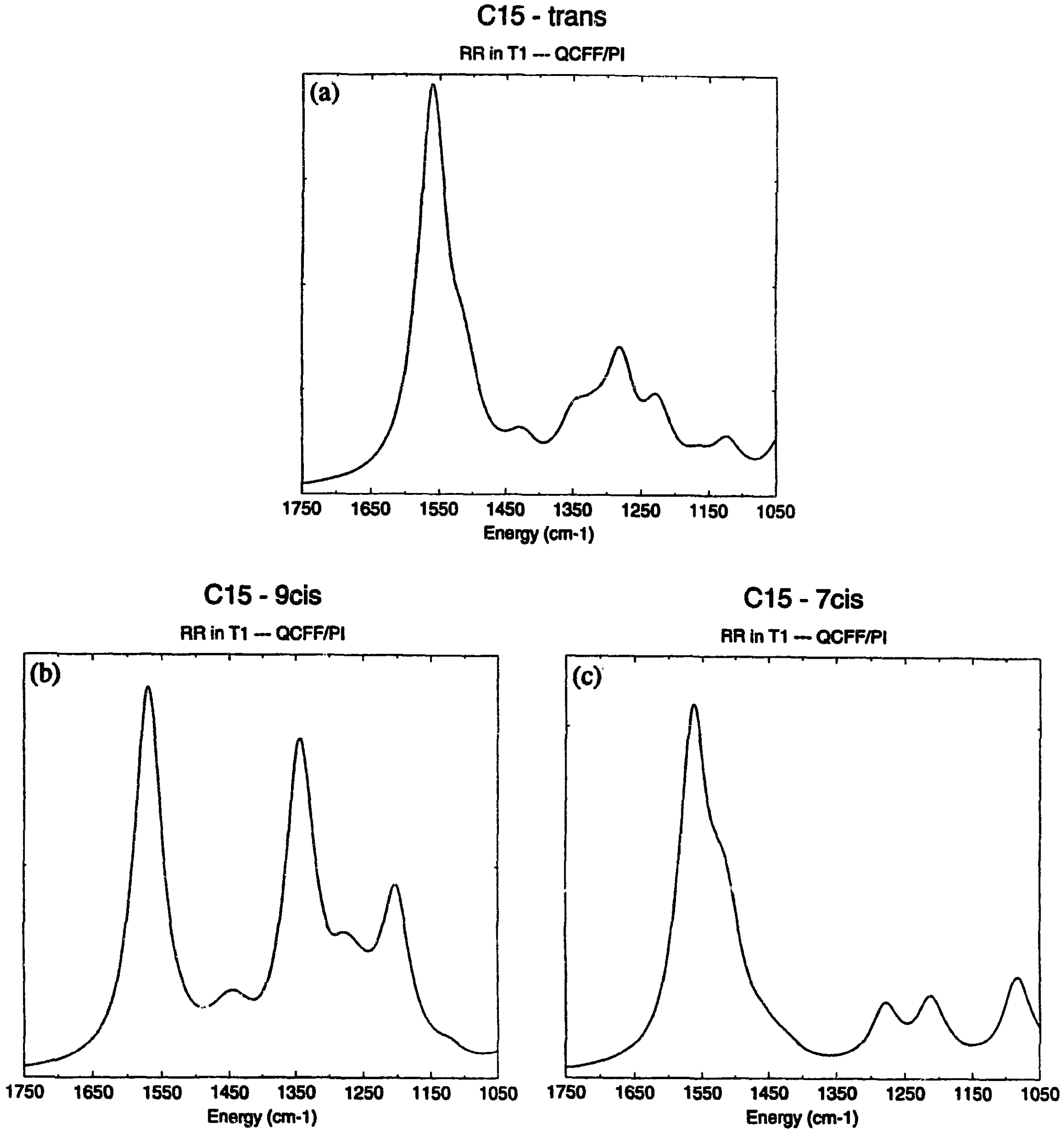

Fig. 6. Simulation of the T_1 resonance Raman spectra of the three isomeric forms of β-ionylidene acetaldehyde (C15 aldehyde).

method, less sophisticated, but applicable to much larger systems, can be reliably employed to simulate the RR spectra of chromophores of this and larger sizes.

Of relevance to the prediction of activity in the T_1 RR spectra is the geometry change upon excitation to the T_n resonant state. The equilibrium structures of the three isomers in T_n were obtained by employing the QCFF/PI hamiltonian and the bond lengths of the conjugated framework are also reported in Fig. 5. It is seen that, similarly to the corresponding resonant triplet state in hexatriene, the bond lengths of the T_n state are comparable with one another. Moreover, it is worth noting that the excitation to the resonant state affects the CC bonds of the entire conjugated skeleton. Thus, it is expected that each isomer will show a unique vibrational structure in the RR spectrum, since the spectrum probes bond configurations located in the molecular region of maximum geometry change.

Accordingly, the RR spectra simulated for the three isomeric forms, presented in Fig. 6, look very different. Interestingly, the spectrum simulated for the *trans*-isomer agrees with the correspondingly observed spectrum [5]. The predicted spectrum shows a triplet of overlapping bands in the region below 1350 cm^{-1}, which is readily assigned to the triplet of bands observed below 1300 cm^{-1} [5]. The frequency overestimate of the simulated spectrum is a typical feature of the QCFF/PI method in this energy region [20]. Above 1350 cm^{-1}, the simulation presents a weak feature at ca 1420 cm^{-1}, which is assigned to the experimental 1398 cm^{-1} band. The intensity of this band is underestimated by the calculations because it is due to a methyl deformation. Since the saturated moieties are described, in the QCFF/PI hamiltonian, with simple empirical functional forms, it is known that their geometry change upon excitation is underestimated by the calculations. The strongest activity is pre-

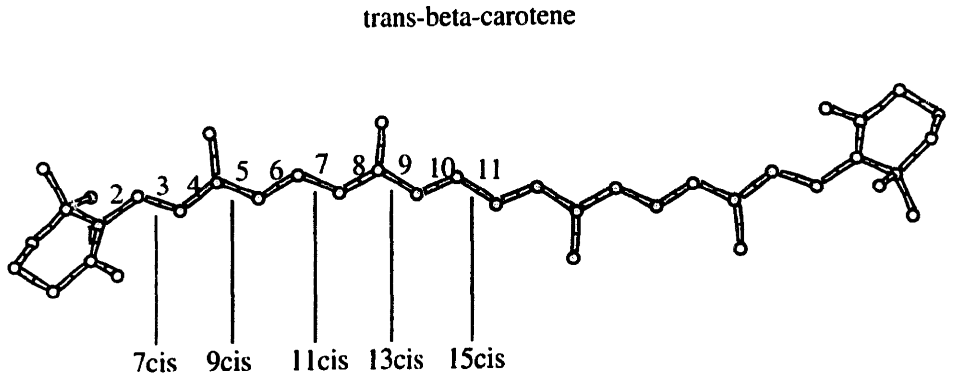

Fig. 7. Bond numbering for the six isomeric forms of β-carotene studied in this work.

dicted at ca. 1550 cm^{-1}, in agreement with the observed spectrum [5]. Finally, the QCFF/PI underestimate of the carbonyl frequency is responsible for the appearance of a shoulder below 1550 cm^{-1}, which must be assigned to the observed 1614 cm^{-1} band. In summary, the main features of the RR spectrum of *trans*-C15 aldehyde are nicely reproduced by the simulations. Thus, it seems unlikely that the dramatic difference between the simulated and observed spectra of the 7 *cis*- and 9 *cis*-isomers (see Fig. 6) is due to inaccuracies of the simulations.

To account for the differences between the observed and simulated spectra we need to understand the dynamics of C15 aldehyde in T_1 during the 5 ns available before the RR spectra of Ref. [5] were measured. To this end we map the T_1 potential energy surface by using the energies obtained from DFT–BLYP calculations. These predict the 9 *cis*- and the 7 *cis*-isomers to be 1.2 and 3.1 kcal mol^{-1} higher, than the *trans*-isomer, respectively. Based on the satisfactory results obtained at the same level of theory for hexatriene and stilbene [21] we believe that the above energy estimates are reliable. Thus, at variance with the conclusions drawn in Ref. [5], DFT–BLYP calculations indicate that the energies of the three intermediates are considerably different and that the most stable structure in T_1 is the *trans*-form.

To establish whether or not isomerization and equilibration has occurred in the spectra of Ref. [5] we need to know the barrier heights for interconversion among the three isomers. In order to get a qualitative estimate (more accurate DFT–BLYP calculations are in progress), we can compare the barriers computed for hexatriene and stilbene in T_1, and make use of a simple correlation between the bond length and the barrier height. The bond length of the central CC bond of HT is predicted by CASSCF and DFT–BLYP calculations to be 1.470 (1.465) Å, respectively, and the barrier height is of the order of half a kcal/mol [17]. Similarly, for stilbene, the central CC bond in T_1 is predicted to be 1.462 Å by DFT–BLYP calculations, and the computed barrier is of the same magnitude or lower than that computed for HT [21]. Back to C15 aldehyde, the bonds No. 8 and No. 10 (see Fig. 3 and Fig. 5) are computed by DFT–BLYP calculations to be 1.452 and 1.441 Å, respectively. These bonds are considerably elongated, compared to the ground state structure, and it appears reasonable to assume that the barriers to the *trans*-form will be lower than 6–7 kcal mol^{-1}. The scenario emerging from the shape of the potential energy surface of T_1 outlined above is that equilibration can take place during the 5 ns before the RR spectra are measured, and that the resulting spectra, very similar to one another, do indeed correspond to the spectrum of the equilibrated intermediates. In agreement with the computed energy differences, the dominant contribution to the RR spectrum is due to the *trans*-form. In the light of the present results, it would be of considerable interest to measure time-resolved T_1 absorption spectra and RR spectra of the isomers of C15 aldehyde with shorter laser pulses and different time delays, in order to monitor the time evolution of mono-*cis*-species.

3.3. β-carotene

Carotenoids in photosynthetic systems have the dual function of light harvesting and photoprotection [4]. All-trans carotenoids are mainly responsible for harvesting the light energy, while 15 *cis*-carotenoids bound to the photosynthetic reaction centre are mainly responsible for quenching singlet oxygen and for dissipating the energy into heat. In the latter function the lowest triplet state of β-carotene is involved.

The resonance Raman spectra of β-carotene were studied by several authors [2]. More recently, beside the spectrum of the *trans*-form, the spectra obtained from several mono-*cis*-isomers were also investigated [6–8]. The spectra obtained from the 7 *cis*-, 9 *cis*-, 13 *cis*- and 15 *cis*-forms look very similar to the spectrum of the *trans*-isomer [8]. Nevertheless, while the spectra of the 13 *cis*- and 15 *cis*-forms are identical to the trans spectrum, the spectra of the 7 *cis*- and 9 *cis*-isomers appear to be slightly different and were attributed to intermediates in T_1 that keep the initial *cis*-configuration [6,8].

Similarly to C15 aldehyde, we employed the QCFF/PI method to compute the equilibrium structures and vibrational force fields of the five mono-*cis*-isomers along with the *trans*-form (see Fig. 7). To simulate the vibrational structure of the T_1 RR spectra, the corresponding equilibrium structures in the resonant state T_n were also obtained. A survey of the geometrical change upon excitation to the T_n state is presented

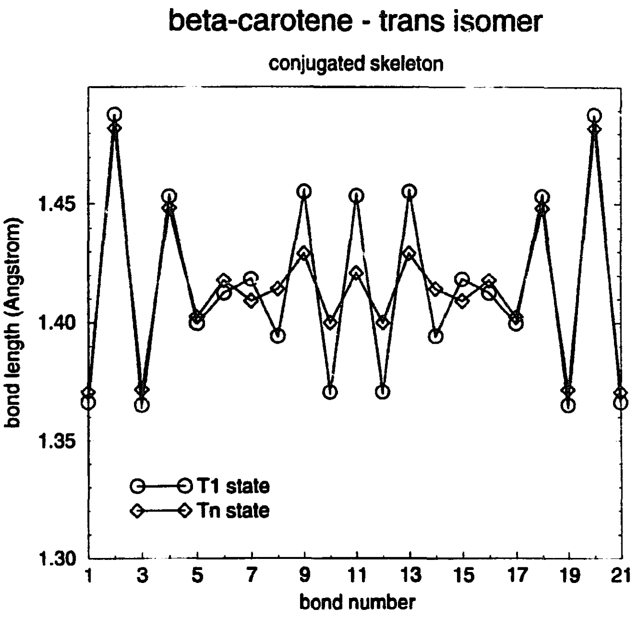

Fig. 8. Bond lengths of the conjugated framework of β-carotene, in T_1 and T_n.

in Fig. 8, where only the bond lengths of the conjugated framework are presented. The bond numbering in Fig. 8 corresponds to the numbering in Fig. 7. At variance with the homologue of retinal discussed in the previous section, the $T_1 \rightarrow T_n$ excitation in β-carotene is localized on the central part of the molecule. This implies that the RR spectrum of the 7 *cis*- and 9 *cis*-species, is expected to be similar to that of the *trans*-species, since the electronic excitation affects molecular regions far from the *cis*-configuration. Conversely, inspection of Fig. 8 suggests that the RR spectrum of the 13 *cis*- and 15 *cis*-structures will be different from that of the *trans*-form. The spectra simulated on the basis of computed molecular parameters are presented in Fig. 9. In agreement with the above discussion, the spectra simulated for the 7 *cis*- and 9 *cis*-forms are very similar to the simulation of the RR spectrum of *trans*-β-carotene A gradual change from the spectra of the 7 *cis*- to the 15 *cis*-isomers is also noticed and considerably different spectra for the 13 *cis*- and especially for the 15 *cis*-isomers are obtained.

The results of the simulations can be preliminarily discussed and compared with the observed spectra on the basis of the CC bond lengths of the T_1 equilibrium structures: As shown in Fig. 8 the CC bonds No. 3, 5, 7, 9 and 11 (see Fig. 7 for bond numbering) are computed to be 1.365, 1.400, 1.419, 1.455 and 1.453 Å, respectively. These bonds have a *cis*-configuration in 7 *cis*-, 9 *cis*-, 11 *cis*-, 13 *cis*- and 15 *cis*-isomers, respectively, and their lengths suggest that very high barrier will exist on the triplet surface for isomerization to the *trans*-form from the 7 *cis*- and 9 *cis*-isomers. Thus, it is plausible to assume that the corresponding T_1 intermediates will have not isomerized before the measurement of the RR spectrum (500 ps of pump-probe delay in Ref. [8]) and the spectra, though similar to the *trans*-form, are different. Conversely, interconversion of the 13 *cis*- and 15 *cis*- to the *trans*-form occurs because the corresponding bond lengths are considerably elongated in T_1 and the resulting spectra are identical for the three species. The simulations also suggest that the contribution of the 15 *cis*-form to the spectrum is negligible.

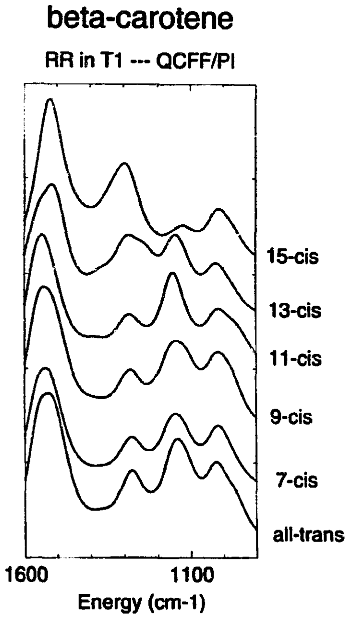

Fig. 9. Simulation of the T_1 RR spectra of the six isomeric forms of β-carotene.

4. Conclusions

The resonance Raman spectra of hexatriene, C15 aldehyde and β-carotene are simulated with the help of quantum chemical calculations of molecular structures and force fields in the lowest triplet state. While ab initio CASSCF and DFT-BLYP methods are used for the smaller molecule studied, a combination of semiempirical and density functional computations are employed for the larger systems.

Simulations based on ab initio CASSCF calculations show that the *trans*-form dominates the resonance Raman spectra of both *cis*- and *trans*-1,3,5-hexatriene. Similarly, it is suggested that the *trans*-species of C15 aldehyde is responsible for the spectra obtained from the three isomers. The preliminary results on β-carotene show that the resonance Raman spectra obtained from photoexcitation of 13 *cis*-, 15 *cis*- and *trans*-β-carotene are due to the equilibrated mixture of the three species and that the contribution of the 15 *cis*-form is negligible.

It is concluded that the combination of semiempirical and density functional calculations provides a reliable portrait of the details of the T_1 potential energy surfaces for chromophores of biological importance.

Acknowledgements

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