J. Am. Chem. Soc. **1981,** *103,* 1036-1041

Time-Resolved Resonance Raman Spectroscopy: The Triplet State of *all-trans*-Retinal

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Received July 14, 1980

Abstract: The resonance Raman spectrum of the short-lived lowest triplet state of *all-trans*-retinal in benzene and toluene, produced by naphthalene-sensitized electron-pulse radiolysis, is reported. Strong transient vibrational bands are found at 1550 and 1186 cm^{-1} and weaker ones at 1137, 1212, 1253, 1305, and 1339 cm^{-1}. The spectra indicate increased π-electron delocalization in the triplet state and suggest that the relaxed excited triplet state exists in either an all-trans or 9-cis conformation. The results are compared with resonance Raman spectra from intermediates in the photochemical cycle of bacteriorhodopsin. Similarities are found between the present spectrum and that of the bL$_{550}$ intermediate. Furthermore, the rate constant for triplet energy transfer from naphthalene to retinal was determined to $k = 5.5 \times 10^9$ M^{-1} s^{-1} and that of triplet–triplet annihilation of retinal to $2k = 6 \times 10^9$ M^{-1} s^{-1}.

Retinal is of importance in the process of vision and in the mechanism of light-to-energy conversion in bacteriorhodopsin. The primary light event in visual excitation involves the photochemical isomerization of the polyene aldehyde,[1,2] 11-*cis*-retinal, which is bound to the opsin protein of rhodopsin via a protonated Schiff base linkage. Photoisomerization may occur directly through the excited singlet state or through a triplet intermediate and leads ultimately, after a series of reactions which are not presently understood in detail, to the *all-trans*-retinal isomer and the opsin. Bacteriorhodopsin on the other hand, named for its structural similarity to rhodopsin, is the only protein present in the purple membrane of *Halobacterium halobium*.[3] The great interest in bacteriorhodopsin is due to its action as a photosynthetic system, pumping protons from the inside of the cell membrane to the outside[4] and thereby forming an electrochemical gradient which is thought to drive the synthesis of ATP. As in rhodopsin, in bacteriorhodopsin a retinal moiety is bound via a protonated Schiff base to a lysine residue on a large opsin protein. However, here the retinal is believed to exist as either the 13-cis or all-trans isomer and the protein is dissimilar, leading to different functions and mechanisms.

The photophysical properties of the triplet state of retinal-like polyenes and their intersystem crossing efficiency have been studied extensively.[5-26] In *all-trans*-retinal a strongly allowed triplet–triplet optical transition with its absorption maximum from 445 to 480 nm[5,6] depending on the solvent polarity is found both upon direct[5,7] and sensitized[8] flash-photolytic or pulse-radiolytic[7,9] excitation. The extinction coefficient in hexane at 450 nm is 7.4×10^4 M^{-1} cm^{-1}[9] and the absorption band is assigned to the $T_1 \rightarrow T_7$ ($\pi \rightarrow \pi^*$) transition[10,11] from the lowest triplet ($\pi\pi^*$) state lying 38 kcal mol^{-1}[8] above the ground state. No vibrational structure is observed either in the triplet–triplet or in the ground state absorption spectra. The decay of the lowest triplet state of the all-trans isomer is indicative of both first- and second-order processes with a solvent-dependent first-order rate constant $k_1 = (0.55\text{–}1.1) \times 10^5$ s^{-1}[5,7] and a second-order rate constant for triplet–triplet annihilation $2k_2 = 1.5 \times 10^{10}$ M^{-1} s^{-1}.[6] The triplet–triplet absorption spectra of different isomers are very similar to each other.[7,12] The yield of photoisomerization and triplet quantum yield upon irradiation has been the subject of a number of studies,[6,7,9,12-26] as the question of whether the path of isomerization proceeds via the singlet and/or the triplet state is of interest in biological systems. The quantum efficiencies of triplet formation Φ_{ISC} are relatively high (0.4–0.82) for retinals[6,7,9,13-16] at room temperature in nonpolar solvents, and in these systems an excitation wavelength dependence[14-16] is observed. The highest value ($\Phi_{\mathrm{ISC}} = 0.82$) was found for the highest excitation wavelength (423 nm).[16] The intersystem crossing efficiency is low in retinols, retinyl Schiff bases, and protonated retinyl Schiff bases.[6,13,20] Also, the Φ_{ISC} of retinals is small in polar solvents; this has been attributed to state ordering effects where the relative position of a low-lying[1] (n,π^*) state is of importance (for a recent discussion see ref 17). The high intersystem crossing yield of retinals makes it conceivable that the triplet state may contribute considerably to photoisomerization.[6,13,18-26] The yield of trans → cis photoisomerization has been shown to be low (0.04–0.2) in nonpolar and even lower (0.006) in polar solvents.[12,13,22-24,26] Unfortunately, the detailed results concerning the distribution of photoproducts[23,24] do not agree with each other. This cis → trans photoisomerization efficiency is considerably higher (0.2–0.25) in nonpolar and polar (0.04–0.05) solvents.[12,22] The question of whether isomerization by direct excitation involves excited singlet and/or triplet pathways has been discussed by several authors,[6,12,18,20] and different models have been proposed. Most recently, it was concluded by one of the authors[12] from laser flash photolysis studies that 11-cis to all-trans isomerization occurs via a nonrelaxed excited triplet state, which partitions between the relaxed 11-cis and all-trans triplet states during the exciting laser

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pulse while a singlet participation was excluded. The trans → cis photoisomerization process does not seem to occur via a triplet mechanism.[18]

Resonance Raman spectroscopy has proved to be a powerful tool in the study of biological molecules and of polyenes in particular. Rimai and co-workers[27-30] showed that the resonance Raman spectra of retinal are characteristic of the different conformations twisted around specific double bonds. Callender et al.,[31] essentially confirming the results of Rimai, pointed out, however, the possibility of photoisomerization prior to Raman scattering, which was eliminated by using a continuous-flow apparatus. Recently Cookingham et al.[32] provided a detailed analysis of the vibrational spectrum of retinal analogues based on substitutions, deuteration, and Schiff base linkages. Theoretical studies, in general agreement with experimental findings, have been reported by Warshel and co-workers.[33-35]

In recent years the technique of time-resolved resonance Raman investigations has been approached by different groups in different ways. Wilbrandt and co-workers[36-38] used pulsed excitation combined with optical multichannel detection in pulse radiolysis for the study of free radical anions and recently[39] reported on the first resonance Raman spectrum from a molecule (*p*-terphenyl) in the lowest excited triplet state. Other groups using various techniques reported on similar types of work.[40-42] Campion, Terner, and El-Sayed[43,44] used both continuous-flow and pulsed methods for the analysis of the photocycle of bacteriorhodopsin, and in the study of the primary photochemical event in visual pigments resonance Raman spectroscopy is able to provide very specific information.[45,46] Mathies[47] published an extensive review of this topic.

The resonance Raman spectrum of the lowest triplet state of β-carotene was reported recently by Dallinger et al.[48] and was reinvestigated by Jensen et al.[49] In this work, the resonance Raman spectrum of the lowest excited triplet state of *all-trans*-retinal is reported and discussed critically with respect to photolability on one hand and possible implications for bacteriorhodopsin photochemistry on the other. Furthermore, the rate of triplet energy transfer from naphthalene to retinal and the decay of the triplet state of retinal are investigated by optical absorption measurements. The work is based on the well-known[50,51] formation of naphthalene triplet states upon electron irradiation of benzene solutions and subsequent triplet energy transfer to solute molecules.

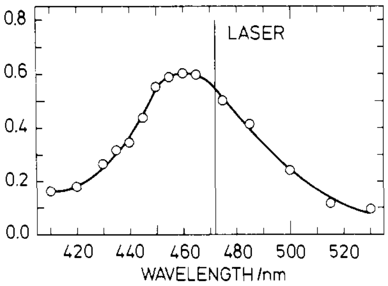

Figure 1. Triplet–triplet absorption spectrum of a 10^{-4} M solution of *all-trans*-retinal in benzene containing 10^{-2} M naphthalene, obtained by electron irradiation (path length = 2 cm).

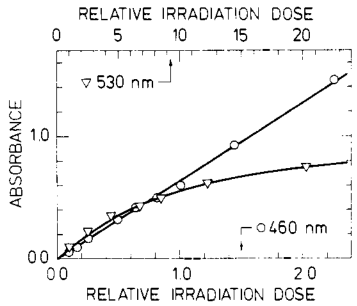

Figure 2. Maximum absorbance as a function of relative irradiation dose measured at 460 and 530 nm (path length = 2 cm).

Experimental Section

The electron irradiation facility and the optical multichannel detection system for optical absorption and resonance Raman experiments have been described in detail.[52] The previously used image intensifier was replaced by a magnetically focused EMI 9912 unit, thereby increasing the optical gain considerably. All Raman experiments were performed at an excitation wavelength of 472.01 nm, to which the dye laser was tuned by using coumarin 102 as laser dye. The laser pulse energy was 5 mJ and the laser beam was moderately focused to a diameter of 2 nm into the sample. The spectral band-pass of monochromator was set to 5 cm^{-1}; the total spectral resolution of the multichannel system, however, was 12 cm^{-1}. The wavenumbers that were determined are estimated to be accurate to ±4 cm^{-1} for the strong and to ±6 cm^{-1} for the weak vibrational bands. Optical absorption measurements were performed in a cell of 2-cm path length.

all-trans Retinal was supplied from Sigma and used without further purification. Naphthalene (scintillation grade) was obtained from Merck. Benzene (Merck p.a.) was passed through a column packed with neutral alumina (Woelm) before use. Toluene (Merck p.a.) was used without further purification. The 10^{-3} (resonance Raman work) or 10^{-4} M (optical absorption measurements) solutions of retinal in benzene or toluene containing 10^{-2} M of naphthalene as sensitizer in order to populate the polyene triplet state efficiently were flushed with high-purity Ar before using. A fresh solution was provided after each exposure to two electron pulses. First, the transient resonance Raman spectrum with and subsequently a background spectrum without electron irradiation were thus recorded twice with each solution. No differences (within experimental error) in the vibration spectra between the first and second pulse could be detected.

Results

Electron irradiation of an Ar-saturated benzene solution containing 10^{-2} M naphthalene and 10^{-4} M *all-trans*-retinal gave an optical absorption (Figure 1) with λ_{max} = 460 nm. The transient

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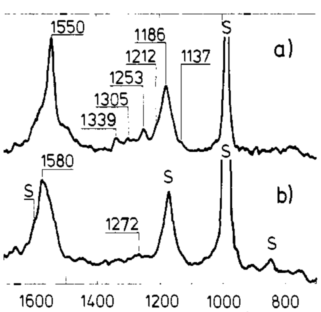

Figure 3. Resonance Raman spectra of a 10^{-3} M solution of *all-trans*-retinal in benzene containing 10^{-2} M naphthalene, Ar-saturated, excitation wavelength 472.01 nm: (a) obtained 1.4 μs after irradiation with an electron pulse; (b) obtained without electron irradiation. Central region (1100-1400 cm^{-1}) is averaged over nine, and other regions are averaged over four laser pulses.

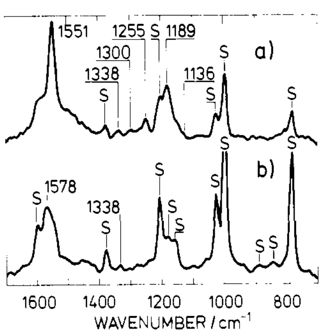

Figure 4. Resonance Raman spectra as in Figure 3 but in toluene.

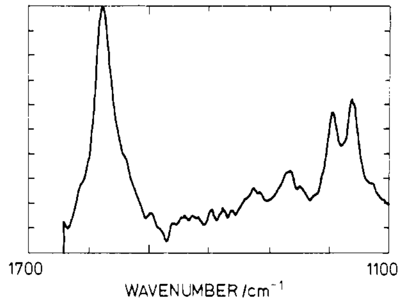

Figure 5. Resonance Raman spectrum of a 10^{-3} M solution of *all-trans*-retinal in CCl_4 without electron irradiation, air-saturated, excitation wavelength 472.01 nm.

decayed at low irradiation doses by a first-order reaction with $k_1 = 1.16 \times 10^5$ s^{-1}. Figure 2 shows a plot of maximum absorbance as a function of relative irradiation dose. The curvature of this plot at high doses indicates either an incomplete energy transfer from naphthalene to retinal and/or a rapid second-order decay process for triplet retinal competing efficiently with the rate of formation. In the first case, dose-dependent processes for the decay of triplet naphthalene such as triplet–triplet annihilation or enhanced spin inversion induced by paramagnetic species formed during the irradiation pulse could, with increasing irradiation dose, begin to compete with the triplet energy-transfer rate. In the second case, a high triplet–triplet annihilation rate for retinal triplet could be a process that partly offsets its formation rate. By numerical integration of a coupled differential equation system we fitted the rate constant (k_2) for triplet–triplet energy transfer from naphthalene to retinal and that ($2k_3$) for the second-order decay of retinal triplet to an experimentally determined plot of absorbance vs. time at a relative irradiation dose of 0.64. The G value and the decay constants for triplet naphthalene were used as previously described.[53] Excellent agreement with experimental findings was obtained by using rate constants $k_2 = 5.5 \times 10^9$ M^{-1} s^{-1} and $2k_3 = 6 \times 10^9$ M^{-1} s^{-1} and an extinction coefficient 6.4×10^4 M^{-1} cm^{-1}.[17] At very high doses the same set of rate constants reproduced the time dependence of the transient absorbance measured at 530 nm, but the simulated yield was ca. 40% lower than the experimental one. A reason for this could be the experimental interference of the absorbance of a benzene exciplex at 530 nm leading to an experimental value for the maximum absorbance which was too high. This point, however, was not studied in detail. At the highest irradiation doses (68 krad as calibrated previously[49]), the experimentally determined maximum absorbance corresponded to a maximum triplet state concentration of 4.7×10^{-5} M, calculated by using an extinction coefficient of 6.4×10^4 M^{-1} cm^{-1}. Our second-order rate constant $2k_3$ is somewhat lower than the value $2k_3 = 15 \times 10^9$ M^{-1} s^{-1} determined by Fisher and Weiss.[6]

Resonance Raman experiments were performed on 10^{-4} and 10^{-3} M Ar-saturated benzene solutions and 10^{-3} M Ar-saturated toluene solutions all containing 10^{-2} M naphthalene. From the decrease of scattered Raman intensity of solvent lines when increasing the retinal concentration from 10^{-4} to 10^{-3} M an increase of roughly a factor of 5 in transient retinal triplet concentration was estimated, thus resulting in a maximum value of 2.4×10^{-4} M of triplet state concentration. Hence approximately 20% of the parent compound was converted into triplets.

The recorded resonance Raman spectra obtained by irradiation of 10^{-3} M solutions of *all-trans*-retinal in Ar-saturated benzene and toluene containing 10^{-2} M naphthalene are shown in Figures 3 and 4, respectively. The upper trace spectra (a) were obtained 1.4 μs after irradiation with an electron pulse while the lower trace spectra (b) were recorded without electron irradiation. Each spectrum is composed of three individual multichannel spectra, centered at different wavenumbers. In the fingerprint region (1100–1400 cm^{-1}), spectra of nine laser pulses (changing solutions after each two electron pulses) were averaged by a computer; in the other regions (700–1100 and 1400–1700 cm^{-1}) only four laser pulses were averaged. All spectra except the low wavenumber region of Figure 3a (for comparison) were smoothed by a procedure subsequently fitting a fifth degree polynomial to each of seven data points, thereby calculating the smoothed value for the central data point. As many vibrational bands of ground-state retinal were masked by solvent lines, a reference spectrum in CCl_4 (air-saturated) was recorded under identical conditions as the transient spectra and is shown in Figure 5. This seemed necessary in order to establish the absence of photoisomerization. The recorded resonance Raman bands together with values for ground-state *all-trans*-retinal and assignments from the literature are summarized in Table I.

Discussion

(a) The Resonance Raman Spectrum of the Triplet State. *all-trans*-retinal in solution is known[54] to have a planar polyene chain with all the single bonds from C7 to C15 in the s-trans conformation. The plane of the β-ionone ring is twisted with respect to the chain in a distorted s-cis conformation about the C6–C7 single bond with the torsional angle in the range 30–70°. The polyene chain shows a distinct bond alternation with π-bond orders of approximately 0.9 for the double and 0.3 for the single bonds. Heyde et al.[29] pointed out the linear correlation which exists in a series of homologues between the optical absorption maximum and the C=C stretching frequency. With increasing delocalization of the π-electrons the absorption maximum shifts to longer wavelengths and the C=C π-bond order decreases.

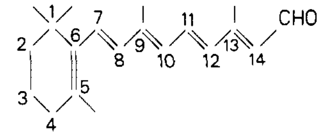

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Table I. Vibrational Wavenumbers (cm^{-1}) for the Ground State and Triplet State of *all-trans*-Retinal

ground state from literature		triplet state from this work		
a	*b,c*	*b*	*d*	description[e]
970 w				C–C–H bend out of plane
1009 m				C–CH$_3$ stretch
		1137 vw?	1136 vw?	C14–C15 stretch
1167 s	1165	1186 vs	1189 vs	C–C (C9–C13) stretch
	1179			
1201 s	1199	1212 w		C–C stretch + C9,C13 Me rock
1272 m	1271	1253 w	1255 w	C–C–H bend + C=C stretch
	1283	1305 vw	1300 vw	
1338 m	1336	1339 w	1338 w	C13 sym Me deform
1397 vw				C9 sym Me deform
1451 vw				C9 and C13 asym Me deform
	1549			
1582 vs	1580	1550 vs	1551 vs	C=C stretch
1671 w	1662			C=O stretch

[a] In CCl$_4$ (from ref 31). [b] In benzene. [c] From ref 29. [d] In toluene. [e] According to ref 32.

Extended use of this relation has since been made for predicting either absorption maxima from vibrational frequencies or vice versa in the intermediates of the biological systems. On the basis of experimental substitution methods, Cookingham et al.[32] gave a detailed vibrational assignment for retinal, which the following qualitative discussion will make use of.

The most prominent wavenumber change of *all-trans*-retinal when going from the ground state to the triplet state is the observed decrease of the C=C stretching frequency by approximately 30 cm^{-1}. A corresponding shift was seen in the spectrum of triplet β-carotene,[48,49] where a decrease of 25 cm^{-1} was measured. This shift in wavenumber from 1580 to 1550 cm^{-1} for retinal fits well into the linear plot of vibrational wavenumber vs. absorption transition energy for *all-trans*-retinal in different solvents;[29] this is somewhat surprising in view of the different mechanisms involved. It indicates, however, that π-electron delocalization is the common cause for the shift in both λ_{max} and C=C stretching frequency when exciting *all-trans*-retinal to the triplet state, as it is the case for solvent-induced bathochromic wavelength shifts. An additional indication of the importance of π-electron delocalization is the fact that the C=C stretching vibrational band is the strongest band in the resonance Raman spectrum of the ground state as well as in the triplet state; this infers a considerable change of C=C bond length both upon $S_0 \rightarrow S_1$ and $T_1 \rightarrow T_7$ excitation. A factor contributing to the higher extent of delocalization of π-electrons may also be a decrease of torsional angle[33] between the plane of the ionone ring and the polyene chain upon excitation, providing a further conjugation between the ring and the chain. This would be in agreement with the findings of Cookingham et al.[32] who found that a removal of the methyl group in position 5 leads to a frequency decrease of the 1580-cm^{-1} band by diminishing the steric repulsion between the 5-CH$_3$ and 8-H and thereby increasing the degree of planarity.

The other strong vibrational feature in the resonance Raman spectrum of the triplet state of retinal is a band at 1186 cm^{-1}. While in the ground state, two vibrational bands of comparable intensity at 1165 and 1199 cm^{-1} are seen; in the triplet state only one strong feature with a frequency between the two ground state bands was found with a possible weak shoulder at 1212 cm^{-1}. Although strong solvent lines in this region of the spectrum partly mask the transient bands, it should be noted that the solvent lines are attenuated approximately by a factor of 3 upon electron irradiation as can be seen from other spectral regions. This leads to the consistent finding of only one strong band, but with possible weak bands eventually masked by the solvents. The question, however, as to what type of normal mode should the band be assigned is less clear. The 1199-cm^{-1} band of the ground state has been attributed[32] to a mixture of C—C single bond stretching and in-plane methyl rocking of the methyl groups at the 9- and 13-positions, while the 1165-cm^{-1} band is probably due to a symmetric stretching motion of the C7 and C13 carbon atoms in the isoprenoid chain. With the assumption that the potential energy distribution of vibrational modes is the same in the excited state as in the ground state and that the contribution from the C—C stretching mode is the dominant one in these bands, a considerable increase in the wavenumber of these modes upon excitation would be expected. Here we must take into account the large decrease in C—C single bond length in the excited states compared with that in the ground state predicted theoretically by several authors.[33,55-57] It seems reasonable to assume, therefore, that the strong band at 1186 cm^{-1} is shifted upward by 19 cm^{-1} with respect to the ground-state band at 1167 cm^{-1} and that the possible transient band at 1212 cm^{-1}, which may be somewhat underestimated in wavenumber, as it is seen only as a shoulder, is shifted by 13 cm^{-1} from the ground state at 1199 cm^{-1}. The change in intensity pattern when going from the ground state to the triplet state can hardly be explained by the present experimental observations. However, it should be noted that the band at 1201 cm^{-1} (in CCl$_4$) has been shown[32] to be sensitive to the nature of the substituents at C9 and C13. When either one of the methyl groups is removed, the band at 1201 cm^{-1} undergoes an increase in frequency and a decrease in intensity. As noted above removal of methyl groups may lead to changes in equilibrium geometry due to the changes in the steric interactions. Thus, the decreased intensity of the 1212-cm^{-1} triplet band compared with the 1199-cm^{-1} ground-state band is most likely due to changes in equilibrium geometry induced by the electronic excitation. On the other hand as the band at 1165 cm^{-1} (ground state) is caused mainly by symmetrical stretching of the central C—C single bonds, it may gain intensity in the triplet state because of the greater extent of π-electron delocalization. As the wavenumber shift of this band in the triplet state is considerably lower than that of the C=C double bond stretching mode, it may indicate that other motions like C—C—H bending may be admixed to this mode as has been noted previously[58] for carotenoids.

Considering the transient band from the triplet state at 1339 cm^{-1}, it should be noted that a corresponding band in the ground state of *all-trans*-retinal is seen at 1336 cm^{-1}. It is markedly changed neither in frequency nor in intensity when going to the triplet state. This mode has been assigned[32] to a symmetric methyl deformation in the 13-position. In different isomers (ground state) it appears at different wavenumbers (13-cis, 1316 and 1352 cm^{-1}; 11-cis, 1345 cm^{-1}; 9-cis, 1329 and 1337 cm^{-1}). With the assumption that the assignment given is adequate, no shift of the band at 1336 cm^{-1} due to the electronic excitation alone would be expected. Thus the position of the band in the triplet spectrum may be used as an indicator of the triplet state configuration. The observed band at 1339 cm^{-1} indicates an all-trans or 9-cis configuration of the triplet state. A 9-cis conformation would be favored by the following argument. A very weak vibrational band seems to be observed in the triplet state at 1137 cm^{-1}. No corresponding vibrational band in the ground state of *all-trans*-retinal is seen in this spectral region, while all of the 9-, 11-, and 13-cis isomers have a band that occurs between 1114 and 1133 cm^{-1}. This band has been attributed[32] to a C—C stretching vibration associated with the C—C single bond adjacent to the cabonyl group, the position of the cis double bond having an influence on the intensity. The appearance of a transient band at 1137 cm^{-1} could, together with the 1339-cm^{-1} band, be an indication of a 9-cis conformation in the triplet state of *all-trans*-retinal. This would infer an upward shift of 20 cm^{-1} for the terminal C—C

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bond stretching frequency and a much stronger shift (from 1147 to 1186 cm^{-1}) for the symmetric single bond stretching frequency discussed above, but in this case compared with the ground state of 9-cis-retinal. We previously found a possible indication for a central cis double bond in the triplet state of *all-trans-β*-carotene[49] and note that theoretical calculations[33,55-59] have predicted such an effect for the triplet states of polyenones. They also predict the largest change in C—C single bond length upon excitation toward the middle of the polyene chain, explaining the much larger shift of the C—C single bond stretching frequency associated with the bonds from C9 to C13 than with the C14—C15 bond.

The remaining two bands at 1253 and 1305 cm^{-1} in the triplet state of retinal are believed to correspond to the ground state bands at 1272 and 1283 cm^{-1}, respectively. Cookingham et al.[32] found that the separation of these modes grows larger with increasing electron delocalization and noted that normal modes with mixed C—C—H deformations with C═C stretching vibrations would be observed at these frequencies. The origin of these bands was located at the central portion of the isoprenoid chain. These authors measured a shift from 1272 to 1266 and 1283 to 1291 cm^{-1} in *all-trans*-retinal upon desmethylation of the *β*-ionone ring at the 5-position; they attributed this to increased planarity and hence *π*-electron delocalization between the *β*-ionone ring and the isoprenoid chain. This assignment would thus infer a shift of 19 and 22 cm^{-1} for these two bands, respectively.

(b) Photolability. As has been pointed out by several authors, care must be taken to avoid substantial photoisomerization of the photolabile visual pigments such as retinal during the recording of a Raman spectrum. A rough estimate of such effects under our experimental conditions shall therefore be made. The fraction F of *all-trans*-retinal photolyzed during a laser pulse with energy E (number of photons) focused in the Raman cell on an area A is given[47] by eq 1,

$$F = 1 - \exp\left(-\frac{2.303 \times 10^3)E\epsilon\Phi}{AN_0}\right) \qquad (1)$$

where ϵ is the molar decadic extinction coefficient at the laser wavelength, Φ is the quantum efficiency of photoisomerization, and N_0 is the Avogadro number. Using our experimental values for the laser pulse, an extinction coefficient of *all-trans*-retinal in the ground state of 120 $M^{-1}\ cm^{-1}$, and a total quantum yield of trans → cis photoisomerization of 0.08,[23] we obtain $F = 0.01$. Hence, it seems that photoisomerization from the ground state can be neglected, in agreement with our experimental results in Figure 5, where no bands from any of the cis isomers are seen.

Another photoinduced process may be an accumulation of molecules in the triplet state, implying some depletion of the ground state at our high irradiation intensity. The stationary triplet state concentration can be estimated from the relation (2),

$$\frac{dR_T}{dt} = I\sigma_0\Phi_{ISC}R_G - R_T/\tau_T \qquad (2)$$

where R_T is the concentration of triplet states, I is the laser intensity (photons/cm^2 s), σ_0 is the absorption coefficient at the laser wavelength, Φ_{ISC} is the intersystem crossing quantum yield, R_G is the concentration of ground state molecules, and τ_T is the triplet lifetime. In the air-saturated solutions (Figure 5) the triplet lifetime is of the order of 100 ns; hence a stationary distribution of molecules between the ground state and the triplet state (neglecting the excited singlet state because of its short lifetime) can be assumed leading to the fraction F_T of molecules in the triplet state.

$$F_T = \frac{1}{1/I\sigma_0\tau_T\Phi_{ISC} + 1} \qquad (3)$$

From $\Phi_{ISC} = 0.82$[16] we obtain $F_T = 0.015$. This corresponds to approximately 8% of the transient triplet state concentration generated by electron irradiation; thus, a considerable number of molecules are excited to the triplet state by the laser pulse itself. This is probably the reason for the observation of shoulders or very weak bands in the background spectra of Figures 3 and 4 at 1550 cm^{-1} and in the spectrum of Figure 5 at 1550, 1255, and 1133 cm^{-1}. All these bands belong to the triplet spectrum discussed above.

Finally, the question of whether some photoisomerization may be induced by triplet–triplet absorption of laser light is difficult to answer. To check this point it would probably be necessary to reduce the laser power by several orders of magnitude; this is because the high extinction coefficient for triplet–triplet absorption even of events with very low quantum yield could play a significant role. Unfortunately, this is beyond the capabilities of our experimental system, as a large number of pulses would be needed in order to record a Raman spectrum; this, however, is prevented by the low repetition rate of our electron accelerator. It should be noted, however, that even in the case of some photoisomerization from the triplet state, probably only those species that absorb strongly at the laser wavelength would be detected. Thus, we feel rather confident that our present results can be ascribed entirely to the triplet state of *all-trans*-retinal.

(c) Implications for the Cycle of Bacteriorhodopsin. In this section we wish to point out some similarities between our triplet state resonance Raman spectrum and some of the intermediates found in the photochemical cycle of bacteriorhodopsin. As mentioned previously, bacteriorhodopsin is believed to contain *all-trans*-retinal as chromophore. El-Sayed and Terner investigated the series of intermediates occurring after the irradiation of bacteriorhodopsin with visible light,[43,44,59] and Lewis[46] and Stockburger et al.[60] reported on similar results.

The light-adapted bR_{570} shows bands at 1255 and 1305 cm^{-1}. In the triplet state, bands are observed at the same positions and they are tentatively assigned to originate from the ground state at 1272 and 1283 cm^{-1}. These bands were shown[32] (in the ground state) to be insensitive to substitutions at the aldehyde end of the isoprenoid chain and therefore have been suggested to be characteristic for the modeling of visual pigments and possibly bacteriorhodopsin. A further feature of bR_{570} which resembles our spectrum is the existense of a band at 1137 cm^{-1}. Both the 1137- and 1255-cm^{-1} bands disappear upon deprotonation in the M_{412} species while the 1305-cm^{-1} band remains unchanged. A single strong band at 1194 cm^{-1} (found at 1190 cm^{-1} by Stockburger et al.[60]) appears in the bL_{550} intermediate, and at the same time a shoulder at 1550 cm^{-1} in the C═C stretching region grows up.

Thus, the number of coincidences (which is not found in any of the transient spectra of rhodopsin) seems to increase in going from the bR_{570} to the bL_{550} intermediate. Indeed, the resonance Raman spectrum of bL_{550} resembles our triplet state spectrum rather well, with the exception of the bands at 1011 and 971 cm^{-1} which could be masked by solvent lines in the spectrum of the triplet state, and of a band at 1381 cm^{-1}, possibly masked in toluene and not found in benzene. The band at 1275 cm^{-1} in bL_{550} is very weak, and some indication of a very weak band at that position is observed in benzene. A participation of the triplet state in the cycle of bacteriorhodopsin has previously been rejected on the basis of the low intersystem crossing efficiencies in protonated Schiff bases of retinal. The interaction with the protein, which is generally assumed to be stronger than in rhodopsin, could possibly induce some triplet-like structure in the chromophore. However, as a discussion in this direction probably would be purely speculative, we restrict ourselves to the descriptive findings given above.

Conclusion

The resonance Raman spectrum of the lowest triplet state of *all-trans*-retinal produced by electron irradiation of benzene and toluene solutions containing naphthalene has been investigated. Tentative assignments were given pointing out an increase in *π*-electron delocalization and noting the possibility of either an

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all-trans or 9-cis conformation the triplet state. The problem of photolability was discussed with emphasis on the present experimental conditions. The triplet state spectrum obtained was compared with those of intermediates occurring in the photocycle of bacteriorhodopsin. A close resemblance was found between the vibrational spectrum of the bL$_{550}$ intermediate as reported by El-Sayed and co-workers and our triplet spectrum. Furthermore, the rate constant for triplet energy transfer from naphthalene to *all-trans*-retinal has been determined to $k_2 = 5.5 \times 10^9$ M^{-1} s^{-1} and the second-order rate constant for triplet–triplet annihilation was found to be $2k_3 = 6 \times 10^9$ M^{-1} s^{-1}.

Acknowledgment. We thank the laboratory of physics II at the Technical University of Copenhagen for generously letting us borrow their EMI image intensifier and are grateful to Anne Nielsen for skillful technical assistance.

Free Radical Induced Nuclear Magnetic Resonance Shifts: Comments on Contact Shift Mechanisms

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Abstract: Experiments designed to test the various mechanisms proposed by Morishima et al. (*J. Am. Chem. Soc.*, 1970–1975) for the interaction of the organic radical di-*tert*-butyl nitroxide (DBNO) with various organic substrates are reported. Cyclohexane shows a strongly solvent-dependent interaction with the radical and is therefore unsuitable as an internal standard. Charge transfer, involving π systems, does not seem to be an important mechanism for induced shifts in benzophenones, as there is no evidence of ketyl formation. However, charge transfer may occur from nitroxide to *m*-dinitrobenzene. The change in bulk magnetic susceptibility of the solutions due to the addition of radical was found to cause significant shifts of NMR signals. The various models for interaction between radical and substrate are reviewed.

The effect of paramagnetic metal ions on the ^{1}H NMR chemical shifts in various organic molecules and metal complexes has been studied by Eaton[1] and by Drago[2] and their respective co-workers in the 1960's and early 1970's. LaMar[3] and Kreilick[4] have also applied NMR to studies of a variety of paramagnetic systems. More recently Roberts and co-workers[5] and Goff[6] have elucidated the effects of paramagnetic ions on ^{13}C spectra of organic ligands. Richards, Dwek, and Poindexter[7-9] and Mueller-Warmuth[10] have studied the unpaired electron distribution in paramagnetic organic systems by unique experiments involving NMR observation of microwave-irradiated molecules. Evidence for both scalar mechanisms (where odd-electron density is transmitted by polarization of bonding electrons) and dipolar mechanisms (through space) was given.

With regard to the ^{13}C mode of investigation, Morishima and co-workers[11] have recently published a series of papers on the chemical shift changes observed in a variety of molecules induced by radicals such as di-*tert*-butyl nitroxide (DBNO). Generally, a Fermi contact (scalar) interaction was invoked because of a Curie law dependence of the chemical shift change with T^{-1}. However, it is unclear whether a Curie law dependence necessarily excludes contributions from pseudocontact shifts. Horrock's work suggests that it does not.[12] In one case, Morishima did in fact propose a pseudocontact interaction.[13]

Several mechanisms were considered by Morishima and co-workers to explain the DBNO-induced shifts: (1) Hydrogen bonding between DBNO and the OH or CH bonds of the substrate; in other work, the term "spin polarization" was applied. The two terms appeared to be used interchangeably.[11a–d] (2) A spin delocalization mechanism, in which the radical donates an electron to an antibonding orbital of the substrate; i.e., to the antibonding orbital of C–X bonds (X = F, Cl, Br, or I).[11c] (3) In later work, a direct interaction between the radical and p orbitals was considered to lead to negative "spin density" on aromatic or olefinic carbons.[11a]

Morishima's work and our own experience have shown that acidic hydrogens (in the hydrogen bonding sense) undergo upfield shifts in the presence of DBNO, whereas nonacidic hydrogens exhibit a complex behavior that is not understood.[14] In some cases, no shift at all for ^{1}H is observed, although the attached ^{13}C is shifted. Due to the broadness of the ^{1}H spectra and the complexity of the spectra obtained in annular-tube experiments, ^{1}H-induced shifts will not be considered in this paper.

Our interest in ^{13}C contact shifts arose from reports by Morishima and co-workers of a correlation between contact shifts and spin–spin coupling constants.[11e] The conformational dependence of certain DBNO-induced shifts was shown to be similar to the well-known W effect in spin coupling.[13] Thus, it seemed possible

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