

## From polyenals to retinal: An electron-spin-echo study of the triplet state

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*Abstract* - The lowest triplet state  $T_0$  of all-trans polyenals dissolved in polyethylene films has been investigated by pulsed laser excitation in combination with electron-spin-echo spectroscopy, both in a magnetic field and in zero field. It is found to be a  $^3\pi\pi^*$  state and the orientation of the fine-structure axes, the zero-field energies of the spin sublevels and their relative populating rates and decay rates have been determined for a number of unsubstituted polyenals, methyl-substituted analogues and retinal. Variations of these properties with the number of carbon-carbon double bonds and the substitution pattern will be discussed. As compared to the unsubstituted conjugated chains, methyl substitution hardly affects the zero-field splitting and the relative populating rates but accelerates the radiationless decay. The near equivalence of the triplet data for retinal and 3,7,11-trimethyl-2,4,6,8,10-dodecapentaenal shows that the triplet excitation in retinal extends over all six double bonds which suggests that the chromophore of retinal is more or less planar in  $T_0$ .

### INTRODUCTION

Molecular triplet states have been successfully studied by electron paramagnetic resonance techniques already for several decades. Initially, states with a lifetime of a tenth of a second or more were investigated for which enough steady-state population can be built up under continuous illumination to allow for the use of conventional continuous-wave EPR techniques. New possibilities arose when it was realized that transitions between spin sublevels can be detected indirectly and with much greater sensitivity through changes in the phosphorescence. Optical detection of magnetic resonance has become a very versatile technique in the study of triplet states and its applicability is not lifetime limited. A class of interesting molecular triplet states, however, combines a short lifetime with a radiationless decay to the ground state. For such cases neither cw EPR, nor phosphorescence detection can be applied and the study of those triplet states benefits from the development of time-resolved EPR.

Besides other techniques, electron-spin-echo (ESE) spectroscopy has become available and applied to photo-excited triplet states [1]. Appreciating this opportunity, we have studied pyridine in an attempt to explain the virtually non-radiative character of its triplet state. Application of ESE spectroscopy in combination with pulsed laser excitation has enabled a detailed study of this until then elusive state and pyridine was found to undergo a profound structural change upon excitation. While being planar in the ground state, pyridine adopts a non-planar boat-like structure in the lowest triplet state and a description of the electronic excitation in terms of  $n\pi^*$  or  $\pi\pi^*$  is no longer meaningful [2].

Inspired by this success we have embarked upon a study of all-trans polyenals. These molecules consist of a chain of alternating single and double carbon-carbon bonds ending in an aldehyde group. From its composition one would judge this chromophore to be a relatively simple one but the characterization of its triplet state still presents a challenge to spectroscopists, not the least because phosphorescence has not been observed [3]. We have recently shown that a study of the electronic structure and the dynamic properties of the lowest triplet state  $T_0$  of polyenals is feasible by the use of electron-spin-echo (ESE) spectroscopy [4]. Here we report and discuss results obtained for the all-trans

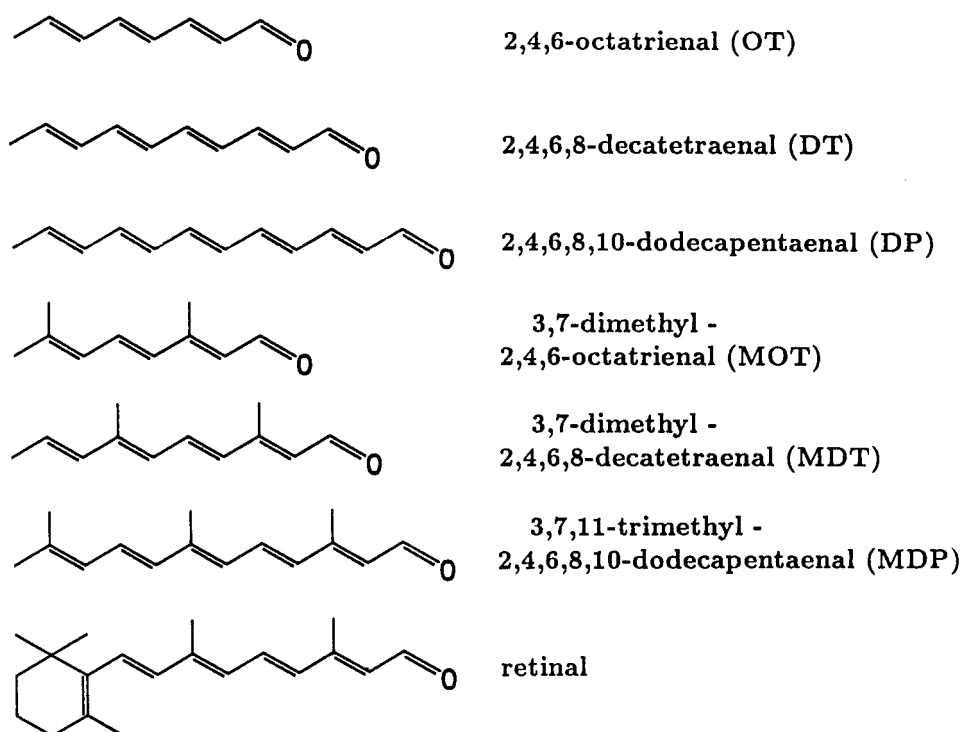


Figure 1: *The all-trans polyenals studied: structures, names and abbreviations.*

isomers of 2,4,6-octatrienal (OT), 2,4,6,8-decatetraenal (DT), 2,4,6,8,10-dodecapentaenal (DP), some methyl-substituted analogues (MOT, MDT and MDP), and retinal (fig.1). While the unsubstituted molecules are of interest because they may be considered model compounds for linear conjugated systems, retinal has been studied because this molecule is involved in fundamental processes in nature. Its protonated Schiff base is the chromophore in rhodopsin (11-cis retinal) and bacteriorhodopsin (all-trans retinal), key proteins in vision and bacterial photosynthesis. The methyl-substituted molecules have been included in order to bridge the gap between the unsubstituted ones and retinal.

#### ELECTRON-SPIN-ECHOES AND ZERO-FIELD ENERGIES

In the ESE experiments a laser flash excites the polyenals and after a certain delay time  $t_d$ , two microwave pulses are applied separated by a time  $\tau$ . If the microwave frequency is resonant with a transition between two of the triplet spin sublevels, the system may respond with an outburst of microwaves, the echo, at another time  $\tau$  after the second pulse. Experiments were performed at 1.2 K in a magnetic field and in zero field. For those in zero field the microwave frequency could be varied between 1 and 4 GHz, for those in a magnetic field this frequency was fixed at 9.35 GHz. Samples were optically excited at 355 nm by the third harmonic of a Quanta Ray DCR-2 Nd:YAG laser or at 308 nm by a Lambda Physik XeCl excimer laser using pulses of 2 to 5 mJ. For details of the experimental set-up we refer to [5].

Because the spin sublevel structure of the triplet state for a molecule in a magnetic field depends on the direction of this field with respect to the molecule, one would like to study an assembly of identically oriented molecules. So far though it has not been possible to incorporate polyenals into host single crystals. We dissolved them into polyethylene films that were stretched to approximately 500% and in this way we achieved partial alignment. The orientation distribution is uni-axial, the unique direction being the stretch direction.

Upon optical excitation of such samples into the singlet manifold, ESE signals have been observed for all polyenals listed in fig.1. Triplet EPR spectra have been recorded by monitoring the echo intensity as a function of the strength of the magnetic field. The polyenal in polyethylene samples have cylindrical symmetry and the EPR spectra only depend on the orientation of the magnetic field  $\vec{B}_0$  with respect to the stretch direction  $\vec{s}$  of the polymer film. Consequently, the study of the

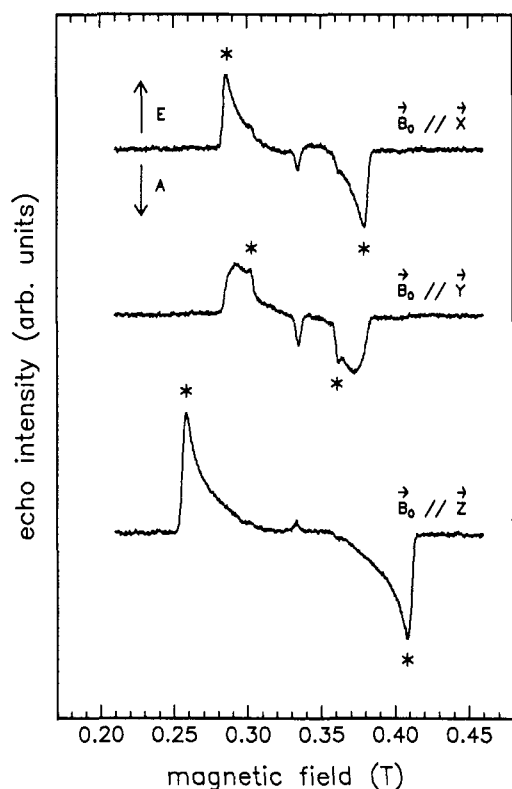


Figure 2: ESE-detected EPR spectra of trimethyldodecapentaenal in a stretched polyethylene film for  $\vec{B}_0$  parallel to the canonical orientations. The resonance fields of the peaks marked with an asterisk are stationary with regard to small variations in the direction of the magnetic field. E denotes emission, A absorption of microwaves. The microwave frequency is 9356 MHz,  $t_d$  is 300 ns and  $\tau$  is 500 ns.

spectra as a function of magnetic-field orientation can be restricted to one plane containing  $\vec{s}$  for which we took the plane of the film. As an example we show in fig.2 the ESE-detected EPR spectra of MDP for three orientations of  $\vec{B}_0$  with respect to  $\vec{s}$ . These spectra resemble those previously obtained for the unsubstituted polyenals [5]. They show emission (at low fields) and absorption (at high fields) of microwaves over a wide range of magnetic-field strength and consist of relatively sharp features (indicated by an asterisk) and broad shoulders. Qualitative considerations [4] and numerical simulations based on a Gaussian orientation distribution of the molecules in the film around  $\vec{s}$  prove that the ESE-detected EPR spectra derive from the triplet state.

The three spectra in fig.2 correspond to the so-called canonical orientations of  $\vec{B}_0$  in the plane of the film, for MDP at angles of  $90^\circ \pm 2^\circ$ ,  $74^\circ \pm 4^\circ$ , and  $16^\circ \pm 2^\circ$  with respect to  $\vec{s}$ . These directions are indicated by  $\vec{X}$ ,  $\vec{Y}$ , and  $\vec{Z}$ , respectively, because in each of these cases the magnetic field is parallel to one of the principal axes of the fine-structure tensor of those perfectly aligned molecules that have this principal axis in the plane of the film. From the three canonical orientations of  $\vec{B}_0$  with respect to  $\vec{s}$  observed for each of the unsubstituted and methyl-substituted polyenals, we have derived the orientation of the fine-structure principal axes with respect to  $\vec{l}$ . Here  $\vec{l}$  represents the direction within the polyenal molecule that is oriented parallel to the stretch direction  $\vec{s}$  of the film for a perfectly aligned molecule. From linear-dichroic absorption spectroscopy it was concluded that for this type of molecules  $\vec{l}$  coincides with the direction of the polyene chain [6], which is in agreement with our observation that the degree of alignment in the stretched films decreases the shorter the chain becomes.

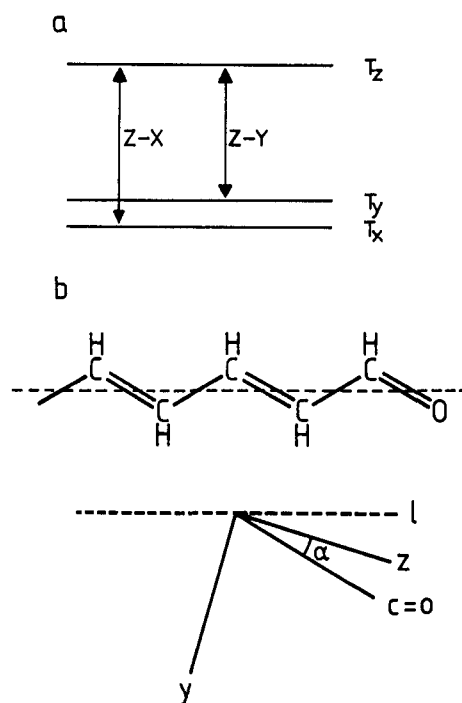
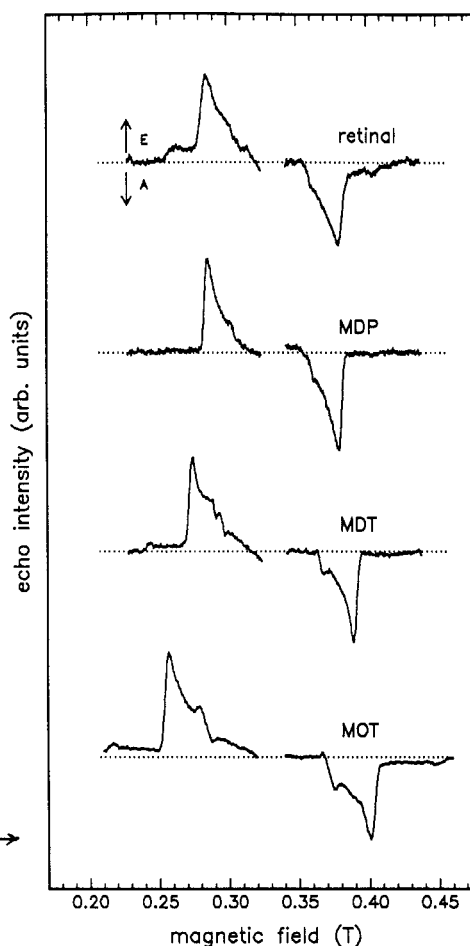


Figure 3: a. Spin sublevel ordering of the  $^3\pi\pi^*$  state for the all-trans isomers of the polyenals and retinal. b. The orientation of the  $\vec{y}$  and  $\vec{z}$  principal axes of the fine-structure tensor with respect to the polyenal chain. The  $\vec{x}$ -axis is perpendicular to the direction  $\vec{l}$  of the polyene chain.

Table 1: Frequencies of the observed zero-field transitions and the angle  $\alpha$  (cf. fig. 3) for the polyenals and retinal.

	(Z-X)/h (MHz)	(Z-Y)/h (MHz)	$\alpha$
OT	3862 $\pm$ 4	3387 $\pm$ 3	10 $^\circ$ $\pm$ 2 $^\circ$
DT	3041 $\pm$ 4	2620 $\pm$ 3	12 $^\circ$ $\pm$ 2 $^\circ$
DP	2496 $\pm$ 3	2125 $\pm$ 3	14 $^\circ$ $\pm$ 2 $^\circ$
MOT	3625 $\pm$ 10	3130 $\pm$ 10	11 $^\circ$ $\pm$ 2 $^\circ$
MDT	2870 $\pm$ 15	2460 $\pm$ 10	12 $^\circ$ $\pm$ 2 $^\circ$
MDP	2360 $\pm$ 10	1995 $\pm$ 10	14 $^\circ$ $\pm$ 2 $^\circ$
Retinal	2370 $\pm$ 15	1990 $\pm$ 40	

Figure 4: ESE-detected EPR spectra of retinal and the methyl-substituted polyenals in a stretched polyethylene film for  $\vec{B}_0$  perpendicular to  $\vec{s}$ , i.e. parallel to the canonical  $\vec{X}$  direction. E denotes emission, A absorption of microwaves. The microwave frequency is 9356 MHz,  $t_d$  is 300 ns and  $\tau$  is 500 ns.



The orientation of the fine-structure axes system in the polyenals is schematically represented in fig. 3. It is found that  $\vec{x}$  is perpendicular to the plane of the molecule,  $\vec{y}$  and  $\vec{z}$  are in this plane while the angle between  $\vec{z}$  and  $\vec{\ell}$  slightly decreases with increasing length of the polyenal chain. Analysis of the relative populating rates of the  $|T_y\rangle$  and  $|T_x\rangle$  sublevels shows that  $\vec{z}$  intersects the smaller angle between  $\vec{\ell}$  and the direction of the carbonyl bond [5]. The angle  $\alpha$  between  $\vec{z}$  and the direction of the carbonyl bond follows from the mutual orientation of  $\vec{z}$  and  $\vec{\ell}$  under the assumption that  $\vec{\ell}$  makes an angle of 30 $^\circ$  with the carbonyl bond. Values for  $\alpha$  are given in table 1.

In fig. 4 we compare the EPR spectra of the methyl-substituted polyenals MOT, MDT, and MDP, and retinal at the same orientation of  $\vec{B}_0$  with respect to  $\vec{s}$ . As regards the main features, the spectra are similar in shape but cover an ever wider range of magnetic field strengths the shorter the polyenal chain which refers to an increasing zero-field splitting. For retinal and MDP, which have the same number of double bonds, the maxima (and minima) in the spectra appear at virtually the same magnetic field.

From the EPR spectra for different orientations of  $\vec{B}_0$  with respect to  $\vec{s}$  estimates of the sublevel splitting have been obtained for all polyenals and subsequently the  $T_z - T_x$  and  $T_z - T_y$  (cf. fig. 3) transitions have been observed in zero field. The corresponding frequencies are summarized in table 1. The sublevel ordering as indicated in fig. 3 results from the dynamic experiments to be described below. We find  $|T_z\rangle$  to be the upper sublevel, and  $|T_y\rangle$  and  $|T_x\rangle$  to be close in energy for all polyenals.

The sublevel ordering, its independence of chain length, and the zero-field splittings indicate that the lowest triplet state of the polyenals is of  $\pi\pi^*$  character. A simple estimation of the contribution

of the spin-spin dipolar interaction to the zero-field splitting, based on a Hückel approximation of the wave function of this  ${}^3\pi\pi^*$  state, shows that the zero-field parameter  $X$  for a series of polyenals is expected to vary inversely proportional to  $(n+1)$ ,  $n$  being the number of carbon-carbon double bonds [5]. This behaviour of  $X$  is indeed observed both for the unsubstituted and the methyl-substituted polyenals, the values for the latter being systematically somewhat smaller (fig. 5).

The frequencies of the zero-field transitions for retinal are nearly identical to those for MDP and approximately 25% smaller than those for MDT. The electronic structure of the lowest triplet state of retinal resembles that of a polyenal with a total number of six double bonds. The double bond of retinal that is part of the methyl-substituted cyclohexene ring (fig. 1) apparently fully participates in the triplet excitation which suggests that the chromophoric part of retinal is more or less planar in  $T_0$ . In the ground state all-trans-retinal was found to have a skewed 6-s-cis conformation with a dihedral angle of  $50^\circ$  to  $60^\circ$  both for single crystals from X-ray diffraction data [7] and for free retinal from quantum-chemical calculations [8]. If this would be the case in the polyethylene film as well, the observed zero-field splitting would indicate a profound geometry change upon triplet excitation towards a much more planar structure.

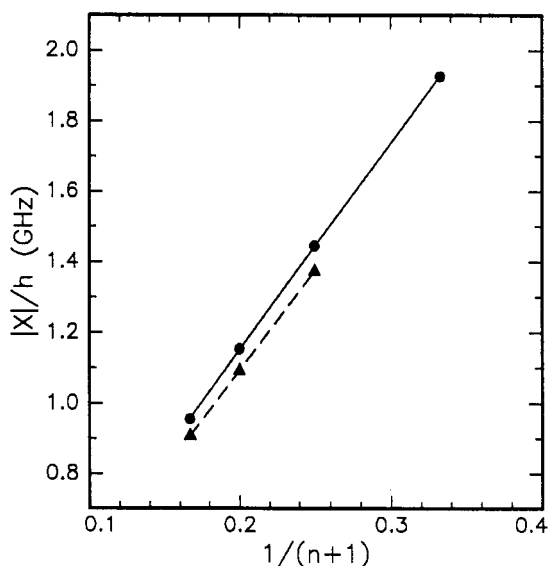


Figure 5: The variation of the magnitude of the zero-field parameter  $X$  with the chain length (total number of double bonds  $n+1$ ) for the unsubstituted (●) and methyl-substituted (▲) polyenals. The value of  $X$  for 2,4-hexadienal ( $n = 2$ ) is taken from [5].

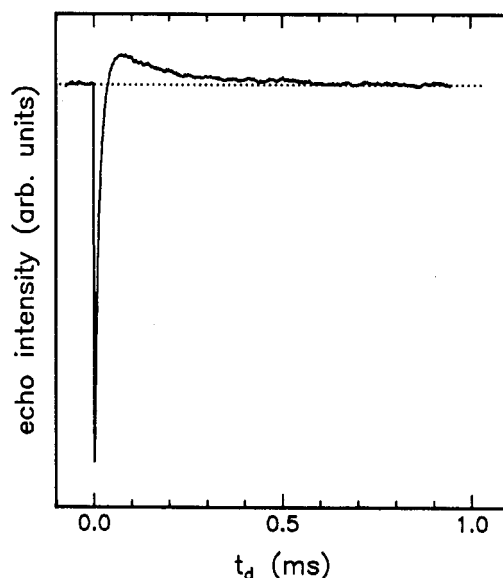


Figure 6: Echo intensity for all-trans retinal as a function of the delay time  $t_d$  between the laser flash and the first microwave pulse: high-field transition for  $\vec{B}_0 \perp \vec{s}$  and  $\tau = 500$  ns.

#### DYNAMICS OF THE TRIPLET STATE

The populating and decay of the spin sublevels of  $T_0$  have been investigated in a magnetic field and in zero field by monitoring the echo intensity as a function of  $t_d$ , the time between the laser flash and the first microwave pulse. A typical example of a decay curve is given in fig. 6.

Qualitatively the echo decay is easy to understand once we realize that the echo intensity is proportional to the population difference between the triplet spin sublevels connected by the microwaves. A curve like the one in fig. 6 thus corresponds to the sum of two exponentials whose time constants and pre-factors refer to the lifetimes and the initial populations of the sublevels. The high-field transition with  $\vec{B}_0 \parallel \vec{X}$  corresponds to the transition between the lower state  $|\frac{1}{\sqrt{2}}(iT_y + T_z)\rangle$  and the upper state  $|T_x\rangle$ . The strong absorptive signal at  $t \approx 0$  results from the preferred population of  $|T_x\rangle$  while the signal crosses zero and becomes emissive because of the slow decay of  $|T_x\rangle$  as compared to that of  $|T_y\rangle$  and  $|T_z\rangle$ .

Table 2: Populating probability of  $|T_z\rangle$ , decay rates of  $|T_x\rangle$ ,  $|T_y\rangle$  and  $|T_z\rangle$ , and lifetime  $\tau = k_{av}^{-1}$  with  $k_{av} = (k_x + k_y + k_z)/3$  for the polyenals and retinal at 1.2 K.

	$p_z$	$k_x(ms^{-1})$	$k_y(ms^{-1})$	$k_z(ms^{-1})$	$\tau(\mu s)$
OT	0.935±0.020	0.6±0.1	1.5±0.2	13±3	200
DT	0.925±0.010	1.9±0.2	8±1	36±3	65
DP	0.910±0.020	4.6±0.5	25±4	73±5	29
MOT	0.946±0.020	2.2±1.0	7±4	26±2	85
MDT	0.938±0.020	6.5±1.5	34±7	55±2	31
MDP	0.845±0.040	12.0±1.5	53±13	95±7	19
Retinal	0.877±0.035	14.0±2.5	59±12	85±4	19

From such decay curves we have obtained the relative populating rates and the decay rates of the individual spin sublevels. Data for the various polyenals, summarized in table 2, reveal that upon optical excitation into the singlet manifold invariably about 90% of the molecules end up in the  $|T_z\rangle$  spin sublevel. The large selectivity of the populating process arises from the fact that intersystem crossing is determined by first-order spin-orbit coupling between  $T_0$ , a  $^3\pi\pi^*$  state, and the  $^1n\pi^*$  state [5]. The dominant contribution comes from the local interaction on the oxygen atom. In a one-centre approximation a spin state is prepared upon intersystem crossing with the spin aligned in the plane perpendicular to the carbonyl bond. If the fine-structure axis  $z$  would be parallel to this bond, population of  $T_0$  via excitation into the singlet manifold would result in the exclusive population of  $|T_z\rangle$ .

The decay of  $T_0$  shows sublevel selectivity as well, albeit less pronounced than the populating process (table 2). The  $|T_z\rangle$  sublevel decays fastest to the ground state for all polyenals studied. The average rate constants correspond to lifetimes varying from 200  $\mu s$  (OT) to 19  $\mu s$  (MDP and retinal). These relatively short lifetimes as compared to those of phosphorescent ketones, aldehydes, and enones are in line with the non-radiative character of the triplet state. For retinal the lifetime at 1.2 K is only about twice as large as the value observed at room temperature [9] which indicates that the radiationless decay is governed by intramolecular processes.

The decay rates of all spin sublevels increase with increasing chain length, both for the unsubstituted and the methyl-substituted polyenals. For the unsubstituted molecules this increase was found to correlate with the decrease of the triplet state energy with respect to the ground state [5] which suggests that the Franck-Condon factors dominate the variation in the decay rates with chain length. Unlike for the zero-field parameters and the relative populating rates, addition of substituents on the polyenal chain significantly influences the radiationless decay. The lifetimes of all spin states become shorter and the sublevel selectivity decreases. The change occurs upon methyl substitution, while the presence of the methyl-substituted cyclohexene ring in retinal does not affect the decay anymore. The decay rates for retinal are about identical to those for MDP. The effect of the methyl groups along the chain on the decay rates may well have to do with a change in vibronic coupling upon addition of these substituents, because methyl groups are known to perturb the skeletal modes of the polyenal chain.

From ab-initio calculations on short-chain polyenals [10] we have deduced a phosphorescence lifetime of the  $|T_z\rangle$  sublevel for octatrienal in the order of  $10^3$  seconds. By comparing this value with the observed lifetime we estimate the quantum yield of phosphorescence for this spin sublevel of OT to be only  $10^{-7}$ . Moreover, for short-chain polyenals the calculated radiative lifetime becomes substantially longer upon lengthening the conjugated chain. On the other hand, the ESE experiments show that the triplet lifetime shortens systematically with increasing chain length which means that we expect the quantum yield of phosphorescence to become even smaller than  $10^{-7}$  for polyenals longer than OT.

## CONCLUSION

The triplet state of all-trans polyenals with three or more carbon-carbon double bonds is found to be of  $\pi\pi^*$  character. Optical methods have as yet largely failed in the study of this state, in particular because no phosphorescence has been observed for these compounds. Pulsed laser excitation of the polyenals into the singlet manifold leads to a high spin polarization in  $T_0$  which allows for the detection of electron-spin echoes on a microsecond time scale. Suitable host crystals for polyenals have not been found but we have shown that polyethylene films offer a suitable alternative. The orientation of the fine-structure axes system, the zero-field energies of the spin sublevels, and their relative populating and decay rates have been determined for a series of unsubstituted polyenals, methyl-substituted polyenals, and retinal. A further characterization of the electronic and geometric structure of these conjugated systems in  $T_0$  depends on the success of hyperfine studies that we presently perform by the use of electron-spin-echo envelope modulation techniques. In addition we have started an ESE study of the triplet state of polyenes, even simpler chromophores than the polyenals but more difficult to investigate because they lack the carbonyl group.

## Acknowledgement

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