

## A Laser Flash Photolysis Study of 11-Cis-Locked Retinal Analogues

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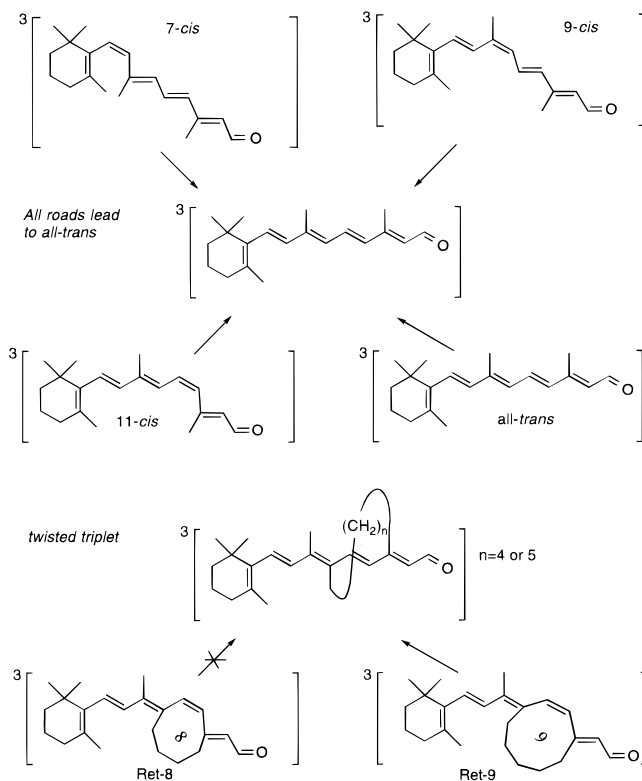
The photophysics and photochemistry of cis-locked retinal analogues have been investigated by steady-state and by picosecond and nanosecond time-resolved techniques. We have found that retinal analogues locked into the 11,12-cis configuration via bridged 8- and 9-membered rings, Ret-8 and Ret-9, respectively, are restricted in twisting about the 11,12 double bond in the first triplet state,  $T_1$ . The twisting is restricted to different extents depending on the size of the ring. In the case of Ret-8, no geometric isomerization occurs following photoexcitation. This contrasts *cis*-cyclooctene derivatives which can be photoisomerized to thermodynamically less favored *trans*-cyclooctene. The result is rationalized by noting that in Ret-8 the ring incorporates four consecutive  $sp^2$  carbons whereas the latter has only two. For Ret-9 which has one extra carbon in the ring, however, “*cis*–*trans* isomerization” becomes possible resulting in shorter-lived excited states and a detectable transient on the nanosecond time scale which is assigned to a short-lived ( $\tau \approx 110$  ns) transoid ground state species.

### Introduction

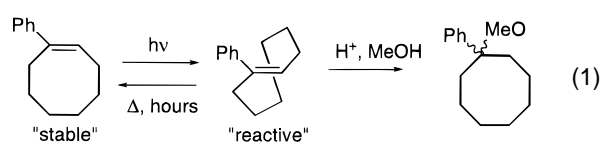
**Use of Retinal Analogues To Elucidate the Mechanism of Visual Transduction.** Retinal analogues of specific design have been extensively used to elucidate the interaction of the visual retinal chromophores and their biopolymer proteins.<sup>1,2</sup> Typically the synthetic analogues are incorporated into the appropriate apoprotein, such as opsin, *in vitro* or *in vivo*, and the structure and mechanism by which rhodopsin triggers the visual process is investigated. The *cis*-locked retinal analogues, Ret-8 and Ret-9 depicted in Scheme 1, were synthesized<sup>3</sup> to investigate the visual transduction process by incorporating them into opsin and also to check whether a rhodopsin transduction intermediate incorporating a bridged transoid ring could be detected. Ret-8, but not Ret-9, could be incorporated into opsin to give the rhodopsin analogue Rh8; flash photolysis of Rh8 solutions in digitonin showed that it formed the first intermediate photorhodopsin in 15 ps and the second intermediate in 1 ns, both having a transient transoid 11-ene, which reverted back to starting Rh8 in 50 ns.<sup>4</sup> Experiments with dihydroretinal analogues and Rh8 incorporated into rhodopsin membrane showed that sudden polarization or charge translocation in the excited state does occur for the latter,<sup>5</sup> but this is insufficient for the visual transduction process which appears to require a full *cis*–*trans* isomerization of the entire  $\pi$ -system. (That is, Rh8 was inactive whereas the enzymatic activities of rhodopsins containing dihydroretinals were greatly reduced or zero.<sup>5</sup>) It has been assumed that the *cis*-locking ring may prevent isomerization and does not introduce new photochemical pathways.<sup>6</sup> This study tests the extent to which twisting about the 11,12-ene is restricted in the *cis*-locked retinal analogues Ret-8 and Ret-9.

**Two Paradigms for the Photochemistry of 11-Cis-Locked Retinal Analogues.** *Cis*–*Trans* Isomerization of Cyclic Alkenes. Strained *trans*-cyclic alkenes which are stable from minutes to hours can be formed through photoisomerization.<sup>7,8</sup> Either the twisted triplet electronic, or ground state *trans* geometric isomer can act as the source of chemical reactivity. In particular, attack by nucleophiles under acidic conditions (e.g.

### SCHEME 1



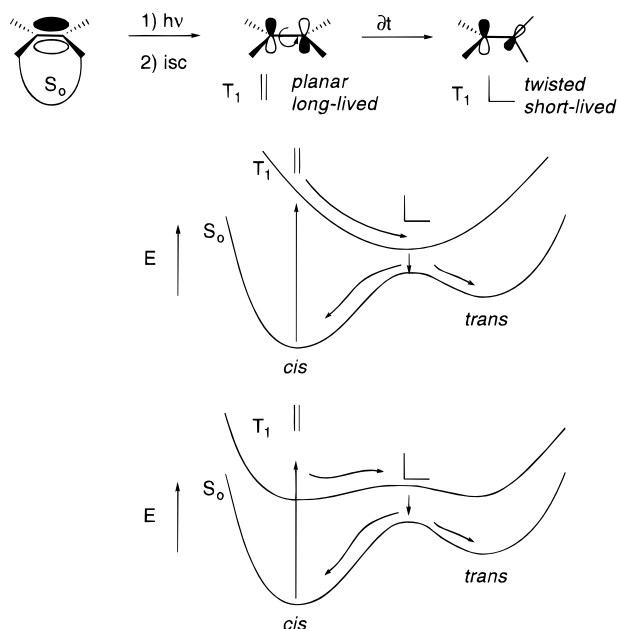
reaction with methanol, eq 1) is diagnostic of a twisted ground state cyclic alkene.<sup>7–14</sup>



The ability to approach a twisted geometry in the excited state affects the lifetime of the first triplet state.<sup>15–17</sup> Apparently the twisted triplet is best described as a 1,2-biradical (Scheme

<sup>⊗</sup> Abstract published in *Advance ACS Abstracts*, December 15, 1995.

## SCHEME 2



2).<sup>17,18</sup> In the case of the retinal analogues, increasing conjugation length gives a higher perpendicular triplet than the planar species as summarized in the lower half of Scheme 2. Note, however, that the ability of the molecule to twist to a perpendicular state in the triplet manifold should still correspond to its ability to undergo rapid relaxation to the ground state as the two surfaces approach more closely around this geometry.

**Solution Photochemistry of 11-cis-Retinal.** In the photolysis of native 11-cis-retinal a very short-lived singlet state decays rapidly, exhibiting weak fluorescence and fast intersystem crossing (isc).<sup>19,20</sup> The photoisomerization of 11-cis-retinal to all-trans-retinal occurs with a quantum yield of 0.2.<sup>21,22</sup> The triplet quantum yield is 0.61.<sup>22</sup> Apparently adiabatic cis-trans isomerization occurs on the triplet surface on the picosecond time scale. The triplet-triplet absorption at early times exhibits a maximum at 430 nm and a few hundred picoseconds later has shifted to 450 nm.<sup>23</sup> Various retinal isomers (such as 7-cis, 9-cis, 11-cis, and all-trans) lead to the same triplet-triplet absorption spectrum on the nanosecond time scale with  $\lambda_{\max} = 450$  nm.<sup>24</sup> The explanation most widely accepted is that the all-trans-triplet is thermally most stable and accessible regardless which retinal geometric isomer is initially excited (Scheme 1).<sup>23-25</sup>

There is a solvent effect on the fluorescence and triplet quantum yields. In polar media the singlet  $n,\pi^*$  and  $\pi,\pi^*$  states invert as the lowest energy excited singlet,  $S_1$ .<sup>20,22</sup> Thus in polar media fluorescence quantum yields are enhanced and triplet quantum yields are diminished. We have found the analogues, Ret-8 and Ret-9, to exhibit very similar behavior.

**Support for Full Cis-Trans Isomerization of the Cis-Locked Retinals.** Employing the known photophysics and photochemistry of retinals and phenylcyclooctene as a basis for comparison with the retinal analogues has been helpful in our explorations. We shall provide evidence that Ret-8 probably has a cis-locked triplet state,  $T_1$ . On the other hand it is quite likely that Ret-9's triplet twists about the 11-12 double bond by at least  $90^\circ$  and relaxes quickly to the ground state to yield a "transoid" species which thermally reverts to starting material. It is remarkable that such extraordinary differences in behavior of these two cis-locked retinal analogues result from the addition of but one extra methylene unit in the cis-locking ring of Ret-9 as compared to Ret-8.

## Experimental Section

**Materials.** Hexane, cyclohexane, methanol, acetonitrile, and benzene were all obtained from Aldrich in the purest form possible (HPLC or spectrophotometric grade) and were used as received.

**Kinetic Absorption Spectroscopy.** The nanosecond flash photolysis system employed has the following characteristics. The third harmonic of a neodymium YAG laser (Quantaray DCR II,  $\lambda = 354.5$  nm, 7-10 mJ/pulse, 5-6 ns pulse duration) was used to excite the sample. Experiments were carried out using a  $1 \times 1$  cm quartz cell. A pulsed 150 W xenon lamp, combined with an ISA H10 monochromator, serves as the monitoring system. The signals from a Hamamatsu R928 photomultiplier tube were terminated into  $93 \Omega$  and into a Tektronix 7912 programmable digitizer (single channel, 200 MHz bandwidth). This digitizer was controlled along with other aspects of the experiment (shutters, pulser, monochromator, etc.) through both a GPIB and a digital/analog input/output interface board (National Instruments NBGPIB and LabNB boards respectively) using a Macintosh IICI with Labview 2 software. A point of interest regarding the single channel transient digitizer (Tektronix R7912) is that the lamp profile and the resulting  $I_0$  calculation was handled by passing the signal directly to an analog input line of the LabNB board of the Macintosh computer. The board is triggered externally by a TTL pulse and can sample the analog port once every  $16 \mu\text{s}$ , which is more than adequate to capture the lamp profile which has a  $\approx 2$  ms pulse duration. Thus the need for a separate sample and hold box or a digital oscilloscope is avoided. The data were processed and stored also using the Macintosh and Labview software. Typically 10-15 laser pulses were averaged to produce each experimental trace. Decay kinetics of the transient absorptions produced by photolysis of the retinal analogues were monitored by direct detection at 430 or 340 nm for Ret-8 and Ret-9 respectively. Transient absorption spectra were produced by obtaining full kinetic traces at each wavelength and sampling the change in optical density at a fixed delay following the laser pulse. The change in absorbance was then plotted as a function of wavelength.

**Picosecond Pump-Probe Spectroscopy.** The picosecond pump-probe apparatus has recently been described in detail.<sup>26</sup> For the picosecond system a typical pump/probe setup with monochromatic detection was employed. The output from an actively-passively mode-locked Quantel YG 501C Nd:YAG laser (1064 nm, fwhm  $\approx 40$  ps, 75 mJ/pulse) was frequency doubled to produce 532 nm pulses which were divided into two equal portions. Half of the green pulse was passed along with the residual fundamental through a KDP crystal to produce the third harmonic 355 nm pulses ( $\approx 7$  mJ/pulse, fwhm  $\approx 30$  ps) while the second half, reflected at right angles, was passed out a hole cut in the laser cover and directed with a dichroic mirror through a second frequency doubling crystal to produce 266 nm light (2-4 mJ/pulse, fwhm  $\approx 26$  ps). The residual green left after fourth harmonic generation ( $\approx 6$  mJ/pulse, fwhm  $36 \pm 4$  ps) was also available after appropriate attenuation of the power to be used as a probe beam. All decay kinetics reported here which correspond to the decay of the triplet-triplet absorption of the retinals employed 396 nm pulses, (which were generated by Raman shifting with cyclohexane) attenuated to less than 1 mJ/pulse as the probe light<sup>27</sup> and 355 nm pulses, concentrated to a 2 mm diameter for excitation of the samples. Pump and probe beams entered the cell at a small angle with the diameter of the probe beam being centered within and slightly smaller than the pump beam. The probe beam was passed along a variable delay such that it could arrive at the

sample at any time between  $-1000$  and  $+15000$  ps with respect to the pump beam. Data collection, shutter control and delay line position were automatically controlled via a Macintosh IIfx computer using Labview 2 software which also provided data processing, storage and hard copy output.

**Fluorimetry.** Fluorescence spectra were recorded on a Perkin-Elmer LS5 spectrofluorimeter. Excitation was usually at 266 or 308 nm and emission was recorded from 300 to 500 nm or from 330 to 600 nm respectively.

**Single-Photon Counting.** The single-photon-counting instrument has been described before.<sup>28a</sup> The unit was used at room temperature and was equipped with an Edinborough 199F ns flash lamp, Ortech Electronics, and a Tracor-Northern TN-1710 multichannel analyzer. The instrument is capable of measuring fluorescence lifetimes between 2 and  $\approx 5000$  ns and was interfaced with an IBM PS/2 Model 70 computer.

**NMR.** A Bruker AF 250 instrument was employed to gather the  $^{13}\text{C}$  NMR spectra and a Varian Model VXR-200 was employed for routine measurements of proton spectra.

**UV-Vis Spectrophotometry.** The absorption spectra were recorded on a Perkin-Elmer 559 A or a Lambda Array 3840 UV-vis spectrophotometer.

**Oxygen Concentrations.** We employed a Spectra Gases mass flow gas mixer system to generate mixtures of oxygen and argon of known composition which in all aspects was similar to that reported by Maillard et al.<sup>28b</sup> Molar  $\text{O}_2$  concentrations under 1 atmosphere pressure were calculated using Henry's law and solubilities found in ref 28c.

**Singlet Oxygen Phosphorescence Detection.** The integrated output from a liquid nitrogen cooled germanium photodiode was employed in a pulsed, time-independent detection of singlet oxygen phosphorescence. The detector employed was a Model 403L from Applied Detector Corp. and has been described before.<sup>29</sup> The signals were captured with a Tektronix TDS 320 digital storage oscilloscope and passed to a Macintosh IIfx computer.

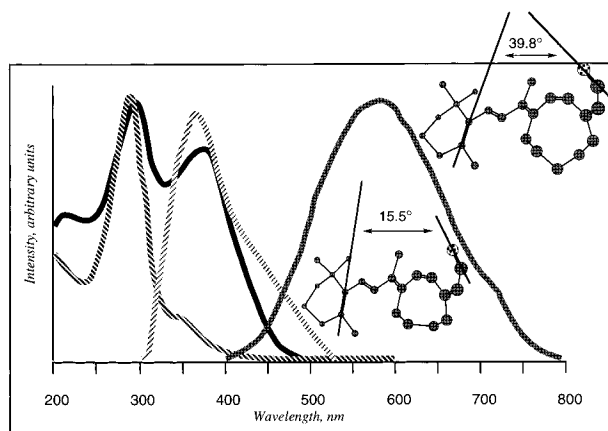
## Results

### First Excited Singlet States of the Retinal Analogues.

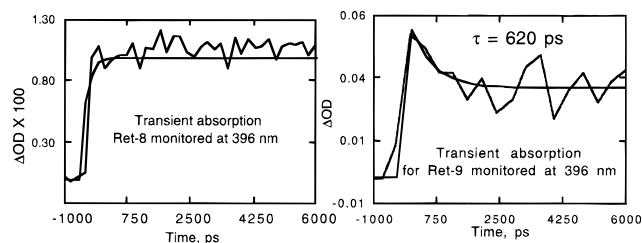
**Fluorescence.** Both retinal analogues, Ret-8 and Ret-9, exhibited very weak fluorescence as is characteristic of highly conjugated polyenes.<sup>30</sup> The fluorescence spectra were broad and structureless. Ret-9 exhibited an emission maximum at 350 nm with a shoulder at a  $\approx 330$  nm onset. If we take this to represent the 0,0 transition for Ret-9, then the upper limit for  $E_s$  is estimated to be 87 kcal mol<sup>-1</sup>. Ret-8 has a smooth structureless Gaussian-shaped emission spectrum with a maximum at 570 nm and an onset at  $\approx 500$  nm. Thus we estimate an upper limit of  $E_s$  for Ret-8 to be  $\approx 57$  kcal mol<sup>-1</sup>. The fluorescence spectra along with the ground-state absorption spectra of the two retinal analogues are displayed in Figure 1.

**Fluorescence Lifetime.** Measurement of the room temperature singlet lifetime for both the analogues was attempted by nanosecond laser flash photolysis and by time-correlated single-photon counting, SPC. In each case the measured decay was limited by the instrument response ( $\approx 20$  ns for LFP and  $\approx 2$  ns for SPC). We measured directly the growth of the triplet-triplet absorption signal on the picosecond time scale to be very fast ( $<40$  ps) and thus estimate  $\tau_s \leq 40$  ps based on the picosecond pump-probe work or  $\tau_s < 2$  ns based on the nanosecond emission techniques. Fluorescence lifetimes for native retinal have been reported in the literature to be between 17 and 30 ps.<sup>19,20</sup>

**The Triplet States of the Cis-Locked Retinals.** The solution spectra of the triplet states of parent 11-*cis*- and 11-



**Figure 1.** Ground-state absorption and fluorescence spectra of Ret-8 (black line and gray line) and of Ret-9 (dark and light hatched lines, respectively), in cyclohexane solution. Molecular mechanics calculations indicate a decrease in conjugation within the  $\pi$ -system of the chromophore of Ret-9 compared to Ret-8. A Monte Carlo conformational search for global minima yielded the geometries for Ret-8 and Ret-9 shown above. The angles noted are between the two terminal double bonds in the chromophore (the  $\beta$ -ionone ring and the carbonyl).

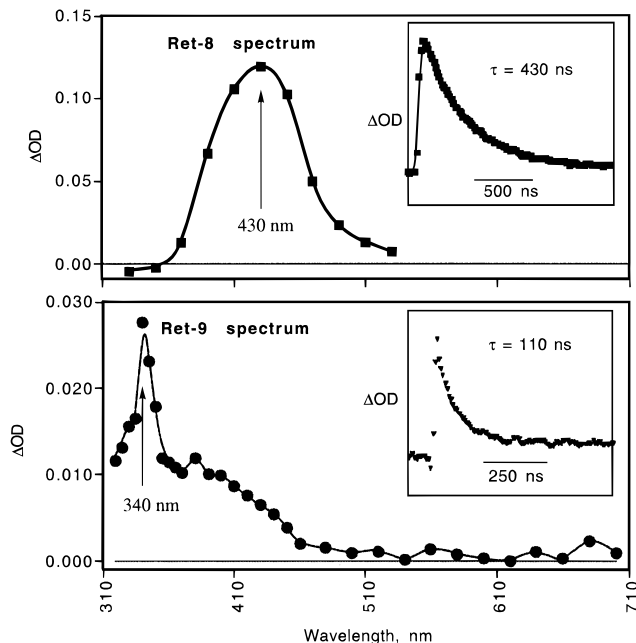


**Figure 2.** Transient absorption kinetics observed for (a) Ret-8 and (b) Ret-9 at 396 nm with excitation at 355 nm in hexane solution obtained with the picosecond pump-probe spectrometer.

*trans*-retinals have been reported in the literature.<sup>22-25</sup> Triplet-triplet absorptions of the retinal analogs were detected by picosecond (Ret-8 and Ret-9) and nanosecond laser flash photolysis. In initial experiments the wavelength of inquiry was 396 nm and corresponded to a region where the parent retinal triplet absorbs.

### Picosecond Pump-Probe Detection of Retinal Triplets.

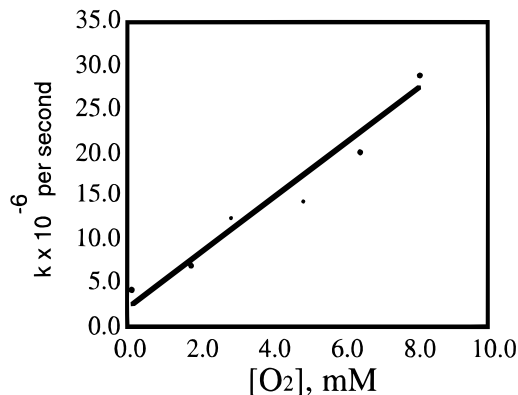
In the solution photochemistry of the native 11-*cis*-retinal intersystem crossing from  $S_1$  to  $T_1$  occurs rapidly with high efficiency. The initially formed triplet has been shown to undergo relatively slow adiabatic relaxation.<sup>23,25</sup> The explanation proposes that *cis*-to-*trans* isomerization occurs on the triplet surface within a few hundred picoseconds and a shift in the absorption maximum initially at 430 to 455 nm is the observable.<sup>23,25</sup> We examined the picosecond kinetics of Ret-8 and Ret-9 at 396 nm. In the case of Ret-9 we found that the transient absorption, which we attribute to the triplet state,  $T_1$ , showed partial fast decay with  $\approx 50\%$  stable residual absorption. The decay was fit to first-order kinetics to yield a lifetime of 650 ps and the residual absorption showed no decay out to 8 ns. In contrast the transient absorption observed for Ret-8 at 396 nm following photolysis at 355 nm exhibited a smooth growth to a stable plateau value on the 8 ns time scale which could be fit to monoexponential kinetics. The growth time constants for both Ret-8 and Ret-9 were estimated by fitting the picosecond data on a shorter time window of  $\approx 500$  ps and were  $31 \pm 6$  ps, respectively. These match the fluorescence decay lifetimes measured for *all-trans*-retinal of between 17 and 30 ps.<sup>19,20</sup> The kinetic traces for Ret-8 and Ret-9 are shown in Figure 2.



**Figure 3.** Nanosecond transient absorption spectra observed following 355 and 308 nm laser flash photolysis of Ret-8 and Ret-9 in argon purged hexane solution, respectively. The insets show the kinetic behavior of the transients observed close to the absorption maxima of the respective spectra.

**Characterization of Reactive Transients in the Nanosecond Time Regime.** *Transient Absorption Spectra.* Laser flash photolysis of the retinal analogues at 355 and 308 nm in methanol and hexane led to readily detectable transient absorptions with signal maxima occurring at 430 and 340 nm for Ret-8 and Ret-9, respectively (see Figure 3). Essentially the same transient absorptions were observed in both solvents. The yield of signal in methanol solution was, however, a factor of 10 lower than that found for hexane solutions. Polar solvents are known to reduce the quantum yield of triplet of the parent retinal chromophore,<sup>22</sup> thus giving us the first indication that the observed transients may be, or are possibly derived from, the triplet state,  $T_1$ . Typical absorption spectra are shown in Figure 3 and were assembled by collecting decay traces at each wavelength from which values of the change in optical density were sampled at 100 ns following the laser flash and plotted against wavelength. The insets to Figure 3 show typical kinetic traces which were used to construct the spectra. Evaluation of the decay of the signal according to monoexponential kinetics yielded lifetimes of 430 and 110 ns for the two transients observed following photolysis of Ret-8 and Ret-9, respectively. We were able to assign these transients to the triplet state,  $T_1$ , in the case of Ret-8 and to a transient ground state for Ret-9 by determining their characteristic reactivity toward triplet and strained alkene scavengers. These experiments are outlined in more detail below.

*Quenching with  $O_2$ .* One of the hallmarks of electronically excited molecules that have a triplet spin multiplicity is their near diffusion controlled reaction with molecular oxygen. Thus oxygen is often employed as a convenient diagnostic tool to characterize triplets. Oxygen also reacts with other species such as free radicals thus it is not conclusive evidence for assignment of a transient absorption to a triplet state but is supporting evidence when coupled with other experiments. In this case we employed singlet oxygen phosphorescence detection where the yield of this species may be conclusively tied to the involvement of a triplet species. Photolysis of Ret-8 with 355 nm light in aerated hexanes gave a transient with identical UV



**Figure 4.** Plot of the observed decay rate constant for 11-*cis* retinal as a function of oxygen concentration. The slope of the least-squares best line fit to the data, representing the bimolecular rate constant for oxygen quenching of the 11-*cis*-retinal triplet, was found to be  $(3.1 \pm 0.6) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ .

absorption properties to that found in an argon purged solution except that the lifetime was drastically reduced. Varying concentrations of oxygen were delivered to the solution by bubbling a variable mixture of oxygen and argon and the decay kinetics of the transient centered at 430 nm were determined. From a plot of the observed rate constant for decay against oxygen concentration a slope of  $(2.9 \pm 0.5) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  was obtained and is equated to the rate constant for oxygen quenching of the 430 nm transient according to eq 2. A plot

$$k_{\text{obs}} = \frac{1}{\tau_0} + k_q[\text{O}_2] \quad (2)$$

according to eq 2 for oxygen quenching of the 11-*cis*-retinal triplet is exhibited in Figure 4. We obtained the bimolecular rate constant for reaction of 11-*cis*-retinal of  $(3.1 \pm 0.6) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ . This matches well with the literature value of for  $O_2$  quenching of  $3.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  for *all-trans*-retinal in *n*-hexane at room temperature.<sup>33</sup> In the case of Ret-9, however, we did not find any significant change in the decay kinetics as a function of oxygen concentration. The amount of the signal (top  $\Delta o.d.$  at 340 nm) was, however, reduced and under conditions where the oxygen concentration exceeded  $\approx 10 \text{ mM}$  no transient absorption was observed. In this case we concluded that oxygen was reacting with the precursor to the detected species and was unreactive toward the detected species itself. If we assumed that the precursor species is a short-lived triplet state then we can estimate the lifetime assuming a similar rate constant for reaction with oxygen as that measured for the 11-*cis*-retinal and Ret-8 triplet states (namely  $3.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). Thus we carried out a Stern–Volmer analysis based upon the depletion of the top absorption signal at 340 nm as a function of oxygen concentration. Unfortunately the data we were able to obtain were very scattered. This was probably due to the short lifetime of the state involved and partly due to the weak signals encountered at the higher oxygen concentrations. Thus only an upper limit for the triplet lifetime of  $< 15 \text{ ns}$  could be determined by this technique.

*Energy Transfer to  $\beta$ -Carotene.*  $\beta$ -Carotene has an excited state with a very low triplet energy ( $E_T = 22 \text{ kcal mol}^{-1}$ ) and has been employed to quench such low energy species as orthoxyllylene triplet biradicals<sup>31</sup> and singlet oxygen ( $^1\Delta_g$ ) via electronic energy transfer to generate triplet  $\beta$ -carotene.<sup>32</sup> The  $\beta$ -carotene triplet–triplet absorption has a characteristically strong absorption at 525 nm and the growth kinetics of this species may be conveniently followed at this wavelength. Quenching the retinal analogue triplet states with  $\beta$ -carotene

would provide further information on the nature of the transients observed and in favorable circumstances would allow us to measure the quantum yield of triplet formation of Ret-8. This would be achieved via comparison of triplet energy transfer (TET) from *all-trans*-retinal, *t*-Ret, ( $E_T = 36 \text{ kcal mol}^{-1}$ ) to  $\beta$ -carotene since the quantum yield of triplet formation for *t*-Ret in benzene is known (0.5).

Thus we have generated the triplet state of *t*-Ret in benzene solution under an argon atmosphere which had a lifetime of  $\tau_T \approx 3 \mu\text{s}$ . Although this lifetime is shorter than a reported value<sup>24</sup> it merely reflects experimental conditions (a residual amount of oxygen is likely due to incomplete removal) and does not interfere with our studies. In the initial effort a 1 cm path length cell was employed. An accurate quenching rate constant, however, could not be measured due to absorption of  $\beta$ -carotene at the laser wavelength (355 nm). We opted to use a shorter path length cell (2 mm) for future studies so that higher concentrations of  $\beta$ -carotene could be employed without interfering with excitation of the retinal. This required rearranging the approach of the laser beam with respect to the monitoring beam so that it was front face (20°) rather than right angle (90°) excitation.

The rate of decay of *t*-Ret triplet was monitored in the presence of varying amounts of  $\beta$ -carotene. A plot of the apparent rate constant for decay vs [ $\beta$ -carotene] yielded a straight line, the slope of which was  $k_q = (1 \pm 1) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ .

Attempts to measure the rates of TET from the two retinal analogue triplets to  $\beta$ -carotene were unsuccessful. This result was not surprising in the case of Ret-9 since all previous results indicated a triplet lifetime < 20 ns and in light of the low concentrations of  $\beta$ -carotene available ( $\approx 1 \text{ mM}$  before absorption of the exciting laser light at 355 nm became important). With Ret-8, however, we had assigned the triplet to a species which exhibited a lifetime of 400 ns and should have been quenchable by the concentrations of  $\beta$ -carotene employed provided  $k_q \approx 1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ , and thus it was surprising that quenching was not observed in this case. When transient kinetics were monitored at 340 nm there was irreversible bleaching of the ground state absorption in the presence of  $\beta$ -carotene which implies that Ret-8 may be reacting irreversibly with  $\beta$ -carotene. After approximately 20 laser "shots" transient absorption at 430 nm was no longer evident but could be recovered by shaking the sample. At this point it remains unclear what the nature of the interaction between these two species is, save to say, that it does not appear to be simple electronic energy transfer and thus is not much use to us in characterizing the photophysics of Ret-8.

**Reaction with Tempo Free Radical.** Tempo free radical is known to quench triplet excited states very efficiently and was another candidate for testing the reactivity of the nanosecond transients detected in the laser flash photolysis of the Ret-8 and Ret-9 analogues. It was verified that Tempo does not catalyze the thermal isomerization of *trans*-1-phenylcyclooctene to the thermodynamically more stable *cis* isomer. In fact flash photolysis of a solution of *cis*-1-phenylcyclooctene in which various amounts of Tempo were added exhibited no acceleration of the thermal reversion of the *trans* species. Thus we concluded that Tempo could be used as a selective quencher of the triplet states of the retinal analogues without interfering with the stability of any *trans* isomers formed in the photolysis of these species.

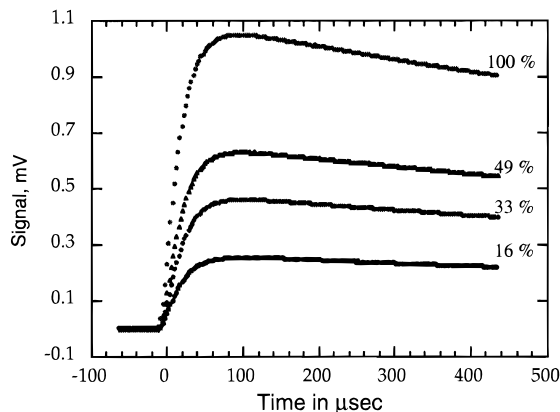
The ability of Tempo as a triplet polyene quencher was verified by measuring the rate for quenching the triplet state of 11-*cis*-retinal in an experiment analogous to that employed to

measure the rate of quenching by  $\beta$ -carotene. In this instance we were able to measure rates of quenching of the triplets of 11-*cis*-retinal and Ret-8 of  $(3.8 \pm 1.4) \times 10^9$  and  $(5.1 \pm 1.1) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ , respectively. No measurable increase in the rate of decay of the transient of Ret-9 could be measured. The top signal of this species was, however, diminished as a function of increasing Tempo concentration. At higher Tempo concentrations Tempo competed with ground state Ret-9 for absorption of the excitation pulse, thus a Stern–Volmer analysis to estimate Ret-9's triplet lifetime via measurement of the top  $\Delta\text{o.d.}$  as a function of [Tempo] was not possible.

**Effect of pH on Transient Kinetics.** One of the characteristic reactions of strained *trans*-cycloalkenes is the acid catalyzed addition of methanol across the strained double bond.<sup>7–14</sup> In the preceding cases the cyclic-*trans* species are reactive intermediates with lifetimes on the microsecond time scale. In the case where a cyclooctene is employed the ring strain of the *trans* species is sufficiently reduced that in the absence of trace impurities it is stable for days before appreciable amounts isomerize to the thermodynamically favored *cis* form. Indeed we have verified that this is the case and have characterized the *trans* isomer of 1-phenylcyclooctene by following steady-state photolyses with <sup>1</sup>H NMR detection. We also measured the lifetime of the *trans* form in a series of nanosecond flash photolysis experiments under conditions of triplet sensitization with xanthone ( $E_T \approx 74 \text{ kcal/mol}$ ), excited at 355 nm in argon purged methanol solutions. The rate of decay of *trans*-1-phenylcyclooctene increased markedly in the presence of trace amounts of acid. We determined a bimolecular rate constant for this reaction in the usual manner to be,  $k_{H^+} = 1.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  methanol at room temperature. The same species could be generated with a lower yield by direct photolysis of the *cis* precursor at 308 nm and showed no change in lifetime in aerated solution. The end result is, that even though the *trans* cyclooctene is stable enough to be characterized by conventional techniques, it remains strained enough to be reactive toward alcoholic solvent in the presence of trace acid. Since the retinal analogues incorporate two extra  $\text{sp}^2$  hybridized carbons in the cycle and which belong to exocyclic double bonds, we expected the putative *trans* isomers, should they be formed, to be less thermally stable and probably more reactive toward acid than the 1-phenylcyclooctene *trans* isomer.

Photolysis of retinals in polar media has been shown to reduce the yield of triplets formed due to a decreased rate of intersystem crossing. This is thought to be due to a change in the ordering of the two lowest lying singlet states  $S_1$  and  $S_2$  which are  $n, \pi^*$  and  $\pi, \pi^*$ , respectively, in nonpolar media.<sup>22</sup> We have observed a similar reduction of signal of the transients of Ret-8 and Ret-9. This implies that these species are either the retinal analogue triplets or they are triplet derived. We estimate the polar solvent to nonpolar solvent ratio of the 340 (Ret-9) and 430 nm (Ret-8) transient yields to be 0.01 and 0.023, respectively, based on a comparative photolysis study. Thus in studying the effects of acid upon the rates of decay of the aforementioned transients exceedingly weak signals were dealt with due to the necessity to work in polar media. Nonetheless it was evident that neither Ret-8 (430 nm) nor Ret-9 (340 nm) transients exhibited any large changes in kinetics in the presence of small amounts of acid in argon purged methanol solution. Ret-9 showed the largest change in kinetics with the rate of decay of the 340 nm transient changing from (approximately  $1 \times 10^7 \text{ s}^{-1}$ ) in pure methanol to ( $5 \times 10^7 \text{ s}^{-1}$ ) in 0.76 M [ $\text{H}^+$ ] in methanol. From these data we estimate an upper limit of  $5.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ .

**Intersystem Crossing Quantum Yields of the Retinal Analogues.** We chose to employ singlet oxygen,  $\text{O}_2 (^1\Delta_g)$  phos-



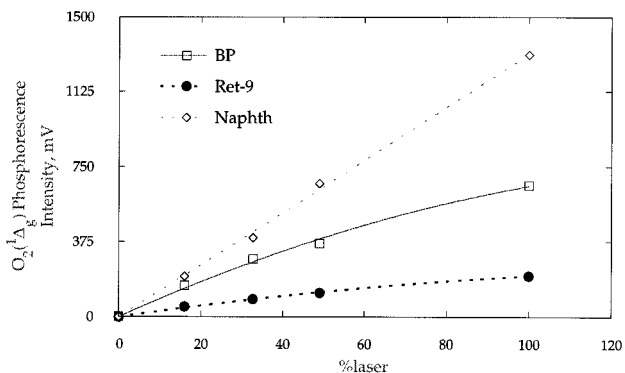
**Figure 5.** Singlet oxygen phosphorescence intensity monitored at 1270 nm following flash photolysis of naphthalene in aerated cyclohexane solution with a 308 nm 20 ns laser pulse at various intensities as marked.

phorescence detection as a probe for intersystem crossing efficiency,  $\phi_{isc}$ , for the retinal analogues Ret-8 and Ret-9 based on the precedence set by Das and co-workers.<sup>33</sup> They showed that the efficiency of singlet oxygen generation from *all-trans*-retinal in oxygen saturated solution equaled the intersystem crossing quantum yield for this species. The same held true for two other polyenes. In the phosphorescence measurements we employed methodology similar to that used by Gorman and Rodgers.<sup>32,34</sup> We employed a xenon chloride excimer laser ( $\lambda = 308$  nm) as the excitation source as opposed to 355 nm laser line employed by Gorman et al. This precluded the possibility of employing the benzophenone/naphthalene standard.<sup>34</sup> Instead we chose direct photolysis of naphthalene which has a  $\phi_{isc}$  of 0.75<sup>35</sup> and an efficiency of singlet oxygen generation,  $S_{\Delta}$ , in alkane solutions of 1.0.<sup>32</sup> To ensure that our apparatus was working correctly we measured singlet oxygen generation efficiencies,  $\phi_{\Delta}$ , for benzophenone and the native 11-*cis*-retinal which could be compared to literature values.

Each sample was diluted in solution until the optical density at 308 nm matched that of the naphthalene standard (0.20). Small differences in optical densities were corrected for via normalization of the data. Both aerated and oxygen purged solutions were employed and yielded similar results. For example, Ret-9 yielded a  $\phi_{isc}$  of 0.16 and 0.18 in aerated and oxygen purged isoctane solutions respectively. The laser pulses were maintained at  $<6.0$  mJ/pulse by using appropriate neutral density filters. Furthermore each sample was excited with four different intensities corresponding to  $\approx 16, 33, 49,$  and 100% of the full laser intensity by employing calibrated neutral density filters. A typical plot of singlet oxygen,  $O_2(^1\Delta_g)$ , phosphorescence intensities in mV against the percent laser dose is shown in Figure 6. Each point represents the average of 16 separate measurements. In the case where the data did not give a linear response as a function of laser dose the data were iteratively fit through least-squares analysis to a second order polynomial function. The slope  $m_{\Delta}$  at zero laser dose was then employed in the calculation of  $O_2(^1\Delta_g)$  generation efficiencies,  $\phi_{\Delta}$ , according to eq 3, where  $\phi_{\Delta x}$  is the efficiency of singlet

$$\phi_{\Delta x} = \frac{m_{\Delta x}}{m_{\Delta N}} \phi_{\Delta N} = (0.75) \frac{m_{\Delta x}}{m_{\Delta N}} \quad (3)$$

oxygen generation by the compound under study,  $\phi_{\Delta N}$  is the  $\phi_{\Delta}$  for naphthalene, and  $m_{\Delta x}/m_{\Delta N}$  is the ratio of the laser dose dependent slopes for the analyte (x) and for naphthalene (N). Finally, on the basis of the work of Das et al., we equate the quantities  $\phi_{\Delta}$  for the retinals to  $\phi_{isc}$  which requires the assumption that  $S_{\Delta}$  for the retinals and analogs is 1.0.<sup>36</sup>



**Figure 6.** Plots of the singlet oxygen phosphorescence intensities monitored at 1270 nm as a function of laser dose for naphthalene, Ret-9, and benzophenone. (Ret-8 which yielded results similar in quality to Ret-9 is not shown since these data were collected on a different day with a separate naphthalene standard.)

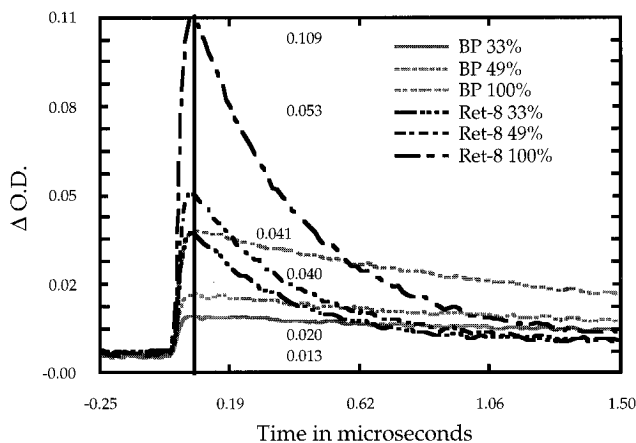
*Retinal Analogue Triplet–Triplet Extinction Coefficients.* With the value of the intersystem crossing efficiency of the Ret-8 in hand, determination of the triplet–triplet extinction coefficient is then conveniently accessible through comparative transient absorption flash photolysis. In the case where the two samples, an analyte and a reference, are identical with respect to geometry of the spectrophotometer and their optical densities at the excitation wavelength then the top  $\Delta OD$  (top absorption signal at the absorption spectrum maximum) is related to the product of their quantum yields for triplet formation,  $\phi_{isc}$ , and their molar extinction coefficients,  $\epsilon_{T-T}$  according to eq 4, where AN refers

$$\frac{\text{Top } \Delta OD_{AN}}{\text{Top } \Delta OD_S} = \frac{\phi_{isc}^{AN} \epsilon_{T-T}^{AN}}{\phi_{isc}^S \epsilon_{T-T}^S} \quad (4)$$

to analyte (Ret-8) and S refers to the standard. As the standard we employed benzophenone whose triplet–triplet absorption at 535 nm in argon purged benzene has an absorption coefficient  $\epsilon_{T-T}$  BP of 7220 L mol<sup>-1</sup> cm<sup>-1</sup> and where  $\phi_{isc}$  is taken to be 1.0. As with the singlet oxygen yield determinations the triplet–triplet  $\Delta OD$ 's were determined as a function of the percent laser dose with calibrated neutral density filters. The results of these experiments yielded  $\epsilon_{T-T} \approx 41$  000 and is the average of 10 separate measurements. Example decay traces of Ret-8 triplet monitored at 445 nm in argon-purged cyclohexane and benzophenone triplets monitored at 535 nm in benzene are displayed in Figure 7. Thus in place of a single value for top  $\Delta OD$  in eq 5 we employed the slope of the least-squares best line fit to the top  $\Delta OD$  data as a function of percent laser dose, which allowed us to avoid contributions to error from saturation effects and power dependent triplet yields.

## Discussion

The Ret-9 analogue has a very different ground state UV–vis and fluorescence spectrum from that of Ret-8 which more closely resembles that of the native retinal chromophores. The likely source of this difference is suggested by molecular mechanics calculations to be a decrease in conjugation within the  $\pi$ -system of the chromophore.<sup>37</sup> A Monte Carlo conformational search for global minima yielded the geometries for Ret-8 and Ret-9 shown in Figure 1. Note that the angle of the carbonyl bond compared to the C–C double bond of the  $\beta$ -ionone ring is much larger for Ret-9 than for Ret-8. If we qualitatively equate the size of the out-of-plane angle of the two terminal unsaturated bonds in these chromophores to the extent of conjugation in the  $\pi$ -system then it is reasonable to assume the ground state absorption for Ret-9 should be blue-



**Figure 7.** Comparative flash photolysis of Ret-8 and benzophenone as a function of laser dose. Benzophenone triplet was monitored at 535 nm and Ret-8 triplet at 430 nm. Based on the relative intensities of the respective triplet-triplet absorptions we estimate a molar extinction coefficient for Ret-8 triplet of,  $\epsilon = 41\,000$ .

shifted compared to Ret-8 in line with our observed spectra.<sup>38</sup> Flash photolysis experiments reveal further differences between these two retinal analogues.

We assigned the absorption detected at 395 nm to the triplet state of Ret-8 since its growth time constant on the picosecond time scale was much larger ( $\approx 40$  ps) than that expected for a singlet excited state jump growth. As well the triplet states of both the native 11-*cis*-retinal and *all-trans*-retinal have significant absorptions at 395 nm and there is no reason to expect that Ret-8 should be different. The singlet state of Ret-8 fluoresces weakly which is in line with prompt intersystem crossing (isc) and fast growth of the proposed triplet-triplet absorption for Ret-8 at 395 nm quoted above.

The dynamics of this absorption were followed over a 6 ns time window and exhibited no measurable decay. For comparison one should consider the picosecond dynamics of the triplet-triplet absorption spectrum of 11-*cis*-retinal. During a 100 ps period the wavelength of maximum absorption shifted from 430 nm to 450 nm. Adiabatic *cis-trans* isomerization on the triplet surface was submitted as the probable reason for the spectral evolution.<sup>23,25</sup> From our data we have no reason to suggest adiabatic relaxation through a twisted triplet. We tentatively suggest that the *cis*-locking ring of Ret-8 limits the twisting in the triplet state such that a perpendicular triplet cannot be reached. This is supported by the absorption spectrum collected on the nanosecond time scale shown in Figure 2. It exhibits a maximum absorption at 430 nm similar to the spectrum of unrelaxed 11-*cis*-retinal triplet mentioned above. In addition the lifetime of the triplet was found to be 500 ns in argon purged hexane solution. Also supporting this conclusion is the fact that triplets which can attain a twisted geometry frequently have much shorter lifetimes<sup>15-17</sup> and have larger rate constants for reaction with  $O_2$ .<sup>39</sup>

The transient absorption with  $\lambda_{max}$  of 430 nm observed on the nanosecond time scale is assigned to the triplet of Ret-8 since it is quenched by molecular oxygen and Tempo free radical at close to the diffusion controlled limit and since it sensitizes singlet oxygen ( $^1\Delta_g$ ) with a quantum efficiency of 0.47. If this value is equated to the quantum yield of isc to give triplet<sup>33</sup> then it implies a dark mode of relaxation from the first excited singlet state since fluorescence is weak and isc is fast and yet the yield of triplet is only  $\approx 50\%$  of the maximum possible value.

These findings imply that the triplet state of Ret-8 retains the *cis* geometry at the 11-12 double bond due to ring strain which limits the amount of possible twisting in the excited

surfaces. The well-known stable *trans*-cyclooctene formed upon photolysis of *cis*-1-phenylcyclooctene is achievable because its 8-membered ring is more flexible than that of Ret-8 (it incorporates only two  $sp^2$  carbons as opposed to four consecutive  $sp^2$  carbons found in the Ret-8 *cis*-locking ring, two of which belong to exocyclic double bonds). Recent work by Leigh et al. provide results which support an intriguing conical intersection mechanism for conjugated diene photochemistry. Apparently *cis-trans* isomerization about one or the other double bond involves simultaneous torsional motions about the central C-C single bond.<sup>40</sup> For Ret-8 it is quite reasonable that motion is restricted about the single bonds adjacent to the 11,12-ene and could stop isomerization on the triplet surface. If the same is true for the excited singlet of Ret-8 then it is unlikely that it will achieve the  $90^\circ$  twist required to effect the "sudden polarization" of Salem's model which occurs between  $89^\circ$  and  $91^\circ$  twist about a central double bond in solution.<sup>41</sup> It may, however, fall within the range  $40-130^\circ$  more recently calculated as the twisting required to enable polarization of  $S_1$  at the 11,12-ene in retinal.<sup>42</sup>

We also detected the triplet-triplet absorption of Ret-9 on the picosecond time scale at 395 nm and make the assignment to the triplet excited state based on reasoning similar to that used above for Ret-8. In this case, however, the absorption exhibited a fast decay over a 6 ns time scale to a plateau value of residual absorption. The experimental data were fit to first-order decay kinetics to yield a lifetime of 650 ps.<sup>26</sup>

One reviewer pointed out that one possibility is that the Ret-9 transient is a triplet confined to geometries corresponding to  $15 < E_T < 20$  kcal/mol such that it undergoes  $O_2$  quenching neither by spin exchange nor by excitation transfer. We tentatively assign the transient absorption centered at 353 nm, however, to a ground-state "twisted" or "transoid" species. This assignment is based upon its absorption spectrum which is similar to the *all-trans*-retinal ground state and due to the lack of reactivity of this transient species toward oxygen and Tempo free radical. Whereas oxygen and Tempo quenched the Ret-8 triplet readily.

The quenching of the Ret-9 transient, according to changes in the decay kinetics was moderate, as stated above. The top signal was, however, reduced markedly at higher concentrations of added quencher. We propose that the triplet state,  $T_1$ , of Ret-9 is short-lived ( $\approx 650$  ps  $< \tau_T < 16$  ns) and decays to produce a ground state *trans* or *transoid* species which has some absorption at 395 nm (explaining the residual absorption on the picosecond time scale) but a maximum absorption at 340 nm. The Ret-9 transient (from hereon referred to as *trans*-Ret-9) exhibits an absorption spectrum similar to *all-trans*-retinal but decays thermally with a 105 ns lifetime to the starting material Ret-9. This short lifetime for a ground-state *transoid* cyclic alkene is not unprecedented, *trans*-cyclohexene derivatives have been reported to have lifetimes of only 650 ns and approximately 130 ns.<sup>43</sup>

We were unable to ascertain through time-resolved spectroscopic means whether or not the growth kinetics of *trans*-Ret-9 matched the decay kinetics of the Ret-9 triplet monitored at 395 nm due to the spectral overlap of these two species at these wavelengths. An attempt to estimate the triplet precursor lifetime of the *trans*-Ret-9 through Stern-Volmer quenching analysis based upon the reduction of the top 355 nm optical density due to *trans*-Ret-9 as a function of oxygen concentration was unsuccessful. Unfortunately our data were not good enough (too much scatter) to provide anything but an estimate of an upper limit of the *trans*-Ret-9 precursor lifetime of  $\leq 16$  ns and thus is neither supportive nor contradictory of our hypothesis.

**TABLE 1: Experimental Values for the Slopes Obtained from Dependence of Singlet Oxygen Phosphorescence Intensities upon Laser Dose for Irradiation of the Indicated Compounds in Aerated Solution (Note the Similarity of the Values for  $\phi_{\Delta}$  and  $\phi_{isc}$  for 11-cis-Retinal)**

compound	slope	$\phi_{\Delta}$	$S_{\Delta}$	$\phi_{isc}$
naphthalene	19.4	0.75	1.0 <sup>a</sup>	0.75 <sup>a</sup>
benzophenone	12.75	0.49	0.29–0.40 <sup>a</sup>	1.0
11-cis-retinal	13.7	0.54	1.0 <sup>b</sup>	0.51 <sup>a</sup>
Ret-8	11.1	0.43	1.0 <sup>b</sup>	
Ret-9	4.13	0.16	1.0 <sup>b</sup>	

<sup>a</sup> Literature values. <sup>b</sup> Assumed based on results of Das et al.

A typical behavior of strained *trans*-cycloalkenes is to seek relief via protonation and subsequent net addition of solvent across the *trans* double bond in acidic methanol.<sup>7,9,10,12,13</sup> In our attempt to measure the rate constant for the reaction of *trans*-Ret-9 with protons in acidic methanol we found a great reduction in signal at 355 nm which is probably due to a reduction in the triplet quantum yield due to a reduced rate of intersystem crossing similar to that found for other retinals when moving from nonpolar to polar solvents. The result is based on the assumption that the lowest energy excited singlet becomes  $\pi,\pi^*$  in character in polar media.<sup>22</sup> This coupled with an already prompt decay ( $\tau = 105$  ns) of *trans*-Ret-9 may explain our failure to accurately measure quenching of this species.<sup>44</sup>

We wished to measure the quantum yields for triplet excited state formation in the retinal analogues and if possible their extinction coefficients. A literature search revealed that the yield of singlet oxygen produced in the energy transfer process from the triplet of a polyene such as *all-trans*-retinal could be used to accurately estimate the triplet quantum yields when comparatively measured against a standard actinometer.<sup>33</sup> We measured the yields of singlet oxygen luminescence in the standard way<sup>32,34</sup> as a function of percent laser dose. From the ratio of the slopes measured for the retinals with the standard, as outlined earlier, we obtained values of  $\phi_{\Delta}$  which in practice can be equated to the quantum yield of triplet states,  $\phi_{isc}$ .<sup>33,36</sup> The measured values are summarized in Table 1.

The value of  $\phi_{isc}$  for Ret-8 is very similar to 11-cis-retinal ( $\approx 0.50$ ) whereas that for Ret-9 is significantly lower (0.16). These numbers coupled with the very similar values for the rates of *isc* encountered in the picosecond transient absorption experiments imply Ret-9 may have a more efficient thermal relaxation pathway for  $S_1$  as compared to Ret-8.<sup>36</sup> This is not surprising since the same is true for  $T_1$  and is most probably due to an increased ability of the 11-cis bond to twist in the respective excited states for Ret-9. What is surprising is that the addition of only one methylene unit in the cis-locking ring is required to effect such a dramatic change in photophysics between Ret-8 and Ret-9.

## Conclusion

Laser flash spectroscopy of two 11-cis-locked retinal analogues has led to the detection of two distinct transient absorptions in alkane solutions at room temperature. The difference of one methylene unit in the cis-locking ring between the two analogues results in a remarkable difference in the transients observed following laser excitation. The 11-cis-retinal analogue with the 11-cis unsaturation included in an eight-membered ring affords a triplet state with a 430 ns lifetime at room temperature in argon purged hexane solution. The analogue with the 11-cis unsaturation included in a nine-membered ring affords a ground-state transoid species with a 110 ns lifetime under the same conditions. The assignments are based upon the spectra, the lifetimes, and the reactivity of

the transients. The different behaviors of the two analogues following photoexcitation is probably a result of their ability to twist about the 11,12-ene.

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(36) This requires the further assumption that all triplets decay via singlet oxygen generating processes. Whereas in the case of Ret-8 this is a reasonable assumption, in the case of Ret-9 the estimated lifetime of  $T_1$  is too short to ensure this criterion. In the latter case  $\phi_{\Delta}$  is undoubtedly less than  $\phi_{isc}$ .

(37) Performed on molecular mechanics software, *Macromodel*.

(38) Cyclononane is known to incorporate substantially more ring strain than cyclooctane. Four  $sp^2$  carbons incorporated into an already strained system should further increase the strain energy content and result in the nonplanar chromophore suggested by the calculations.

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