rative VPC (5 ft × 0.25 in. 5% SE-30 on Chromosorb G, 105 °C) furnished 81.6 mg of pure hydrocarbon. The ^{1}H NMR spectrum ($CDCl_3$) clearly shows the presence of only one exo proton at δ 1.88–1.81 and two endo protons at δ 1.53–1.31; integration indicated 98% D_1. Mass spectrum: m/e calcd for $C_{10}H_{11}D$, 133.1002, obsd 133.0998.

(−)-(1*S*)-(2-*endo*-^{2}H)-2,3-Dihydrotriquinacene (11a). Analogous treatment of **9b** (1.7 g, 11 mmol, $[\alpha]^{22}_D$ +55°) with methanesulfonyl chloride yielded 2.28 g (93%) of **10b**. Its ^{1}H NMR spectrum is essentially identical with that of **10a** except for the absence of the doublet of triplets at δ 5.29–4.80. Mass spectrum: m/e calcd for $C_{11}H_{13}DO_3S$ 227.0726, obsd 227.0732.

Lithium aluminum hydride reduction of **10b** (340 mg, 1.5 mmol) as previously described gave 145 mg (72%) of distilled **11a** (86 mg after further VPC purification). Its ^{1}H NMR spectrum (in $CDCl_3$) shows the presence of two exo protons at γ 1.93–1.67 and a single endo proton at 1.53–1.20; integration indicated 98% d_1. Mass spectrum, m/e calcd 133.1002, obsd 133.0997.

(+)-(1*S*)-(2,2-$^2H_2$)-2,3-Dihydrotriquinacene (11c). Reduction of **10b** (340 mg, 1.50 mmol) with lithium aluminum deuteride as before afforded 154 mg (77%) of **11c** (94.5 mg after VPC purification). The single exo and endo saturated protons were seen (in $CDCl_3$) at δ 1.85–1.83 and 1.51–1.32, respectively (ca. 98% d_2). Mass spectrum m/e calcd for $C_{10}H_{10}D_2$ 134.1064, obsd 134.1060.

(+)-(1*R*,4*S*,7*R*,10*S*)-(2-^{2}H)-Triquinacene (12). A 200-mg (0.88-mmol) sample of **10b** in dichloromethane was treated with 4.4 g of alumina (Woelm N-Super I, activated as before) and stirred vigorously under argon for 2 days. Workup in the predescribed manner followed by Kugelrohr distillation at 100 °C (30 mm) furnished 60 mg (52%) of **12** as a colorless oil: IR (neat, cm^{-1}) 3045, 2955, 2875, 2260; ^{1}H NMR ($CDCl_3$, δ) 5.61 (s, 5 H), 3.72 (s, 4 H) with d incorporation = 97 ± 2%; mass spectrum m/e calcd for $C_{10}H_9D$ 131.0845, obsd 131.0840.

(1*S*)-2-(Hydroxymethyl)triquinacene (13). To an ice-cold stirred slurry of lithium aluminum hydride (492 mg, 12.9 mmol) in anhydrous ether (90 mL) was added aluminum chloride (574 mg, 4.3 mmol) in one portion, followed by (−)-**3** (1.00 g, 5.75 mmol, $[\alpha]^{22}_{365}$ −398°) in 30 mL of the same solvent. The reaction mixture was stirred overnight at room temperature, quenched with excess methanol, and washed with 5% hydrochloric acid (100 mL) and saturated sodium bicarbonate solution. The alkaline aqueous layer was back-extracted with ether, and the combined organic layers were dried and evaporated to leave 920 mg (100%) of a colorless oil. VPC analysis (10 ft × 0.12 in. 15% SE-30 on Chromosorb W, 150 °C) showed one component to be heavily dominant. For **13**: IR (neat, cm^{-1}) 3340, 3055, 2965, 2880; ^{1}H NMR ($CDCl_3$, δ) 5.8–5.3 (m, 5 H), 4.07 (s, 2 H), 3.8–3.6 (m, 4 H), 1.8 (br s, 1 H). This alcohol proved labile to gas chromatographic conditions.

(+)-(1*S*)-2-(Chloromethyl)triquinacene (14). To a cold (0 °C) solution of *N*-chlorosuccinimide (777 mg, 5.84 mmol) in dry dichloromethane (16 mL) was added 480 μL (6.53 mmol) of freshly distilled (from CaH_2) dimethyl sulfide. After the mixture was cooled to −20 °C, 850 mg (5.31 mmol) of **13** (84% ee) dissolved in 8 mL of dichloromethane was added. The reaction mixture was stirred at room temperature for 1 h and shaken with ice-cold brine (40 mL). The aqueous phase was extracted with dichloromethane (2 × 10 mL), and the combined organic layers were washed with cold brine (20 mL), dried, and evaporated. Chromatography of the yellow oil on Florisil (25 g) by using hexane–ether (3:1) as eluant afforded 657 mg (70%) of **14** which was pure by VPC analysis: IR (neat, cm^{-1}) 3060, 2965, 2885, 730, 683; ^{1}H NMR ($CDCl_3$, δ) 5.8–5.3 (m, 5 H), 4.11 (s, 2 H), 3.98–3.53 (m, 4 H); mass spectrum, m/e calcd for $C_{11}H_{11}Cl$ 178.0549, obsd 178.0542; $[\alpha]^{22}_D$ +55°, $[\alpha]^{22}_{365}$ +101.4° (c 0.91, $CHCl_3$).

(−)-(1*S*)-2-Methyltriquinacene (15). To a solution of **14** (600 mg, 3.37 mmol) in 15 mL of dry tetrahydrofuran was added 128 mg (3.37 mmol) of lithium aluminum hydride, and the mixture was heated at the reflux temperature for 2 h, cooled, diluted with ether, and quenched with saturated sodium sulfate solution. Following drying and filtration, the clear filtrate was carefully concentrated and the residual oil was purified by preparative VPC (6 ft × 0.25 in. 5% SE-30 on Chromosorb G, 120 °C). There was obtained 208 mg (43%) of **15**: IR (neat, cm^{-1}) 3010, 2920, 2850, 1600, 990, 943, 877, 726, 715; ^{1}H NMR ($CDCl_3$, δ) 5.69 (s, 2 H), 5.62 (s, 2 H), 5.36 (m, 1 H), 3.8–3.4 (m, 4 H), 1.7 (s, 3 H); mass spectrum, m/e calcd 144.0939, obsd 144.0940.

Anal. Calcd for $C_{11}H_{12}$: C, 91.61; H, 8.39. Found: C, 91.22; H, 8.59.

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Resonance Raman Spectra of Excited Triplet State *all-trans*-Retinal

G. H. Atkinson,* J. B. Pallix, T. B. Freedman, D. A. Gilmore, and R. Wilbrandt†

Contribution from the Department of Chemistry, Syracuse University, Syracuse, New York 13210. *Received March 23, 1981*

Abstract: The time-resolved resonance Raman (TR^3) spectra of the lowest-energy excited triplet state of *all-trans*-retinal are reported (Figure 2 and Table I). The triplet state was formed photolytically as a result of intersystem crossing from the lowest energy excited singlet state which was populated by 354.7 nm pulsed excitation. The TR^3 spectra of the excited triplet state were obtained with pulsed laser radiation tuned into resonance with the transient triplet–triplet absorption band of *all-trans*-retinal near 470 nm. Time delays between the two pulsed lasers ranged from 40 ns to 20 μs. The experimental conditions were chosen to ensure that the triplet state monitored by TR^3 spectroscopy derived from the all-trans isomer of retinal.

The major contribution made by the retinal chromophore to the activity of rhodopsin in visual processes is well-recognized and has been the subject of extensive study.[1-3] The photoisomerization of retinal itself, of course, underlies its function as the chromophoric group in rhodopsin. The mechanism by which photoisomerization occurs in retinal may be viewed in terms of two sequential steps: (1) photolytic population of an excited electronic state followed by some degree of photophysical decay and (2) molecular isomerization. The latter step, photolytically induced interconversion of retinal isomers, also has been examined in numerous studies.[4-7] In the specific case of retinal transformations in rhodopsin and bacteriorhodopsin, resonance Raman spectroscopy has been used to identify the conformations of ground-state intermediates.[8,9] It has remained difficult, however, to correlate

† Chemistry Department, Risø National Laboratory, DK-4000 Roskilde, Denmark.

(1) Wald, G. *Nature (London)* **1968**, *219*, 800–807.
(2) Honig, B.; Ebrey, T. G. *Annu. Rev. Biophys. Bioeng.* **1974**, *3*, 151–177.
(3) Ebrey, T. G.; Honig, B. *Q. Rev. Biophys.* **1975**, *8*, 129–184.
(4) Kropf, A.; Hubbard, R. *Photochem. Photobiol.* **1970**, *12*, 249–260.
(5) Waddell, W. H.; Crouch, R.; Nakanishi, K.; Turro, N. J. *J. Am. Chem. Soc.* **1976**, *98*, 4189–4192.
(6) Waddell, W. H.; Hopkins, D. L.; Uemura, M.; West, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 1970–1972.
(7) Rosenfeld, T.; Honig, B.; Ottolenghi, M.; Hurley, J. J.; Ebrey, T. G. *Pure Appl. Chem.* **1977**, *49*, 341–351.
(8) Mathies, R. In "Chemical and Biochemical Applications of Lasers"; Moore, C. B., Ed.; Academic Press: New York, 1979; Vol. IV, pp 55–104.

0002-7863/81/1503-5069$01.25/0

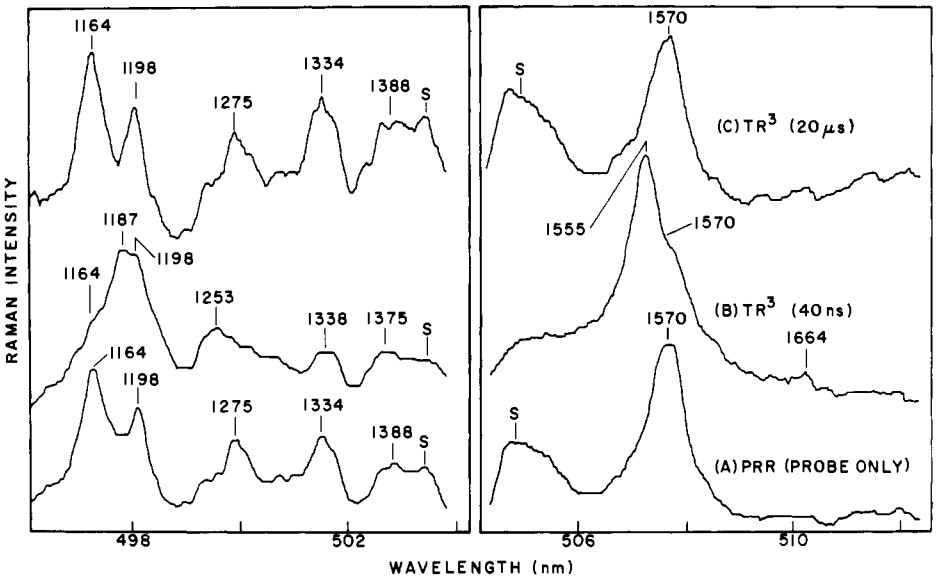

Figure 1. Resonance Raman spectra of a 2.5×10^{-4} M *all-trans*-retinal sample in methanol obtained under rapid flow conditions and with 470-nm laser radiation. (A) Pulsed resonance Raman (PRR) spectrum obtained with probe laser (470 nm) only. (B) Time resolved resonance Raman (TR^3) spectrum at a time delay of 40 ns after 354.7-nm excitation. (C) TR^3 spectrum at a time delay of 20 μs after 354.7-nm excitation. The measured wavenumber (cm^{-1}) position of each Raman is shown (see Table I for error limits). Raman bands from the solvent (methanol) are labeled S. The intensity scale in the right panel (1550-cm^{-1} region) is approximately 2.5 times that in the left panel (1250-cm^{-1} region).

a specific isomerization pathway with the first step, namely the excited electronic state which acts as a precursor to isomerization. The precursor may be the excited electronic state photolytically populated or it may be an excited electronic state populated by subsequent photophysical relaxation. Such a correlation would significantly increase our understanding of the excited-state processes which control both isomerization in retinal itself as well as the related photolytic activity of retinal in rhodopsin.

It has been shown recently that time-resolved resonance Raman (TR^3) techniques can be used to obtain vibrational resonance Raman spectra not only of short-lived photochemical intermediates (e.g., isomers), but also of excited electronic states.[10-15] In this paper, we report the TR^3 spectrum of the excited triplet state of *all-trans*-retinal. This species was recently studied by TR^3 spectroscopy after its formation by energy transfer from a sensitizer generated through pulsed radiolysis.[16] In the work presented here, the excited triplet state was formed more directly by photolytic excitation and the experimental conditions were controlled in order to ensure that an excited state of the all-trans isomer was being monitored.

Experimental Section

The lowest-energy triplet state of retinal was populated by laser excitation into the $S_0 \rightarrow S_1$ absorption band near 380 nm in conjunction with $S_1 \rightarrow T_1$ intersystem crossing. The S_1 state of retinal was populated by direct excitation with the frequency-tripled output (1.5 mJ at 354.7 nm, 17 ns pulse width) of a Nd:YAG laser (International Laser Systems, Model LL-103). After a variable time delay ranging from 40 ns to 20 μs, the output (1 mJ, 8 ns pulse width) from a dye laser (Molectron Corp., Model DL-II) pumped by a frequency tripled Nd:YAG laser (Quanta Ray, Model DCR) was tuned into resonance (between 430 and 520 nm) with the transient triplet–triplet absorption spectrum of *all-trans*-retinal. Intersystem crossing to the lowest-energy triplet state occurs faster than the shortest (40 ns) time delay used.[17] Both laser systems were operated at repetition rates of 10 pulses per second and the time jitter between laser pulses was minimized to 5 ns by external triggering of the Q switches for the two Nd:YAG lasers.

The two laser beams were focused colinearly to a beam waist of about 1 mm in the sample cell. The collection optics were adjusted to view only the top 2 mm of the irradiated sample and to focus the image of the Raman scattering onto a 500 μm slit of a 1-m spectrograph. Raman scattering was detected by an intensified silicon photodiode array cooled to −10 °C (EG and G, Princeton Applied Research, Model 1420 detector and 1218 OMA-2 controller). The detector was gated on (5 ns fwhm) only during the laser pulse used to generate resonance Raman scattering. This pulsed mode greatly reduced the background signal of retinal fluorescence caused by excitation of 354.7 nm.

With the multichannel detection system, scattering from a 340 cm^{-1} wide region was recorded by accumulating approximately 3300 laser pulses for each spectrum. The digitized spectra were then treated with a 17-point smoothing routine, and when necessary, a polynomial expression simulating background was substracted from the data to produce a linear, horizontal baseline.

all-trans-Retinal (Eastman) and methanol (MC&B anhydrous reagent ACS) were used without further purification. Benzene (MC&B reagent ACS) was distilled prior to use. Deaerated samples were prepared by passing nitrogen through the sample for approximately 30 min. The optimal retinal concentration was found to be about 2.5×10^{-4} M. At higher concentrations, the 354.7 nm laser pulse did not penetrate far enough into the sample for optimum Raman scattering. The retinal sample was studied under both static and rapid flow conditions. In the flow apparatus, the sample was drawn through a rectangular quartz cell (2 mm × 4 mm i.d.), using a variable speed pump at a flow rate of approximately 5 mL/s. Assuming that the 1 mm diameter laser pulse irradiates a 3×10^{-3} mL sample volume, each spectrum reproduced here (3300 pulses) exposes 10 mL of sample to laser radiation. This means that by recycling 1 L of solution and recording ten consecutive spectra, a maximum of 10% of the bulk sample could have undergone permanent isomerization.[8]

Results

Both the pulsed resonance Raman (PRR) and TR^3 spectra obtained from samples of *all-trans*-retinal dissolved in methanol are presented in Figure 1. Significant changes in the resonance Raman spectrum were observed primarily in the 1100–1700 cm^{-1} region shown. Resonance Raman scattering from the ground state of *all-trans*-retinal was observed (Figure 1A) when only the probe

(9) Eyring, G.; Curry, B.; Mathies, R.; Fransen, R.; Palings, I.; Lugtenberg, J. *Biochemistry* **1980**, *19*, 2410–2518.
(10) Wilbrandt, R.; Jensen, N. H.; Pagsberg, P.; Sillesen, A. H.; Hansen, E. B. *Nature (London)* **1978**, *276*, 167.
(11) Dallinger, R. F.; Woodruff, W. H. *J. Am. Chem. Soc.* **1979**, *101*, 4391.
(12) Dallinger, R. F.; Guanci, J. J.; Woodruff, W. H.; Rogers, M. A. J. *J. Am. Chem. Soc.* **1979**, *101*, 1355.
(13) Atkinson, G H.; Dosser, L. R. *J. Chem. Phys.* **1980**, *72*, 2195.
(14) Asano, M.; Koningstein, J. A.; Nicollin, D. *J. Chem. Phys.* **1980**, *73*, 688.
(15) Jensen, N. H.; Wilbrandt, R.; Pagsberg, P. B.; Sillesen, A. H.; Hansen, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 7441.
(16) Wilbrandt, R.; Jensen, N. H. *J. Am. Chem. Soc.* **1981**, *103*, 1036.
(17) Hochstrasser, R. M.; Narva, D. L.; Nelson, A. C. *Chem. Phys. Lett.* **1976**, *43*, 15–19.

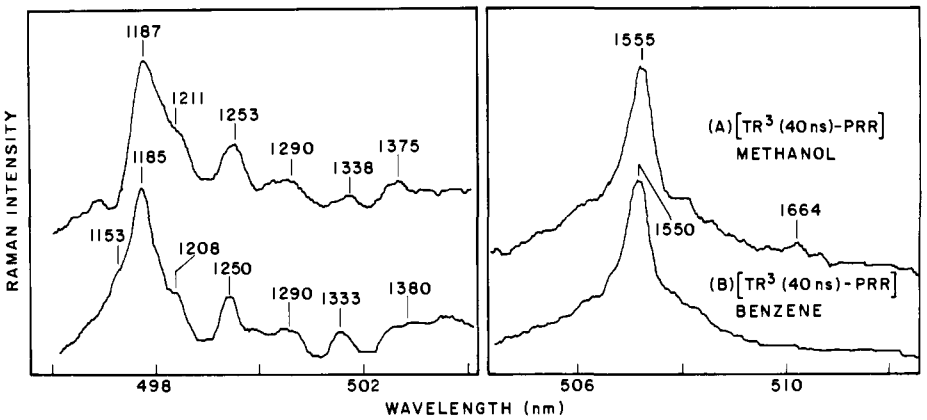

Figure 2. Resonance Raman spectra of the excited triplet state of *all-trans*-retinal obtained at 40 ns after 354.7 nm excitation in methanol (A) and benzene (B) solutions. The concentration of ground state *all-trans*-retinal was 2.5×10^{-4} M in both samples. The spectra shown in A and B were obtained after subtraction of Raman bands from the solvent and ground-state *all-trans*-retinal (see text). The measured wavenumber (cm^{-1}) position of each Raman band is shown (see Table I for error limits). The intensity scale in the right panel (1550-cm^{-1} region) is approximately 2.5 times that in the left panel (1250-cm^{-1} region).

laser at 470 nm was used. The resonance enhancement arises from the strong *all-trans*-retinal absorption band having a maximum near 380 nm.[18,19] When the probe laser follows the 354.7 nm excitation laser pulse by 40 ns, new Raman bands are observed which are not identifiable with either ground-state *all-trans*-retinal or the methanol solvent (Figure 1B). When the delay between the excitation and probe lasers is extended to 20 μs, these new Raman bands disappear to be replaced by a resonance Raman spectrum (Figure 1C) which duplicates the probe spectrum originally measured (Figure 1A).

The Raman spectrum of the transient alone was obtained by subtracting the probe laser spectrum (containing Raman bands from the solvent and, in the case of methanol, ground-state *all-trans*-retinal) from the TR^3 spectrum at a specific time delay. Such quantitative subtraction is feasible only when the probe spectrum can be properly scaled with respect to the TR^3 spectrum. In principle, scaling might be obtained by comparing the intensities of Raman bands from either the solvent or ground-state *all-trans*-retinal. Scaling of this type is made difficult by time dependence of the (triplet–triplet) absorption properties of the reacting solution and by the large initial depletion of the ground-state population of *all-trans*-retinal caused by optical pumping. The transient absorption properties of the solution will affect the measured intensities of Raman bands from both the solvent and ground-state *all-trans*-retinal. Optical depletion will influence only the intensity of bands from the latter. Thus, the intensities of solvent bands might be expected to exhibit a different time dependence than those of ground-state *all-trans*-retinal. With this potential difficulty recognized, the intensities of the solvent bands in each probe spectrum were scaled to match the corresponding solvent bands in each TR^3 spectrum prior to subtraction. In benzene solvent, this procedure is straightforward since no Raman bands from ground-state *all-trans*-retinal are observed. For samples in methanol, however, a more complicated difference spectrum might be expected since (1) the probe and TR^3 spectra both contain Raman bands from ground-state *all*-trans-retinal as well as the solvent and (2) a large percentage (~90%) of the ground-state population is initially pumped into the lowest-energy excited singlet state by laser excitation at 354.7 nm.

The difference spectra from the benzene and methanol samples are identical, however, aside from slight frequency shifts comparable to the error in the measurements. The similarity of the difference spectra can be understood by noting that photophysical decay (primarily internal conversion) of the excited singlet state is rapid (<10 ns) relative to the time scale over which Raman scattering is generated (40 ns to 20 μs). Since the quantum yield for intersystem crossing ($S_1 \rightarrow T_1$) is small (~0.1[25]) in methanol, most of the *all-trans*-retinal returns to its ground electronic state prior to the measurement of the TR^3 spectrum. The degree to which the ground state of *all-trans*-retinal appears to be depleted in these data, therefore, is found to be negligible.

The use of two solvents allowed us to identify several weak bands of the transient in one solvent which were obscured by solvent or ground state *all-trans*-retinal Raman scattering in the other solvent. The intensities of these bands are found to be increased in the TR^3 spectra measured at short (e.g., 40 ns) delay time, relative to the probe spectra and the spectra taken at 20 μs delay. The bands are present before subtraction of solvent or ground-state spectra, and are not artifacts of the subtraction procedures. For example, in benzene solution, a weak shoulder appears at 1153 cm^{-1} (obscured by the 1164 cm^{-1} ground-state band of *all-trans*-retinal in methanol). In methanol, a 1010-cm^{-1} shoulder to the 1030-cm^{-1} solvent band and a weak band at 1665 cm^{-1} are observed. Both of these weak Raman bands are obscured by intense solvent scattering in benzene. The measured Raman frequencies are presented in Table I together with the values reported previously.[16]

The experimental conditions were chosen to ensure that the major isomer present in the sample was *all-trans*-retinal and that, therefore, the TR^3 spectra could be directly associated with this one isomer. This conclusion was confirmed by two observations. First, the resonance Raman bands appearing in the 470 nm probe spectrum of a static sample (in methanol) could be assigned primarily to *all-trans*-retinal. This is the same spectrum obtained at the 20 μs delay. It is well-documented[20-23] that resonance Raman spectra can uniquely identify the ground states of the other retinal isomers. For example, 13-*cis*-retinal (the isomer shown to be the principal photolysis product from *all-trans*-retinal[5,6]) has a strong band at 1221 cm^{-1} which distinguishes it from the all-trans, 9-cis, and 11-cis isomers.[20-23] A weak Raman band at 1221 cm^{-1} was observed in the static *all-trans*-retinal samples exposed to 354.7 nm radiation. The relative intensities of the Raman bands at 1221 and 1198 cm^{-1} (from ground-state *all-trans*-retinal) remain fixed (even with continued exposure to 354.7 nm radiation) indicating that the relative concentration of 13-*cis*-retinal in the static samples remained constant and could be estimated to be no more than 10% of the total retinal sample. Second, the resonance Raman spectra taken with 470-nm exci-

(18) Doukas, A. G.; Aton, B.; Callender, R. H.; Honig, B. *Chem. Phys. Lett.* **1978**, *56*, 248–252.
(19) Rimai, L.; Heyde, M. E.; Heller, H. C.; Gill, D. *Chem. Phys. Lett.* **1971**, *10*, 207–211.
(20) Rimai, L.; Gill, D.; Parsons, J. L. *J. Am. Chem. Soc.* **1971**, *93*, 1353–1357.
(21) Gill, D.; Heyde, M. E.; Rimai, L. *J. Am. Chem. Soc.* **1971**, *93*, 6288–6289.
(22) Callender, R. H.; Doukas, A.; Crouch, R.; Nakanishi, K. *Biochemistry* **1976**, *15*, 1621–1629.
(23) Cookingham, R. E.; Lewis, A.; Collins, D. W.; Marcus, M. A. *J. Am. Chem. Soc.* **1976**, *98*, 2759–2763.

Table I. Vibrational Frequencies (cm^{-1}) in the Lowest Energy Excited Triplet State of *all-trans*-Retinal Observed by Time-Resolved Resonance Raman Spectroscopy[e]

electron irradiation in benzene[a]	laser photolysis	
	in benzene	in methanol
	b	1010[c] (w)
1137[c] (vw)		*b*
	1153?[c] (vw)	*b*
1186 (vs)	1185[d] (s)	1187[d] (s)
1212?[c] (w)	1208[c] (w)	1121[c] (w)
1253 (w)	1250 (m)	1253 (m)
1305 (vw)	1290 (w)	1290 (w)
1339 (w)	1333 (w)	1338 (w)
	1380 (w)	1375 (w)
1551 (vs)	1550[d] (vs)	1555[d] (vs)
	b	1664? (vw)

[a] Wilbrandt, R.; Jensen, N. H. *J. Am. Chem. Soc.* 1981, *103*, 1036. [b] Overlaps Raman band from the solvent or ground state of *all-trans*-retinal. [c] Appears as a shoulder overlapping a Raman band from either the solvent or the excited electronic state of *all-trans*-retinal. [d] ±1 cm^{-1}, sharp spectral feature. [e] Wavenumber positions are accurate to within ±3 cm^{-1} except for sharp spectral features.

tation of a sample under flow conditions which maximize the concentration of *all-trans*-retinal (Figures 1 and 2) contain no band at 1221 cm^{-1} demonstrating that no 13-*cis*-retinal was present.

The identification of these time-dependent Raman bands with the excited triplet electronic state of *all-trans*-retinal derives from three observations. First, the time-dependent decay of the transient was measured quantitatively from the integrated area of bands in the TR^3 spectra. The decay followed first-order kinetics between 5 and 18 μs and gave a lifetime of 8 μs. This value corresponds well with a lifetime reported in the literature for the decay of triplet-state retinal.[24-26] The literature values were obtained from triplet–triplet absorption spectra and therefore they may be representative of isomeric mixtures of retinal. [The room-temperature, optical-absorption spectra of the various isomers of retinal are indistinguishable except for small changes in the maximum absorption coefficient.[24-27]] Second, the TR^3 bands were very sensitive to the presence of molecular oxygen. The decay of the 1550-cm^{-1} band, for example, was at least 50 times faster in an air-saturated benzene solution than in a benzene solution which had been deaerated.[26] Third, the resonance Raman excitation profile for the strongest TR^3 band at 1550 cm^{-1} was found to correlate very well with the triplet–triplet absorption spectrum of *all-trans*-retinal.[24-27] The excitation profile exhibited a one-to-one correspondence with the absorption spectrum in the 430–520-nm region. The detailed analysis of the kinetic data and the excitation profiles are described elsewhere.[28]

Discussion

The contribution of photoisomerization between ground-state isomers must be analyzed in detail in order to properly interpret the TR^3 spectroscopy of excited electronic states in retinal. In the case of *all-trans*-retinal, it is thought that small yields of both the 13-cis and 9-cis isomers are photolytically generated at the excitation wavelengths used in this work.[5,6] These small yields do not necessarily apply to the relative concentrations of these isomers throughout the photoisomerization process. Rather, the measurements refer to the stable concentration of each isomer after photoisomerization has been completed. Since these isomers are themselves photolytically interconvertible (e.g., 13-cis + $h\nu$ → all-trans), the relative concentration of each isomer during photoisomerization remains unknown. This latter, time-dependent concentration of isomers is, of course, the quantity which can be examined by a TR^3 experiment. To study the properties of *all-trans*-retinal therefore, it was necessary to select excitation conditions which ensure that the sample being examined contains primarily the all-trans isomer. The results obtained with the flowing and static samples confirm that primarily *all-trans*-retinal is present under the excitation conditions used here. Furthermore, the reproduction at long (20 μs, Figure 1) reaction times of the resonance Raman spectrum obtained with the probe laser only establishes the reversibility of the photophysical processes being monitored by TR^3 spectroscopy. These results lead us to conclude that the TR^3 spectra reported here are those of the lowest energy triplet state of *all-trans*-retinal as are the kinetic and spectroscopic properties derived from these TR^3 spectra.[28]

A comparison of the TR^3 spectra observed in this work with those reported previously[16] (Table I) demonstrates that the excited triplet state populated by electron irradiation and subsequent energy transfer is the same as that formed photolytically. Since the TR^3 spectrum obtained at 40 ns (this work, Figure 2) is essentially the same as that reported at times long enough to ensure complete electronic and vibrational relaxation (1 μs[16]), we also conclude that both experiments examine the vibrationally relaxed lowest-energy triplet state of *all-trans*-retinal.

The detection of both excited and ground electronic states of specific isomeric forms of retinal by TR^3 spectroscopy suggests that it is now feasible to experimentally correlate isomerization pathways with the excited electronic states of the particular isomer from which it originates. Work directed toward this goal is currently underway.

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(24) Azerad, R.; Bensasson, R.; Cooper, M. B.; Dawe, E. A.; Land, E. S. In "Excited States of Biological Molecules", Birks, J. B., Ed.; Wiley: London, 1976.
(25) Das, P. K.; Becker, R. S. *J. Am. Chem. Soc.* **1979**, *101*, 6348–6353.
(26) Truscott, T. G.; Land, E. J.; Sykes, A. *Photochem. Photobiol.* **1973**, *17*, 43–51.
(27) Veyret, B.; Davis, S. G.; Yoshida, M.; Weiss, K. *J. Am. Chem. Soc.* **1978**, *100*, 3283–3290.
(28) Pallix, J. B.; Freedman, T. B.; Atkinson, G. H., unpublished results.