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Activation Barriers for the Trans → Cis Photoisomerization of *all-trans*-Retinal

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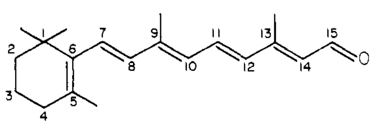
As the light-absorbing component of the visual protein rhodopsin and of bacteriorhodopsin, the cis → trans and trans → cis photoisomerizations of 11-*cis*- and *all-trans*-retinal (structure 1) have been widely studied since the retinyl chromophores of these proteins undergo such reactions following photon absorption.[1]



all-trans-retinal (1)

Experimental measurements of the quantum yields of photoisomerization (ϕ_{PI}) of the isomeric retinals have been made upon direct excitation[2-6] and triplet sensitization,[3,4] and primary photoproducts and product ratios have been determined.[4,5,7-10] Mechanistic details of these reactions have been based upon laser-induced kinetic and spectroscopic methods,[3,6,11-13] and upon theoretical calculations of the potential-energy surfaces of simple polyenes[14-17] and retinylpolyenes.[18-20]

Since only limited experimental information on the energetics of these excited-state isomerizations is available,[2] but is necessary before a description of the photochemical and spectroscopic properties of the retinals can be complete, we have made quantitative investigations of the photoisomerization of *all-trans*-retinal as a function of temperature in a polar and a nonpolar solvent. Using high-pressure liquid chromatographic (LC) techniques the primary photoproducts and their relative ratios were determined at each temperature. The energy barrier of the excited-state isomerization of *all-trans*-retinal, including the barrier of each component isomerization, is calculated on the basis of a proposed isomerization mechanism.

all-trans-Retinal (Sigma Chemical) was purified to >99% isomer pure by using high-pressure LC methods. 3-Methylpentane (3MP; Phillips Petroleum, 99.4%) was refluxed over and distilled from Dri-Na (Baker Chemical) immediately prior to use. Ethyl alcohol (EtOH; 200 proof, Publicker Industries) was distilled from calcium chloride immediately prior to use. Spectrophotometric grade *n*-hexadecane (99+%), *n*-undecane (99%), and *n*-decane (99+%) were purchased from Aldrich Chemical and used as received.

Table I. Quantum Yields of the Trans → Cis Photoisomerization of *all-trans*-Retinal as a Function of Temperature

		ϕ_{PI}[b]		
solvent	temp, K[a]	trans → 9-cis	trans → 11-cis[c]	trans → 13-cis
ethanol	298	0.020	0.090	0.22
	253	0.014	0.071	0.15
	230	0.011	0.079	0.12
	206	0.0052	0.052	0.081
	183	0.0024	0.031	0.059
	160	0.0018	0.023	0.053
3-methylpentane	298	0.015		0.10
	273			0.079
	253	0.0065		0.053
	233	0.0086		0.041
	213	0.0030		0.026
	193	0.0014		0.018
	173	0.00036		0.010

[a] ±2 K. [b] 10^{-4}–10^{-5} M in aerated solutions; ϕ_{PI} ± 15% relative to that measured at 298 K; λ_e = 350 nm, 7-nm bandpass. [c] 11-*cis*-retinal is not a primary photoproduct in 3MP.

LC purifications and separations were accomplished by using the system and chromatographic conditions previously described.[5,21] Room and low temperature absorption spectra were recorded on a Perkin-Elmer Model 575 spectrophotometer using long-stemmed, flat-faced 2-mm cells in conjunction with a flat-faced, double-jacketed liquid nitrogen Dewar. Temperatures between +25 and −180 °C (±1 °C) were obtained by heating nitrogen vapors that were delivered from a reservoir of liquid nitrogen into the double-jacketed Dewar.

Monochromatic radiation was obtained by using a Hanovia 1000-W Hg–Xe lamp and Bausch & Lomb High Intensity Grating Monochromator. ϕ_{PI} was determined at room temperature by using the method we have previously employed.[4,5,21,22] Relative ϕ_{PI}'s were determined between 0 and −125 °C by comparison to the room temperature ϕ_{PI} value obtained under otherwise identical experimental conditions. A correction for the difference in absorption intensity resulting from varying temperature was applied. All high-pressure LC analyses were performed at room temperature. All experimentation was performed under red lighting.

Electronic absorption spectra of *all-trans*-retinal were recorded between 25 and −125 °C; at room temperature in 3MP λ_{max} = 368.5 nm, ϵ_{max} 50 000 M^{-1} cm^{-1} and h (bandwidth at half-height) = 5300 cm^{-1} and in EtOH λ_{max} = 382 nm, ϵ_{max} 47 900 M^{-1} cm^{-1} and h = 5980 cm^{-1}. With decreasing temperature for each solvent, λ_{max} undergoes a bathochromic shift and ϵ_{max} and h increase; however, the increase in ϵ_{max} with decreasing temperature has been shown to be due to a pure solvent contraction effect.[23]

Upon irradiation of *all-trans*-retinal in 3MP at room temperature, 13-*cis*- and 9-*cis*-retinal are formed as primary photoproducts in the relative ratio of 7:1; ϕ_{PI} = 0.12 ± 0.02. ϕ_{PI} obtained in *n*-decane, *n*-undecane, and *n*-hexadecane are somewhat lower (10%), ϕ_{PI} = 0.10; however, these results are within experimental reproducibility of that measured in 3MP. In the polar solvent EtOH, 7-*cis*- and 11-*cis*-retinal are formed as primary photoproducts;[5,7] however, since 7-*cis*-retinal is present in only trace amounts, ca. 1% of the observed products, it was not analyzed. The 13-cis/11-cis/9-cis product ratio was determined to be 11:4:1 which is similar to the ratios previously reported.[5] ϕ_{PI} = 0.33 in EtOH, a value notably higher than that measured in MeOH.[4]

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(1) For a review, see: Rosenfeld, T.; Honig, B.; Ottolenghi, M.; Hurley, J.; Ebrey, T. G. *Pure Appl. Chem.* **1977**, *49*, 341.

(2) Kropf, A.; Hubbard, R. *Photochem. Photobiol.* **1970**, *12*, 249.

(3) Rosenfeld, T.; Alchalal, A.; Ottolenghi, M. *J. Phys. Chem.* **1974**, *78*, 336.

(4) Waddell, W. H.; Crouch, R.; Nakanishi, K.; Turro, N. J. *J. Am. Chem. Soc.* **1976**, *98*, 4189.

(5) Waddell, W. H.; Hopkins, D. L. *J. Am. Chem. Soc.* **1977**, *99*, 6457.

(6) Veyret, B.; Davis, S. G.; Yoshida, M.; Weiss, K. *J. Am. Chem. Soc.* **1978**, *100*, 3283.

(7) Denny, M.; Liu, R. S. H. *J. Am. Chem. Soc.* **1977**, *99*, 4865.

(8) Tsukida, K.; Kodama, A.; Ito, M. *J. Chromatogr.* **1977**, *134*, 331.

(9) Maeda, A.; Shichida, Y.; Yoshizawa, T. *J. Biochem.* **1978**, *83*, 661.

(10) Sperling, W.; Carl, P.; Rafferty, C. N.; Dencher, N. A. *Biophys. Struct. Mech.* **1977**, *3*, 79.

(11) Bensasson, R.; Land, E. J. *Nouv. J. Chim.* **1978**, *2*, 503.

(12) Menger, E. L.; Kliger, D. S. *J. Am. Chem. Soc.* **1976**, *98*, 3975.

(13) Hochstrasser, R. M.; Narva, D. L.; Nelson, A. C. *Chem. Phys. Lett.* **1976**, *43*, 15.

(14) Warshel, A.; Karplus, M. *J. Am. Chem. Soc.* **1972**, *94*, 5612.

(15) Kushick, J. N.; Rice, S. A. *J. Chem. Phys.* **1976**, *64*, 1612.

(16) Salem, L.; Bruckmann, P. *Nature (London)* **1975**, *258*, 526. Salem, L. *Acc. Chem. Res.* **1979**, *12*, 87.

(17) Ohmine, I.; Morokuma, K. *J. Chem. Phys.* **1980**, *73*, 1907.

(18) Suzuki, H.; Nakashi, K.; Komatzu, T. *J. Phys. Soc. Jpn.* **1974**, *37*, 751.

(19) Warshel, A.; Karplus, M. *J. Am. Chem. Soc.* **1974**, *96*, 5677.

(20) Birge, R. R.; Schulten, K.; Karplus, M. *Chem. Phys. Lett.* **1975**, *31*, 451.

(21) Waddell, W. H.; West, J. L. *J. Phys. Chem.* **1980**, *84*, 134.

(22) Waddell, W. H.; Dawson, P. M.; Hopkins, D. L.; Rach, K. L.; Uemura, M.; West, J. L. *J. Liq. Chromatogr.* **1979**, *2*, 1205.

(23) Schaffer, A. M.; Waddell, W. H.; Becker, R. S. *J. Am. Chem. Soc.* **1974**, *96*, 2063.

0002-7863/81/1503-7389$01.25/0

Quantum yields relative to those measured at room temperature were determined to 160 K in EtOH and to 173 K in 3MP. Table I is a summary. Plots of log ϕ_{PI} vs. T^{-1} yield straight lines for each component isomerization in each solvent. In *n*-decane, the ϕ_{PI} values and the relative product ratios measured at 298, 273, and 248 K were identical within experimental reproducibility.

Activation barriers of the excited-state isomerization reactions can be obtained if the quantum yield data are treated as kinetic parameters.[24] This can be accomplished by assuming a specific reaction pathway consistent with the data reported here and the following results obtained for *all-trans*-retinal: (a) triplet sensitization in a polar[4] or nonpolar[3] solvent yields no detectable isomerization ($^3\phi_{\mathrm{PI}} < 0.003$);[4] (b) upon direct excitation only one carbon–carbon double bond is isomerized per photon;[4,21] (c) nanosecond laser photolysis of *all-trans*-, 11-*cis*-, or 13-*cis*-retinal at room temperature gives rise to a distinct fluorescence spectrum for each isomer ($\phi_{\mathrm{F}} \sim 10^{-4}$);[6] (d) room temperature picosecond laser excitation reveals that the excited molecules instantaneously relax to two or more different singlet excited states ($\tau_{\mathrm{S}} \sim 20$ ps);[13] (e) rotation of the retinoid skeleton by twists of 90° leads to a sudden polarization of the lowest singlet excited state;[16] (f) three excited singlet states are of comparable energies,[25] and on the basis of two-photon spectroscopy the Ag^--like state is thought to be of lowest energy in EPA,[26] while fluorescence studies in extensively dried 3MP conclude that the n,π^* state is the lowest singlet excited state.[27,28]

A pathway for the trans → cis photoisomerization of *all-trans*-retinal that is based upon a rate-determining activated torsion to twisted states is presented in Scheme I. The essential features of Scheme I are excitation to the Bu-like excited singlet state, internal conversion to the photoreactive excited singlet state, torsion of the appropriate bond while the molecule is an excited singlet species (e.g., 9-cis-P*), and decay of this species to the ground-state cis or trans isomer. Using $k_{\mathrm{i}} = A_{\mathrm{i}}e^{-E_{\mathrm{i}}/RT}$, assuming that the twisted states (e.g., 9-cis-P*) decay with equal probability to cis and trans double bonds ($\alpha = \alpha_{\mathrm{i}} = 0.5$), and using the approximation that $(A_ie^{-E_{\mathrm{i}}/RT})/(\sum A_{\mathrm{i}}e^{-E_{\mathrm{i}}/RT}) \sim A_{\mathrm{i}}/\sum A_{\mathrm{i}}$ we can show that

Scheme I

$$\text{trans} \xrightarrow{h\nu} \text{trans}^* \text{ (Bu-like)} \tag{1}$$

$$\text{trans}^* \text{ (Bu)} \xrightarrow{k_{\mathrm{IC}}} \text{trans}^* \text{ (Ag}^-\text{-like or } n,\pi^*) \tag{2}$$

$$\text{trans}^* \xrightarrow{k_{\mathrm{D}}} \text{trans} \tag{3}$$

$$\text{trans}^* \xrightarrow{k_9} \text{9-cis-P}^* \tag{4}$$

$$\text{trans}^* \xrightarrow{k_{11}} \text{11-cis-P}^* \tag{5}$$

$$\text{trans}^* \xrightarrow{k_{13}} \text{13-cis-P}^* \tag{6}$$

$$\text{9-cis-P}^* \rightarrow \alpha \text{ 9-cis} + (1 - \alpha) \text{ trans} \tag{7}$$

$$\text{11-cis-P}^* \rightarrow \alpha \text{ 11-cis} + (1 - \alpha) \text{ trans} \tag{8}$$

$$\text{13-cis-P}^* \rightarrow \alpha \text{ 13-cis} + (1 - \alpha) \text{ trans} \tag{9}$$

$$\ln [(\alpha/\phi_{\mathrm{i}}) - (1/f_{\mathrm{i}})] = \ln (k_{\mathrm{D}}/A_{\mathrm{i}}) + E_{\mathrm{i}}^*/RT \tag{10}$$

Table II. Activation Barriers of the Excited-State Isomerization of *all-trans*-Retinal

solvent	product[a] isomer	ϕ_{PI}[b]	E^*, kcal/mol	r^2 [c]
3-methylpentane	13-cis	0.10	2.1	0.994
	9-cis	0.015	3.2	0.944
ethanol	13-cis	0.22	1.6	0.915
	11-cis	0.09	1.6	0.934
	9-cis	0.02	2.5	0.966

[a] Determined by high-pressure liquid chromatographic methods. [b] 10^{-4}–10^{5} M in aerated solutions; $\phi_{\mathrm{PI}} \pm 15\%$; $\lambda_{\mathrm{e}} = 350$ nm, 7-nm bandpass. [c] Coefficient of determination for the linear least-squares fit of the data.

where $f_{\mathrm{i}} = A_{\mathrm{i}}/\sum A_{\mathrm{i}}$ and can be calculated from the ratio of the room temperature quantum yields. Plots of the data according to eq 10 yield straight lines, the slopes of which yield E_{i}^* values (Table II).

A correlation of the ϕ_{PI} values of the component isomerization processes with the magnitude of its excited-state barrier (E^*) may be possible. In 3MP the trans → 13-cis isomerization process has a notably higher ϕ_{PI} value and measurably lower E^* value than that of the trans → 9-cis process. Parallel results are obtained in EtOH for these two component isomerization processes. One exception is that in EtOH the ϕ_{PI} values of the trans → 13-cis and the trans → 11-cis processes are notably different; yet they have essentially identical E^* values. Although not currently understood, this difference in behavior might be related to the fact that 11-*cis*-retinal exists as a temperature-dependent mixture of 12-s-cis and 12-s-trans conformers;[25] hence the assumption that $\alpha_{11} = 0.5$ may not be valid. Another complication is that a dependence of ϕ_{PI} on solvent viscosity is expected and while the ϕ_{PI} values measured in *n*-decane, *n*-undecane, and *n*-hexadecane are approximately 10% lower than that measured in 3MP, these values are within experimental reproducibility. Although it is known that there is an increase in dispersive interactions between retinal and hydrocarbon solvents upon changing from 3MP to *n*-decane to *n*-hexadecane,[29] its effects upon the measured ϕ_{PI} values is not understood.[30]

Finally, Scheme I may account for the notable differences in the photochemical properties (ϕ_{PI} and the number and ratio of the primary photoproducts) of *all-trans*-retinal in 3MP and EtOH if photoisomerization were to occur from the Ag^--like singlet excited state and intersystem crossing from the n,π^* singlet excited state. In 3MP the n,π^* is thought the lowest excited singlet state;[27] $\phi_{\mathrm{ISC}} = 0.6$[3,6,11,31,32] and $\phi_{\mathrm{PI}} = 0.12$. In ethanol, the Ag^--like excited singlet state is thought lowest in energy;[26] $\phi_{\mathrm{ISC}} = 0.1$[6,11,32] and $\phi_{\mathrm{PI}} = 0.33$. That photoisomerization is observed in 3MP or intersystem crossing is observed in EtOH may be due to the proximity effects of the two states.[33]

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(24) Saltiel, J.; Rousseau, A. D.; Sykes, A. *J. Am. Chem. Soc.* **1972**, *94*, 5903.

(25) For a recent review, see: Birge, R. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315.

(26) Birge, R. R.; Bennet, J. A.; Fang, H. L-B.; Leroi, G. E. *Springer Ser. Chem. Phys.* **1978**, *3*, 347–354.

(27) Takemura, T.; Das, P. K.; Hug, G.; Becker, R. S. *J. Am. Chem. Soc.* **1976**, *98*, 7099; **1978**, *100*, 2626.

(28) Retinal fluorescence observed in 3MP (Waddell, W. H.; Schaffer, A. M.; Becker, R. S. *J. Am. Chem. Soc.* **1973**, *95*, 8223.) is attributed to the presence of hydrogen-bonding species.[27] That the photochemical properties of *all-trans*-retinal in 3MP is not being dominated by adventitious hydrogen-bonding species can be seen upon comparison of ϕ_{PI} and the number and ratio of primary photoproducts in 3MP and EtOH, which are notably different. In addition, it is thought that hydrogen-bonding does not measurably affect the intersystem crossing quantum yields measured in nonpolar solvents,[11] since the equilibrium constant for hydrogen bonding is small at room temperature.[27]

(29) Becker, R. S.; Hug, G.; Das, P. K.; Schaffer, A. M.; Takemura, T.; Yamamoto, N.; Waddell, W. H. *J. Phys. Chem.* **1976**, *80*, 2265.

(30) An alternative mechanism can be proposed, one based upon the Bu-like excited singlet state undergoing a temperature dependent, activated crossing to the photoreactive trans excited singlet state (reaction 2, Scheme I), with the subsequent torsional modes being temperature independent, e.g., $E_{13} \sim E_{11} \sim E_9$. Following this mechanism and the assumptions used to derive eq 10 it can be shown that

$$\ln [(f_{\mathrm{i}}\alpha/\phi_{\mathrm{i}}) - 1] = \ln (k_{\mathrm{D}}/A) + E^*/RT \tag{11}$$

Plots of the data according to eq 11 do not reasonably fit one straight line for either solvent. Better fits of straight lines are obtained if component isomerizations are treated individually which is not consistent with the assumptions used to derive eq 11.

(31) Bensasson, R.; Land, E. J.; Truscott, T. J. *Photochem. Photobiol.* **1973**, *17*, 53.

(32) Fisher, M. M.; Weiss, K. *Photochem. Photobiol.* **1974**, *20*, 423.

(33) For example, see: Madej, S. L.; Okajima, S.; Lim, E. C. *J. Chem. Phys.* **1976**, *65*, 1219. Wassam, W. A.; Lim, E. C. *Ibid.* **1978**, *68*, 433.