**Preparation of exo-5-Norbornenyl Bromide.**[38] Dry hydrogen bromide was passed at -70 °C through a solution of norbornadiene (21.6 mL, 200 mmol) in methylene chloride containing 5 g of silica gel. The saturated solution was washed with water, saturated sodium bicarbonate solution, and water. The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure. The residue was distilled in vacuo to give the product, 16.9 g, (49% yield), bp 68-70 °C (20 Torr). The product composition was determined by GC and $^1$H NMR and was found to be 75% *exo*-5-norbornenyl bromide and 25% 3-nortricyclyl bromide. *endo*-5-Norbornenyl bromide was not detectable. The desired *exo*-5-norbornenyl bromide was isolated by using preparative gas chromatography (75 °C): $^1$H NMR $\delta$ = 1.59-1.64 (m, 1 H), 1.81-2.01 (m, 3 H), 2.88 (s, 1 H), 3.08 (s, 1 H), 3.73-3.78 (m, 1 H), 5.96 (dd, $J$ = 3.3 Hz, $J$ = 5.1 Hz, 1 H), 6.17 (dd, $J$ = 3.3 Hz, $J$ = 5.1 Hz, 1 H).

**Preparation of Deuterated Nortricyclene.** 5-Nortricyclyl bromide (173 mg, 1 mmol) was dissolved in ether, cooled to -70 °C and treated with a 1.5 M solution of *tert*-butyllithium in pentane (2.5 mmol, 1.7 mL). The reaction mixture was stirred for 1 h at -70 °C and quenched with methanol-*O*-*d*, washed with water, and dried over anhydrous magnesium sulfate and chloroform (5 mL) added. The solvents were removed by distillation through a spinning band column until the boiling point of chloroform was reached. The residual solution was analyzed by GC-MS and $^2$H NMR: MS (EI) $m/e$ 95 ($M^+$); $^2$H NMR $\delta$ = 1.0 ppm.

**Grignard Reaction of exo-5-Norbornenyl Bromide with Rieke Magnesium.** A mixture of *exo*-5-norbornenyl bromide (350 mg, 2 mmol) and *tert*-butyl alcohol-*O*-*d* (8 mmol) was added to the slurry of Rieke magnesium in ether at -70 °C, and the reaction mixture was stirred at this temperature for 4 h. The mixture was allowed to warm to -40 °C within 0.5 h, and the excess Rieke magnesium was destroyed with saturated ammonium chloride solution. The reaction mixture was then washed with water and dried and chloroform (5 mL) added. The solvents were removed by distillation through a spinning band column until the boiling point of chloroform was reached. The products were identified by GC comparison with authentic samples, and the product ratio was determined by GC and $^2$H NMR: $^2$H NMR $\delta$ = 1.4 ppm (*exo*-5-deuterionorbornene, 65%), $\delta$ = 1.0 ppm (deuterionortricyclene, 35%).

**Grignard Reaction of exo-5-Norbornenyl Bromide with Rieke Magnesium in the Presence of Deuterated Dicyclohexylphosphine.** The Grignard reaction was carried out under the conditions described above using 2 mmol of *exo*-5-norbornenyl bromide, 8 mmol of *tert*-butyl alcohol, and 20 mmol of deuterated dicyclohexylphosphine. After the usual workup the sample was analyzed by GC-MS. Two products were found: 65% norbornene (2% deuterium incorporation) and 35% nortricyclene (22% deuterium incorporation).

(38) Grob, C. A.; Günther, B.; Hanreich, R. *Helv. Chim. Acta* **1982**, *65*, 2288.

**Registry No.** Mg, 7439-95-4; *t*-BuOD, 3972-25-6; *t*-BuOH, 75-65-0; $MgBr_2$, 7789-48-2; *exo*-2-norbornyl bromide, 2534-77-2; *endo*-2-norbornyl bromide, 13237-87-1; *exo*-5-bromo-2-norbornene, 5889-54-3; dicyclohexylphosphine, 829-84-5; deuterated dicyclohexylphosphine, 91523-73-8; *exo*-2-deuterionorbornane, 22642-76-8; *endo*-2-deuterionorbornane, 22642-75-7; 2,5-norbornadiene, 121-46-0; *endo*-5-norbornenyl bromide, 5810-82-2; 3-nortricyclyl bromide, 695-02-3; *exo*-5-deuterio-2-norbornene, 37907-31-6; 3-deuterionortricyclene, 38570-13-7.

# Photoisomerization of Polyenes. 30.† Quantum Chain Processes in Photoisomerization of the All-Trans, 7-Cis, and 11-Cis Isomers of Retinal

**Srinivasan Ganapathy and Robert S. H. Liu***

*Contribution from the Department of Chemistry, University of Hawaii, 2545 The Mall, Honolulu, Hawaii 96822.* *Received July 15, 1991. Revised Manuscript Received December 16, 1991*

**Abstract:** Quantum chain processes in the photoisomerization of retinal isomers (all-trans, 7-cis, and 11-cis) are reflected in the dependence of quantum yield of isomerization on concentration of retinal (to values exceeding unity), the presence of one-photon multiple-bond isomerization, and changes of product distribution on retinal concentration. The processes are believed to take place exclusively from the triplet states. Reaction schemes involving participation of 12-(*S*)-cis conformers in the quantum chain processes have been advanced to account for all of the results.

## Introduction

The concept of propagation of light quanta (quantum chain process) in an isomerization process, first postulated in 1969,[1] was firmly established in dienes by Saltiel and co-workers,[2] followed by cases of trienes.[3] The involvement of the quantum chain process was characterized by increasing quantum yield of isomerization at higher substrate concentrations (to greater than unity) and by the presence of one-photon two-bond isomerization. Since then, similar phenomena have been demonstrated in hindered styryl derivatives[4] and in diphenylbutadiene.[5]

Photoisomerization of retinal has been investigated in great detail by many workers. Those studies preceding 1988 are covered in an extensive review by Becker.[6] More recently, Jensen et al.[7] reexamined the triplet-state reaction in detail, emphasizing the relation between rates of triplet sensitization (energy transfer) with quantum yields of isomerization and photostationary-state compositions. They further clarified some of the ambiguities from conflicting reported data. No attempts were made to examine the effects of retinal concentration although some of their quantum yield data, particularly those from the hindered 11-cis isomer, nearly exceeded unity. The latter situation would have suggested possible involvement of quantum chain processes in retinal isomerization, a possibility that was then demonstrated in the hindered

† For previous paper in the series, see ref 25.

(1) Hyndman, H. L.; Monroe, B. M.; Hammond, G. S. *J. Am. Chem. Soc.* **1969**, *91*, 2852-2859.

(2) (a) Saltiel, J.; Townsend, D. E.; Sykes, A. *J. Am. Chem. Soc.* **1973**, *95*, 5968-5973. (b) Saltiel, J.; D'Agostino, J.; Megarity, E. D.; Metts, L.; Neuberger, K. R.; Wrighton, M.; Zafiriou, O. C. *Org. Photochem.* **1973**, *3*, 1-113.

(3) (a) Liu, R. S. H.; Butt, Y. C. C. *J. Am. Chem. Soc.* **1971**, *93*, 1532-1534. (b) Butt, Y. C. C., Ph.D. Thesis, University of Hawaii, Honolulu, HI, 1973. (c) Butt, Y. C. C.; Singh, A. K.; Baretz, B. H.; Liu, R. S. H. *J. Phys. Chem.* **1981**, *85*, 2091-2097.

(4) (a) Karatsu, T.; Arai, T.; Sakuragi, H.; Tokumaru, K. *Chem. Phys. Lett.* **1985**, *115*, 9-15 and papers cited therein. (b) Sundahl, M.; Wennerstrom, O.; Sandros, K.; Norinder, U. *Tetrahedron Lett.* **1986**, *27*, 1063-1066. (c) Ito, Y.; Uozu, Y.; Dote, T.; Ueda, M.; Matsuura, T. *J. Am. Chem. Soc.* **1988**, *110*, 189-198.

(5) Yee, W. A.; Hug, S. J.; Kliger, D. S. *J. Am. Chem. Soc.* **1988**, *110*, 2164-2169.

(6) Becker, R. *Photochem. Photobiol.* **1988**, *48*, 369-399.

(7) Jensen, N.; Wilbrandt, R.; Bensasson, R. V. *J. Am. Chem. Soc.* **1989**, *111*, 7877-7888.

0002-7863/92/1514-3459$03.00/0 

7-cis isomer.[8] Subsequently Mukai et al.,[9] in a continued resonance Raman study of triplets of retinal isomers and its homologues, also reported that the quantum yield of sensitized isomerization of 11-*cis*-retinal was dependent on retinal concentration, increasing from ~1 at $1 \times 10^{-4}$ M to 5.5 at $4.8 \times 10^{-3}$ M.

Following our earlier results on 7-*cis*-retinal,[8] we have extended similar studies to two other more common isomers of retinal (all-trans and 11-cis). The current findings not only confirm the reported results[9] but also demonstrate the generality of the quantum chain process. In this paper, we further explore the possible causes for the concentration effect.

It should be mentioned that product dependence on retinal concentration was first described in the literature in 1962, detected during preparation of 11-*cis*-retinal by direct irradiation of the all-trans isomer in ethanol.[10] However, the results in hydroxylic solvents, where retinal triplet yields are low (<0.1),[6] are probably unrelated to the current results obtained in hexane, a solvent giving high intersystem crossing efficiency of retinal.[6]

## Experimental Section

**Materials.** *all-trans*- and 13-*cis*-retinal were obtained from Sigma, and 9-*cis*-retinal was obtained from Eastman Kodak. 11-*cis*-Retinal was provided as a gift sample from Hoffmann-La Roche. 7-*cis*- and 7,9-*dicis*-retinal, obtained as byproducts from the synthesis of other polycis isomers of retinal,[11] were provided by Dr. A. Trehan. All compounds were purified by preparative HPLC before use (to >99.5%). All samples were handled under nitrogen in red light. Zinc octaethylporphyrin (ZnOEP) was used as received (Aldrich, 98%). Palladium octaethylporphyrin (PdOEP) was prepared in the same way as described by Mercer-Smith.[12] Hexane (Fischer, HPLC grade) was distilled over $CaH_2$, and benzene (Fischer, ACS certified grade) was dried via azeotropic distillation. The dried solvents were stored over 3A molecular sieves (Aldrich).

**Irradiation Procedure.** Quantum yield measurements were carried out on a small merry-go-round apparatus (Applied Photophysics) at 23 °C. The potassium ferrioxalate actinometer system[13] was used to monitor the light intensity. All samples were degassed by five freeze–pump–thaw cycles at $<10^{-5}$ Torr, sealed, and stored at −37 °C until ready for use. A 150-W Hanovia Xe–Hg arc lamp coupled with an Instrument SA H-20 monochromator (slit width 2 mm) giving light of 365 ± 4 nm was used. Conversion was carried to ~2% for samples of concentration $2 \times 10^{-4}$ M and 4–7% for $<6 \times 10^{-5}$ M. For each experiment, a control sample of equal retinal concentration was kept in the dark to ensure the absence of thermal isomerization during the irradiation period.

Sensitized irradiation of 7-*cis*-retinal was carried out with similarly degassed samples, using $5.2 \times 10^{-5}$ M ZnOEP and $2.2 \times 10^{-4}$ M retinal in benzene at 532 nm. For quantum yield measurements (546 ± 4 nm), PdOEP ($E_T$ = 44.8 kcal/mol[14] compared to 40.6 kcal/mol for ZnOEP)[7] was used to ensure exothermic energy transfer. Variation of light intensity during direct irradiation of 7,9-*dicis*-retinal was achieved by varying the sample distance from the light source (Xe–Hg arc lamp, above).

**Conditions for HPLC Separation.** HPLC analyses were carried out on a 25 × 0.46 cm Dynamax Microsorb Si 60 (5 μm) column, using either 8% ethyl ether in hexane or 1.2% *tert*-butyl methyl ether in 1,1,2-trichloro-1,2,2-trifluoroethane[15] solvent mixtures. The latter is particularly helpful for separating the 7,13-dicis or 11,13-dicis isomer from the 13-cis. The following extinction coefficients[16] were used for the calculation of isomer compositions at 360 nm: all-trans, 45 400; 13-cis, 37 500; 11-cis, 24 800; 9-cis, 36 600; 7-cis, 44 000; 11,13-dicis, 11 800; 9,13-dicis, 34 100; 7,13-dicis, 36 000; 7,9-dicis, 39 800.

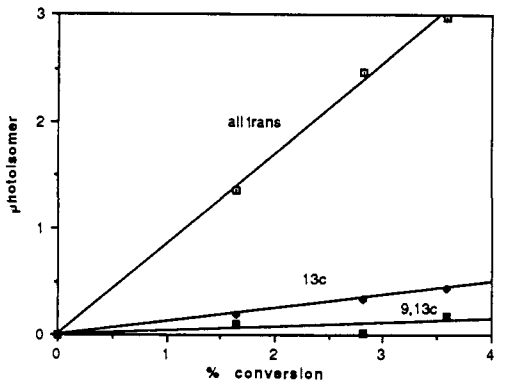



**Figure 1.** Progress of product formation during direct irradiation of 9-*cis*-retinal (365 nm, 23 °C).

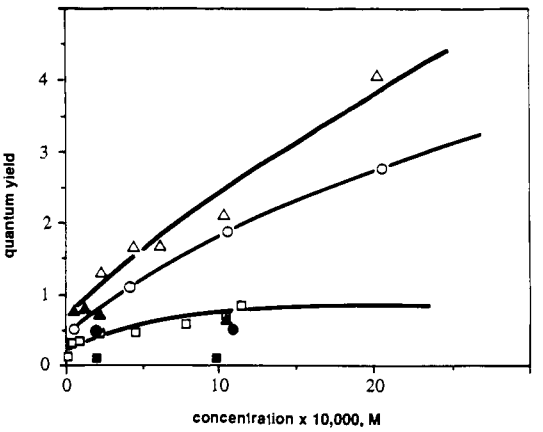



**Figure 2.** Quantum yield of photoisomerization by direct irradiation (365 nm) as a function of hexane solutions of *all-trans*-retinal (□, degassed; ■, aerated), 7-*cis*-retinal (△, degassed; ▲, aerated), and 11-*cis*-retinal (○, degassed; ●, aerated).

## Results

We have determined quantum yields of isomerization of *all-trans*-retinal in hexane (at 365 nm) as a function of retinal concentration under direct irradiation (aerated and degassed). For comparison, relative quantum yields, as well as composition in photostationary-state mixtures, from PdOEP-sensitized isomerization of this isomer were also determined. The data are listed in Tables I and II. Similar data for 7-*cis*-retinal are shown in Table III; the quantum yield data for 11-*cis*-retinal are in Table IV. The presence of the 7,13-dicis and 11,13-dicis isomers among the initial products from direct irradiation of 7-*cis*- and 11-*cis*-retinal, respectively, was confirmed by co-injection of 7,13-*dicis*-retinal and by its UV/vis spectrum recorded on a diode array detector (HP-1040).

To examine the possibility that the two-bond-isomerized products might be due to two-photon excitation, we determined product distribution as a function of intensity (up to a 64-fold variation) of the incident beam. The 7,9-dicis isomer was chosen for this experiment because the larger amounts of the two-bond-isomerized product make it a more sensitive test system. The results are listed in Table V. We have also reinvestigated the initial product distribution in sensitized (ZnOEP) isomerization of 9-*cis*-retinal. We found that 9,13-*dicis*-retinal, which separated from the 13-cis isomer only under special HPLC conditions, was formed as a minor primary photoproduct (Figure 1) that was not mentioned in the earlier investigation.[7] This finding is relevant to the shape of the excited torsional potential surface of triplet retinal (see below).

## Discussion

**All-Trans Isomer.** Several interesting features are evident from the data of *all-trans*-retinal. First, it is clear (Table I and Figure

(8) Ganapathy, S.; Trehan, A.; Liu, R. S. H. *J. Chem. Soc., Chem. Commun.* **1990**, 199–200.
(9) Mukai, Y.; Hashimoto, H.; Koyama, Y. *J. Phys. Chem.* **1990**, *94*, 4042–4051.
(10) Brown, P. K.; Wald, G. *J. Biol. Chem.* **1956**, *222*, 865–877.
(11) (a) Trehan, A.; Liu, R. S. H. *Tetrahedron Lett.* **1988**, *29*, 419–422. (b) Trehan, A.; Mirzadegan, T.; Liu, R. S. H. *Tetrahedron* **1990**, *46*, 3769–3780.
(12) Mercer-Smith, J. A. Ph.D. Thesis, University of Rochester, Rochester, NY, 1987. We thank Professor D. Whitten for providing the detailed experimental procedure.
(13) Nicodem, D. E.; Cabrel, M. L. P. P.; Ferrira, J. C. N. *Mol. Photochem.* **1977**, *8*, 213–238.
(14) Hopf, F. R.; Whitten, D. G. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier Scientific Pub. Co.: Amsterdam, 1975; p 667–700.
(15) Bruening, R.; Derguini, F.; Nakanishi, K. *J. Chromatogr.* **1986**, *361*, 437–441.
(16) Liu, R. S. H.; Asato, A. E. *Methods Enzymol.* **1982**, *88*, 694–698.

**Table I.** Quantum Yield of Photoisomerization of *all-trans*-Retinal in Hexane by Direct[a] Irradiation

| conc ($\times 10^4$ M) | irradiation condition | Quantum yield ($\Phi$)[b] total | 13C | 9C | product ratio 13C/9C |
|---|---|---|---|---|---|
| 2.0 | aerated | 0.12 ± 0.01 | 0.10 | 0.02 | 1/0.20 |
| 2.3 | aerated | 0.12 ± 0.01 | 0.09 | 0.03 | 1/0.28 |
| 9.8 | aerated | 0.11 ± 0.01 | 0.09 | 0.02 | 1/0.20 |
| ~1.0[c] | aerated | 0.12 ± 0.02 | 0.105 | 0.015 | 1/0.14 |
| 0.11 | degassed | 0.13 ± 0.01 | 0.10 | 0.03 | 1/0.26 |
| 0.44 | degassed | 0.32 ± 0.01 | 0.24 | 0.08 | 1/0.35 |
| 0.91 | degassed | 0.36 ± 0.01 | 0.25 | 0.11 | 1/0.44 |
| 2.2 | degassed | 0.47 ± 0.08 | 0.33 | 0.14 | 1/0.44 |
| 4.5 | degassed | 0.47 ± 0.01 | 0.30 | 0.17 | 1/0.59 |
| 7.8 | degassed | 0.59 ± 0.04 | 0.31 | 0.28 | 1/0.89 |
| 10.4 | degassed | 0.69 ± 0.5 | 0.35 | 0.34 | 1/0.95 |
| 11.4 | degassed | 0.84 ± 0.08 | 0.44 | 0.40 | 1/0.91 |

[a] At 365 nm, 23 °C. [b] Average of three samples. [c] Data of Waddell et al., 1980, at 350 nm in 3-methylpentane.

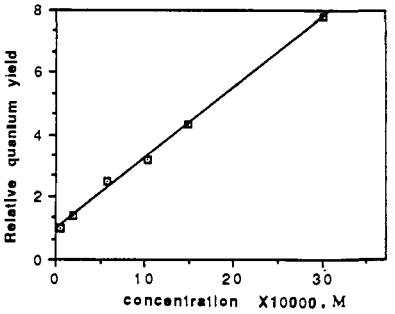



**Figure 3.** Relative quantum yield of isomerization of PdOEP-sensitized isomerization of *all-trans*-retinal in benzene at 24 °C, with 545-nm light as a function of retinal concentration.

2) that for degassed solutions, quantum yields of isomerization are dependent on retinal concentration. No such dependence was observed for aerated solutions (Table I and Figure 2). The combined results, reinforced by the linear plot of relative quantum yields of sensitized isomerization on retinal concentration (Figure 3), suggest that the concentration effect originates from the retinal triplets. Secondly, the ratio of 13-cis to 9-cis among the primary photoproducts was found to be dependent on retinal concentration, reminiscent of a similar dependence upon triplet sensitizer energy.[7] Thirdly, only one-bond-isomerized products were detected as initial products whether under aerated or degassed conditions.

The reaction scheme recently proposed by Jensen et al.[7] in their detailed spectroscopic and chemical study of porphyrin-sensitized isomerization of retinal isomers did not consider the retinal concentration effect. In an expanded scheme, we now include secondary triplet–triplet energy transfer from retinal triplets to the cisoid conformer as the possible cause of the concentration effect. Their involvement is suggested by the following facts. *all-trans*-Retinol acetate and related compounds undergo Diels–Alder reaction or formation of stable organometallic complexes at C11–14,[17] implying that 12-(*S*)-cis conformers are readily available at room temperature. Furthermore, such cisoid conformers are likely to have lower triplet excitation energies, as shown by model compounds of dienes (~60 kcal/mol for (*S*)-*trans*-dienes and 53 kcal/mol for (*S*)-*cis*-dienes).[18,19] Therefore, cisoid conformers of retinal, while not competitively important in the direct excitation process, might act as selective acceptors in secondary energy-transfer processes. The scheme given here includes the minimum steps needed to account for the chemistry at the initial

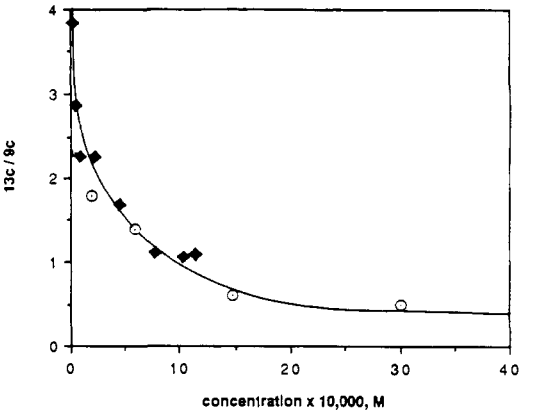


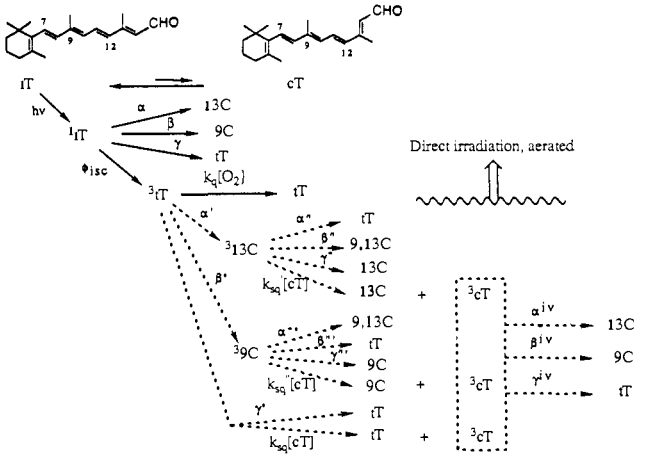

**Figure 4.** Dependence of 13C/9C isomer ratio on retinal concentration: ◆, from direct irradiation of *all-trans*-retinal; ○, from PdOEP-sensitized irradiation of *all-trans*-retinal.

**Table II.** PdOEP-Sensitized Isomerization of *all-trans*-Retinal in Degassed Benzene Solution at 532 nm

| [retinal] (M) | $\Phi_{relative}$ | primary product ratio 13C/9C/11C |
|---|---|---|
| $5.08 \times 10^{-6}$ | 1.00 | 1.00/0.44/0.12 |
| $1.95 \times 10^{-4}$ | 1.4 | 1.00/0.57/0.10 |
| $5.85 \times 10^{-4}$ | 2.5 | 1.00/0.72/0.10 |
| $10.3 \times 10^{-4}$ | 3.2 | 1.00/0.95 |
| $14.8 \times 10^{-4}$ | 4.3 | 1.00/1.70/0.10 |
| $30.1 \times 10^{-4}$ | 7.8 | 1.00/2.03/0.09 |
| $2 \times 10^{-4}$ [a] | | 1.0/0.47/0.06 |

[a] From Jensen et al., 1989.

stage of direct irradiation of *all-trans*-retinal. The symbols in the scheme are self-explanatory, with transoid *all-trans*-retinal designated by tT and its cisoid conformer by cT (small letters designating conformation, capital letters configuration).




Under aerated conditions, the decay of the all-trans triplet ($^3$tT) formed after intersystem crossing is probably dominated by the oxygen-quenching term ($k_q[O_2]$) giving the starting all-trans isomer, thus negating possible involvement of the 13-cis or 9-cis triplet in oxygen quenching. It follows that there will be no contribution from the triplet state to the isomerization process, and no concentration dependence nor two-bond isomerization as shown by the data in Table I. Quantum yields of isomerization simply equal the partition coefficients of excited retinal singlets: $\Phi_{iso}$(total) = $\alpha + \beta$, and 13-cis/9-cis = $\alpha/\beta$. From Table I, $\alpha$ and $\beta$ are found to be 0.10 ± .005 and 0.021 ± .005, respectively. The absence of two-bond-isomerized products also shows that isomeric excited singlets do not mutually interconvert.

Under degassed conditions, conversion of $^3$tT to the 13-cis and, to a lesser extent, the 9-cis triplets causes reactions from the triplet as well as the singlet state. Under the limiting condition of infinite retinal concentration where self-quenching ($k_{sq}[cT]$) dominates

(17) (a) Robson, C. D.; Cawley, J. D.; Weisler, L.; Stern, M. H.; Eddingen, C. C.; Chechak, A. J. *J. Am. Chem. Soc.* **1955**, *77*, 4111–4119. (b) Nakamura, A.; Tsutsui, M. *J. Med. Chem.* **1964**, *7*, 335–337. (c) Birch, A. J.; Fitton, H. *J. Chem. Soc. C* **1966**, 2060–2061. (d) Liu, R. S. H.; Asato, A. E.; Denny, M. *J. Am. Chem. Soc.* **1983**, *105*, 4829–4830.

(18) Liu, R. S. H.; Turro, N. J.; Hammond, G. S. *J. Am. Chem. Soc.* **1965**, *87*, 3406–3412.

(19) Ni, T.; Caldwell, R. A.; Melton, L. A. *J. Am. Chem. Soc.* **1989**, *111*, 457–464.

**Table III.** Quantum Yield of Photoisomerization of 7-*cis*-Retinal in Hexane by Direct Irradiation at 365 nm and 23 °C

| conc ($\times 10^4$ M) | irradiation condition | quantum yield ($\Phi$)[a] total | T | 7.13C | 13C | 9C | 7.13C/13C/9C |
|---|---|---|---|---|---|---|---|
| 1.2 | aerated | 0.77 ± 0.02 | 0.64 | 0.12 | | | 1/0.16/0/0 |
| 10.4 | aerated | 0.65 ± 0.04 | 0.56 | 0.09 | | | 1/0.16/0/0 |
| 0.44 | degassed | 0.76 ± 0.08 | 0.51 | 0.06 | 0.13 | 0.06[b] | 1/0.12/0.25/0.11 |
| 0.58 | degassed | 0.77 ± 0.08 | 0.54 | 0.03 | 0.14 | 0.06 | 1/0.05/0.25/0.11 |
| 2.3 | degassed | 1.30 ± 0.26 | 0.96 | 0.03 | 0.19 | 0.12 | 1/0.03/0.20/0.12 |
| 4.4 | degassed | 1.66 ± 0.33 | 1.28 | 0.05 | 0.19 | 0.14[b] | 1/0.04/0.15/0.11 |
| 10.4 | degassed | 2.10 ± 0.63 | 1.67 | 0.03 | 0.18 | 0.18 | 1/0.04/0.11/0.11 |
| 20.2 | degassed | 4.05 ± 0.50 | 3.34 | 0.18 | 0.24 | 0.29 | 1/0.05/0.07/0.09 |

[a] Average of three samples. [b] Determined from the 13C/9C ratio of Figure 5a.

**Table IV.** Photoisomerization of 11-*cis*-Retinal in Hexane by Direct Irradiation[a]

| conc ($\times 10^4$ M) | irradiation condition | quantum yields ($\Phi$) total | T | 11,13C | 13C | 9C | T/11,13C/13C/9C |
|---|---|---|---|---|---|---|---|
| 2.0 | aerated | 0.49 ± 0.01 | 0.31 | 0.18 | | | 1/0.55/0/0 |
| 10.9 | aerated | 0.51[b] | 0.30 | 0.21 | | | 1/0.68/0/0 |
| 1.0 | aerated | 0.25 | 0.25 ± 0.05 | | | | all-trans sole product |
| 0.52 | degassed | 0.51[b] | 0.31 | 0.09 | 0.07 | 0.03 | 1/0.30/0.23/0.10 |
| 4.2 | degassed | 1.12 ± 0.10 | 0.79 | 0.14 | 0.12 | 0.07 | 1/0.18/0.13/0.09 |
| 10.5 | degassed | 1.89 ± 0.17 | 1.47 | 0.19 | 0.12[d] | 0.11 | 1/0.13/0.08[d]/0.08 |
| 20.5 | degassed | 2.76 ± 0.35 | 2.19 | 0.18 | 0.15[d] | 0.24 | 1/0.08/0.07[d]/0.11 |
| 0.06[e] | degassed | 0.25 ± 0.03 | 0.25 | | | | |
| 0.2[f] | degassed | 0.23 ± 0.03 | 0.23 | | | | |
| 0.5[g] | degassed | 0.42 ± 0.5 | | | | | |
| 48.0[h] | degassed | 5.5 | | | | | |

[a] 365 nm at 23 °C; $\Phi$, average of three samples. [b] One sample only. [c] Waddell et al., 1980, at 350 nm, HPLC method. [d] 13C calculated from 13C/9C plot in Figure 5b. [e] Veyret et al., 1978, at 347 nm, UV method. [f] Becker et al., 1986, at 355 nm, UV method. [g] Jensen et al., 1989, at 254 nm, HPLC method. Products are 13-cis, 9-cis, and all-trans (11,13-dicis and 13-cis might have been co-eluted). [h] Mukai et al., 1990, sensitized.

**Table V.** Product Distribution from Direct Irradiation of 7,9-*dicis*-Retinal in Hexane at 366 nm, Varying Intensity[a]

| distance[b] (cm) | relative intensity | product ratio 9C/7,9,13C/T |
|---|---|---|
| 88 | 1 | 3/10/86 |
| 22 | 4 | 4/9/87 |
| 11 | 64 | 3/12/85 |

[a] $1 \times 10^{-4}$ M, aerated, 23 °C. [b] Distance from focal point.

the decay of $^3$tT, $\Phi_{iso}(\text{total}) = \alpha + \beta + \Phi_{isc}(\alpha^{iv} + \beta^{iv})$ and 13-cis/9-cis = $(\alpha + \alpha^{iv})/(\beta + \beta^{iv})$. In principle, all of these quantities can be determined from plots of 13C/9C (13-cis/9-cis) versus retinal concentration (such as in Figure 4). However, the present data are not sufficiently extensive (failed to level off at high retinal concentrations) for extrapolation to infinite retinal concentration. Nevertheless, it is clear that $\beta^{iv}/\alpha^{iv}$ (the decay ratio to 9-cis and 13-cis isomers from cisoid triplets) must be greater than $\beta/\alpha$ (the same decay ratio for retinal singlets) in order to account for the 9-cis-rich product distributions at high retinal concentrations (Table II). That selective sensitization also produces product mixtures richer in the 9-cis isomer[7] is in agreement with the involvement of cisoid triplets.

At zero retinal concentration, both $\Phi_{iso}$(total) and 13C/9C, while constant, are functions of three sets of partition coefficients ($\alpha$, $\alpha'$, $\alpha''$; $\beta$, $\beta'$, $\beta''$; $\gamma$, $\gamma'$, $\gamma''$; ...). For intermediate retinal concentrations, the situation is even more complex. The curve in Figure 4 is therefore not surprising.

That quantum yields of sensitized isomerization are linearly dependent on retinal concentration (Figure 3), in contrast to the curve of Figure 2, which is in agreement with the involvement of a single reactive excited state for the concentration-dependent process. It should be noted that the early studies on photoisomerization of retinal were conducted at retinal concentrations equal to or lower than $(1-2) \times 10^{-4}$ M,[20] accounting for (see Figure 2) the early claim of no concentration dependence.

(20) (a) Waddell, W. H.; Chihara, K. *J. Am. Chem. Soc.* **1981**, *103*, 7389–7390. (b) Rosenfeld, T.; Alchalel, A.; Ottolenghi, M. *J. Phys. Chem.* **1974**, *78*, 336–341. (c) Veyret, B.; Davis, S. G.; Yoshida, M.; Weiss, K. *J. Am. Chem. Soc.* **1978**, *100*, 3283–3290.

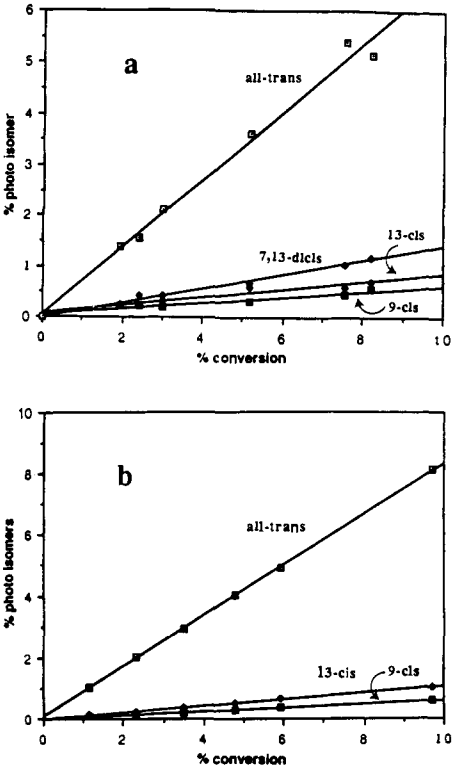



**Figure 5.** Progress of product formation during direct irradiation of 7-*cis*-retinal in hexane (365 nm at 23 °C) (a); progress of product formation during ZnOEP-sensitized isomerization of 7-*cis*-retinal (532 ± 4 nm) (no detectable amounts of 7,13-*dicis*-retinal) (b).

**Hindered 7-Cis and 11-Cis Isomers.** Examination of quantum yield data (Tables III–V and Figure 2) for direct irradiation of 7-*cis*- and 11-*cis*-retinal revealed some trends identical to those of *all-trans*-retinal: quantum yields of isomerization and the 13-cis to 9-cis ratio both depend on retinal concentration. The concentration effect is, however, more pronounced for the hindered

**Table VI.** Photostationary-State Composition from PdOEP-Sensitized Isomerization of *all-trans*-Retinal in Benzene at 546 nm

| | % isomer at photostationary state | | | | |
|---|---|---|---|---|---|
| [retinal] (M) | T | 13C[a] | 11C | 9C | 7C |
| $5.0 \times 10^{-5}$ | 75.9 ± 0.3 | 18.2 ± 0.3 | 0.8 ± 0.2 | 5.0 ± 0.2 | 0.3 ± 0.1 |
| $2.0 \times 10^{-4}$ [b] | 72.9 ± 0.2 | 20.2 ± 0.2 | 0.6 ± 0.2 | 5.9 ± 0.2 | 0.3 ± 0.1 |
| $3 \times 10^{-3}$ | 64.8 ± 0.4 | 22.5 ± 0.7 | 0.1 ± 0.1 | 12.5 ± 0.3 | 0.2 ± 0.1 |

[a] Including 9,13-dicis, not separated under the HPLC conditions. [b] From Jensen et al., 1989.

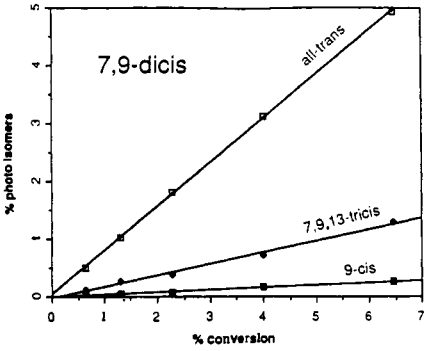



**Figure 6.** Progress of product formation during direct irradiation of 7,9-*dicis*-retinal in hexane (365 nm at 23 °C).

isomers, leading to quantum yields exceeding unity.

One-photon two-bond-isomerized products (i.e., from 7-cis directly to 13-cis or 9-cis) also became more prevalent (Figure 5) under direct or sensitized irradiation. Formation of the 13-cis isomer (a two-bond-isomerized product) in the sensitized reaction of 11-*cis*-retinal was also reported by Jensen et al.[7] and Mukai et al.[9] The plots in Figures 5 and 6 show that the two-bond-isomerized products are formed along with one-bond-isomerized products at early stages of irradiation. Additionally, we have shown that the relative amounts of such products were invariant upon changes of light intensity (Table V) in direct irradiation of 7,9-*dicis*-retinal, a system more sensitive for studies of multiple-bond-isomerized products, thus dispelling any notion of the possible involvement of two-photon excitation. Instead, the results are consistent with the involvement of unquenchable triplets from these hindered isomers. Thus, for 7-*cis*-retinal, its triplets, too short lived to participate in bimolecular quenching processes, undergo rapid torsional relaxation to trans triplets. Subsequent conversion to other unhindered isomeric triplets initiates formation of the multiple-bond-isomerized products.

The selective decay of the 7-cis (or 11-cis) triplets to the all-trans form suggests a skewed torsional surface with no, or a small, barrier surrounding the 7-cis (11-cis) triplet (Figure 7). This is not unexpected because the Franck–Condon triplets for the hindered isomers should be higher in energy as a result of steric crowding (of the same order as isomers in the ground state)[21] while the 90° structures (the torsional barrier) should be much less sensitive to such steric effects. In fact, Koyama and co-workers have demonstrated such characteristics through time-resolved UV–vis[22] and resonance Raman studies.[23] They showed that the triplets of these two isomers had extremely short lifetimes (of the order of picoseconds), readily converting to a mixture of all-trans-like and longer lived (ca. microseconds) isomeric triplets. The present chemical data are therefore in agreement with the conclusions from spectroscopic studies.[9,22-24] The only difference appears to be the exact composition of the equilibrated mixture. Mukai et al. concluded that only 13-cis was present with all-trans, while the 9-cis triplet was considered to be the same as the hindered isomers, rapidly giving way to the all-trans and the 13-cis isomeric triplets. However, our photochemical result (Figure 1) showing

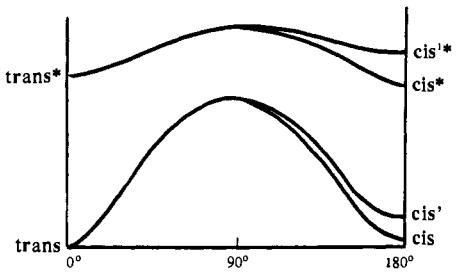
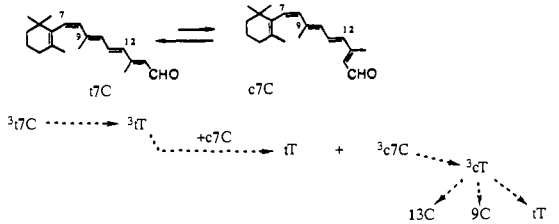



**Figure 7.** Postulated triplet torsional potential curves for hindered (cis' for 7-cis and 11-cis) and unhindered (cis for 9-cis and 13-cis) isomers of retinal. The absence of common triplets between the isomers is consistent with the time-resolved spectroscopic data of Mukai et al.[9] Conversion of the hindered cis triplets to the trans, and subsequently a mixture of the unhindered, should be facile. The hindered cis'* triplets are too high in energy to be present in significant amounts in an equilibrated mixture.

the presence of a small amount of the 9,13-dicis isomer among other primary photoproducts suggests that the 9-cis triplet is also present in the equilibrated mixture, albeit of an amount (5% or less) probably too small to be detected by time-resolved spectroscopic methods (Table VI). Thus, the mixture of retinal triplets appears to be determined by their relative stability, consisting of unhindered isomers only.

For 7-*cis*-retinal, the polyene conformation near the aldehyde group is not expected to differ from that of the all-trans. Therefore, the 12-(*S*)-cis conformer should also be present in sufficient amounts in equilibrium with the transoid conformer to serve as triplet acceptor. The scheme for the initial stage of direct irradiation of 7-*cis*-retinal should, therefore, parallel that of all-trans, except that a step reflecting conversion from the 7-cis triplet to the trans triplet should be inserted. The resulting cisoid 7-cis triplet should decay via the same cisoid trans.




It follows that, for direct irradiation in aerated solutions, the quantum yield of isomerization becomes $\Phi_{iso}(\text{total}) = \alpha + \beta + \gamma + \Phi_{isc}$. Since isomerization takes place from the triplet as well as the singlet state, it is not surprising that for these isomers larger quantum yields of isomerization are observed (Tables III and IV).[29] The initial product ratio becomes $7,13C/7,9C/tT = \alpha/\beta/(\gamma + \Phi_{isc})$.

Isomerization at the terminal double bond seems to be more favored; thus, 7,13 and 11,13 become the early major products. This observation was also noted in a recent study of hindered polycis isomers of retinal leading to a photochemical method for preparing the novel all-cis isomer.[25]

For degassed solutions at high retinal concentrations, the value for the quantum yield of isomerization should range from $\alpha + \beta + \gamma + 2\Phi_{isc}$ to higher values (thus larger than unity) if $^3$cT participates in further quantum chain propagation. And, for degassed solutions at zero retinal concentration, $\Phi_{iso}(\text{total}) = \alpha + \beta + \gamma + \Phi_{isc}$. It is the same as that from direct irradiation in

(21) (a) Rando, R. R.; Chang, A. *J. Am. Chem. Soc.* **1983**, *105*, 2879–2882. (b) Liu, R. S. H.; Mirzadegan, T. *J. Am. Chem. Soc.* **1988**, *110*, 8617–8623.

(22) Mukai, Y.; Koyama, Y.; Hirata, Y.; Magata, N. *J. Phys. Chem.* **1988**, *92*, 4649–4653.

(23) Hamaguchi, H.; Okamoto, H.; Tasumi, M.; Mukai, Y.; Koyama, Y. *Chem. Phys. Lett.* **1984**, *107*, 355–359.

(24) Harriman, A.; Liu, R. S. H. *Photochem. Photobiol.* **1977**, *26*, 29–32.

(25) Ganapathy, S.; Liu, R. S. H. *Tetrahedron Lett.* **1990**, *31*, 6957–6960.

aerated solutions, in agreement with the data in Figure 2.

For 11-*cis*-retinal, a possible complication is the higher amount of the 12-(*S*)-cis conformer (one estimate being ~60% in acetone),[26] which makes direct light absorption of this conformer with ensuing photochemistry of its own no longer negligibly small.

**Additional Comments.** So far, we have overlooked bimolecular association as a possible explanation for the observed concentration effects, even though such phenomena were discussed in the literature concerning retinal fluorescence properties.[27] The reasons for our preference for the involvement of isomeric triplets are the following.

Firstly, the concept of isomeric triplets with different chemical properties has been well documented in simple dienes and trienes.[18,19] Their involvement in pentaenes seems to be unavoidable. Secondly, the retinal triplets produced under selective sensitization at low retinal concentrations[7] have the same chemical properties (favoring formation of 9-cis rather than 13-cis) as those produced under non-selective sensitization and high retinal concentration, in agreement with identical cisoid triplets. Thirdly, examples of singlet exciplexes are well known, many with high fluorescence yields. Such was also the case for retinal dimer (zero fluorescence yield for retinal monomer).[27] The phenomena described here are not related because they involve the triplet state. Fourthly, the concept of dimeric association will be difficult to account for quantum yields greater than 2 as observed in the hindered isomers. Nevertheless, it will be desirable to obtain independent corroborative evidence to support the involvement of isomeric triplets, such as their direct detection by time-resolved resonance Raman spectroscopy. An ideal condition for favorable population of cisoid triplets could be the selective sensitization condition employed by Jensen et al.[7] Results from designed ring-fused retinoids (many already in the literature in visual pigment analogue studies)[28] that impede conformational interconversion will also be interesting.

**Acknowledgment.** The work was supported by a grant from the National Science Foundation (CHE-16500).

**Registry No.** *all-trans*-Retinal, 116-31-4; 13-*cis*-retinal, 472-86-6; 9-*cis*-retinal, 514-85-2; 7-*cis*-retinal, 24315-14-8; 11-*cis*-retinal, 564-87-4; 7,9-*dicis*-retinal, 56085-53-1; 7,13-*dicis*-retinal, 56085-54-2; 11,13-*dicis*-retinal, 564-88-5; 7,9,13-*tricis*-retinal, 56085-55-3; 9,13-*dicis*-retinal, 23790-80-9.

(26) Rowan, R., III; Warshel, A.; Sykes, B. D.; Karplus, M. *Biochemistry* **1974**, *13*, 970-974.

(27) (a) Moore, T. A.; Song, P. S. *Nature (London)* **1973**, *243*, 30-32. (b) Takemura, T.; Hug, G.; Das, P. K.; Becker, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 2631-2634.

(28) (a) Balogh-Nair, V.; Nakanishi, K. In *Chemistry and Biology of Synthetic Retinoids*; Dawson, M. I., Okamura, W. H., Eds.; CRC Press, Inc.: Boca Raton, FL, 1990; pp 147-167. (b) Liu, R. S. H.; Asato, A. E. *Ibid.*, pp 51-76.

(29) One referee notes that the determined valued of ~0.5 for 11-*cis*-retinal is not larger than that reported for intersystem crossing efficiency,[6] but the error limits for both numbers are greater than 10%, making a direct comparison unreliable.

# Mechanistic Studies of Enzymic and Nonenzymic Prolyl Cis-Trans Isomerization

**Richard K. Harrison and Ross L. Stein***

*Contribution from the Department of Enzymology, R80N-A54, Merck Sharp and Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065.*
*Received September 27, 1991*

**Abstract:** The cyclosporin A binding protein, cyclophilin (CyP), and the FK-506 binding protein, FKBP, catalyze the cis-to-trans isomerization of Xaa-Pro bonds in peptides. To probe the mechanism of these reactions and their nonenzymatic counterparts, we determined the following: (1) substrate specificities of CyP and FKBP; (2) dependencies of $k_c/K_m$ on pH and solvent deuterium; (3) secondary deuterium isotope effects; and (4) temperature-dependencies. The results indicate that (1) for cis-to-trans isomerization of Suc-Ala-Xaa-*cis*-Pro-Phe-pNA, values of $k_c/K_m$ for the CyP-catalyzed reactions show little dependence on Xaa. In contrast, for FKBP, $k_c/K_m$ displays a marked dependence on Xaa with a preference for hydrophobic residues. (2) For both enzymes, $k_c/K_m$ is independent of both pH and isotopic composition of the solvent. (3) For the CyP-catalyzed cis-to-trans isomerization of Suc-Ala-Gly(L,L)-*cis*-Pro-Phe-pNA (L = H, D), $^{H}(k_c/K_m)/^{D}(k_c/K_m) = 1.13 \pm 0.01$ and is independent of temperature between 2 and 30 °C (2 °C, $^{H}k/^{D}k = 1.14 \pm 0.02$; 10 °C, $^{H}k/^{D}k = 1.13 \pm 0.01$; 30 °C, $^{H}k/^{D}k = 1.14 \pm 0.03$). A secondary deuterium isotope effect of 1.13 suggests that, in the transition state of this reaction, the Xaa-Pro bond is twisted out of planarity. This value is inconsistent with mechanisms involving nucleophilic catalysis. (4) Eyring plots of ln $[(k_c/K_m)/T]$ vs $1/T$ for reactions of FKBP are linear, while Eyring plots for CyP catalysis are curved and display a maximum around 30 °C. The data for CyP were fit to a model involving a temperature-dependent, reversible isomerization of active to an inactive enzyme. This model is supported by the temperature-independence of the secondary deuterium isotope effect (see 3 above) and the independence of the equilibrium of the two enzyme forms on substrate structure. The activation parameters that were calculated from the Eyring plots indicated enthalpy-entropy compensation for both CyP and FKBP with the critical temperature, $T_c$, equal to 287 and 260 K, respectively. We interpret the compensation in terms of a simple mechanism in which stronger transition-state interactions between enzyme and substrate (more positive $\Delta H^{\ddagger}$) are accompanied by greater restrictions of translational and rotational freedom (more negative $\Delta S^{\ddagger}$).

## Introduction

The cis-trans isomerization of Xaa-Pro bonds (Scheme I) is a reaction of biochemical interest due to its often rate-limiting role in protein folding. The central role that this reaction plays in biochemistry is further highlighted by the existence of an ubiquitous enzyme in nature that catalyzes this reaction. This enzyme, peptidyl prolyl cis-trans isomerase (PPI),[1] was first described in 1984;[2] it catalyzes prolyl isomerization in both peptides[2] and proteins.

(1) Abbreviations: CyP, cyclophilin; FKBP, FK506 binding protein; rhFKBP, recombinant human FK506 binding protein; PPI, peptidyl prolyl cis-trans isomerases; Suc, *N*-succinyl; pNA, *p*-nitroanilide; α-CT, α-chymotrypsin; DMA, *N*,*N*-dimethylacetamide.

0002-7863/92/1514-3464$03.00/0 