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Triplet-Sensitized Cis–Trans Isomerization of the Protonated Schiff Base of Retinal Isomers

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Abstract: Pulsed (N_2 laser) and continuous excitation methods are applied to ethanol solutions of the protonated Schiff bases of retinal isomers (PRSB). PRSB's triplet states, produced via energy transfer from the triplet state of phenanthrene, are found to undergo cis–trans isomerization processes. The observed yields of the triplet-sensitized isomerization ($\phi^T{}_{ISO}$) for the 9-cis, 13-cis, 11-cis, and all-trans isomers are, respectively, 0.5 ± 0.2, 0.2 ± 0.1, 1.0 ± 0.2, and <0.05. The large yield for the 11-cis isomer is rationalized in terms of the destabilization of the first excited triplet of this isomer due to torsion around its 12–13 single bond. Calculations indicate that this leads to a substantial decrease in the barrier for twisting around the double bond and thus may be responsible for the exceptionally high $\phi^T{}_{ISO}$ value observed. The relevance of this analysis to the photochemistry of visual pigments is discussed.

It is generally accepted that, in visual pigments, 11-cis retinal is covalently bound to the protein opsin via a Schiff base linkage.[1] The large bathochromic shifts, relative to the retinal Schiff base (RSB) observed upon pigment formation, are not fully understood, but it now seems likely that they result from the formation of a protonated Schiff base (PRSB).[1d,2] Thus, the photochemical properties of PRSB's are of considerable interest since they may bear directly upon the 11-cis → all-trans photoisomerization that is believed to initiate visual excitation.[3]

Previous studies have considered the primary photophysical processes of molecules related to the visual chromophore. These include retinal,[4] RSB,[5] retinol,[6] retinyl acetate, retinyl-*n*-butylamine, and retinal oxime.[7] In the present work, we selectively populate the lowest triplet state of PRSB (previously undetected),[4a,b] via inter-molecular energy transfer from a triplet donor molecule, focusing our attention on isomerization processes in the triplet manifold of the acceptor. The experimental photosensitized isomerization yields are related to theoretically calculated energy barriers in the lowest triplet state.

In carrying out the theoretical calculations, we have been interested primarily in the specific effects introduced by the twisting in the polyene chain of the 11-cis isomer, where the major deviation from planarity involves significant torsion about the 12–13 single bond (dihedral angle θ_{12-13}).[2,8-11] The fact that the 11-cis isomer is a highly twisted molecule has been used to interpret spectroscopic results[8,12,13] but has not been considered in previous calculations of barriers to isomerization of retinals and related molecules.[12,14,15] In this study, we calculate the effect of this twisting on the potential energy surfaces of the lowest triplet states of PRSB. It is shown that our photosensitization data may be rationalized in terms of this effect.

Experimental Section

All-trans, 9-cis, 13-cis (Sigma Chemical Co.), and 11-cis retinal (a gift from Hoffmann-La Roche, N.J.) were used without further purification. The corresponding Schiff bases were prepared at room temperature by dissolving the aldehydes (~10^{-4} M) in *n*-butylamine (Fluka, puriss). Acidified ethanol (Fluka, spectrograde) solutions were prepared by evaporating the original *n*-butylamine solvent under a stream of nitrogen and dissolving the residual Schiff base in acidified (10^{-3} M $HClO_4$) ethanol. The ab-

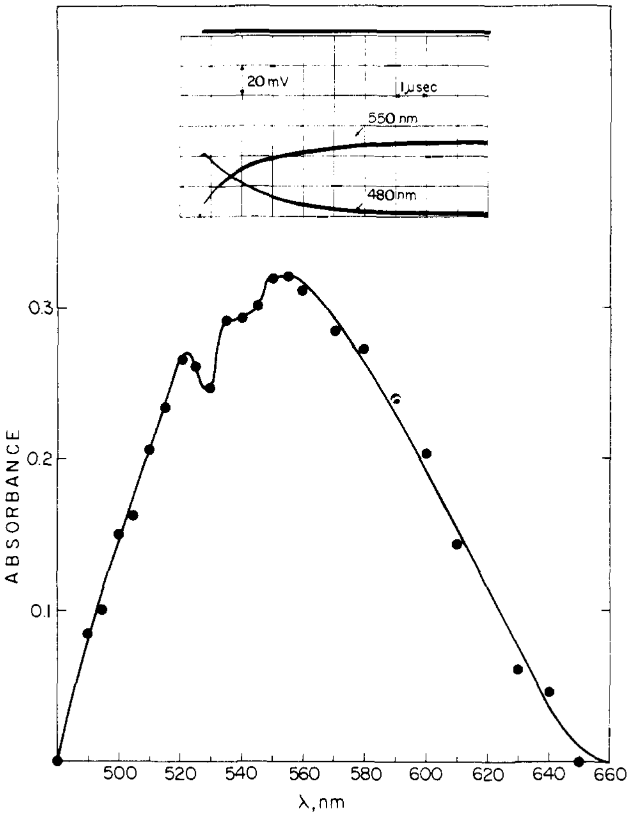

Figure 1. Characteristic oscillograms and transient spectrum in the N_2-laser photolysis of phenanthrene (2×10^{-2} *M*) and 11-cis PRSB (4×10^{-5} *M*) solutions in methanol. The oscillograms represent the decay of $^3P^*$ at 480 nm and the matching growing-in of $^3PRSB^*$ at 550 nm. Upper trace is recorded in the absence and lower traces in the presence on the monitoring light beam. The absorption curve was recorded 9 μsec after pulsing.

sorption spectra of retinal RSB and PRSB were identical with those previously reported.[17,18] All solutions were stored under nitrogen in the dark at 0°. Eastman Kodak phenanthrene was zone refined.

Absorption spectra were recorded using a Cary-14 spectrophotometer. Continuous irradiations around 320 nm were performed using the emission of a Cd-Lamp (Philips) filtered by a $NiSO_4$, $CoSO_4$, potassium biphthalate solution, with an additional 7-54 Corning glass filter. Uranyl oxalate actinometry was employed.[4d]

The pulsed photolysis technique using the 337.1-nm (10 nsec, 0.5 mJ) pulse of an Avco-Everett laser has been previously described.[19]

Solutions were deaerated by bubbling nitrogen. All experiments were carried out at room temperature under deep-red light using freshly prepared solutions.

Results

a. Triplet State and Photosensitized Isomerization of PRSB. Previously published work utilizing direct flash excitation of PRSB with microsecond time resolution gave no evidence of an intermediate attributable to the triplet state, suggesting that little or no intersystem crossing takes place in this molecule.[4a,b] These early findings are consistent with our failure to observe any transient change in absorbance when submitting aerated or deaerated ($\sim 10^{-4}$ *M*) solutions of PRSB in acidified ethanol (λ_{max} 443 nm) to the (337.1 nm, 10 nsec) pulsed N_2-laser excitation, with nanosecond time resolution. In this respect, PRSB differs from rhodopsin,[20-22] retinal,[4,20] retinol,[6] and related molecules[7] which all exhibit transient absorbance changes following direct laser excitation. We have thus turned to indirect triplet excitation, choosing phenanthrene as a triplet energy donor (E_T = 62.7 kcal/mol) in acidified ethanol solutions of PRSB. (The addition of phenanthrene did not affect the ground-state spectrum of PRSB in aerated or deaerated systems.) Figure 1 shows characteristic oscillograms recorded in deaerated acidified (10^{-3} *M* $HClO_4$) ethanol solutions of 11-cis PRSB in the presence of 2×10^{-2} *M* phenanthrene. Pulsing leads to the generation of the phenanthrene triplet state ($^3P^*$) peaking around 480 nm. The presence of PRSB shortens the lifetime of $^3P^*$ (e.g., from 1.35 μsec in the absence of PRSB to 0.68 μsec in the presence of 2.2×10^{-5} *M* PRSB). As shown in Figure 1, the quenching of $^3P^*$ by PRSB is associated with a growing-in above 500 nm which matches the $^3P^*$ decay. The transient spectrum recorded after the termination of the growing-in stage (Figure 1) decays exponentially with a rate constant of 2×10^4 sec^{-1} and is assigned to the PRSB triplet state, $^3(PRSB)^*$. The formation of $^3(PRSB)^*$ in aerated solutions is completely inhibited by molecular oxygen which efficiently competes with PRSB on $^3P^*$. After the decay of $^3P^*$, the transient change in absorbance is given by $\Delta D = \Delta C(\epsilon_{^3(PRSB)^*} - \epsilon_{(11\text{-}cisPRSB)})$, where ΔC is the decrease in the ground-state concentration associated with pulsing. Thus, since the absorption of PRSB in ethanol extends up to ~550 nm, the spectrum presented in Figure 1 represents $^3(PRSB)^*$ only on its low-energy side (an isosbestic point is observed around 480 nm). It is therefore probable that the true maximum of the $^3(PRSB)^*$ band lies further to the blue than the apparent 550-nm peak. The actual spectrum of $^3(PRSB)^*$ was not obtained because of the difficulties associated with the determination of ΔC.

Photosensitized isomerization experiments were carried out in deaerated ethanol solutions as previously described,[4,5] using continuous excitation at 320 nm, at concentrations ([phenanthrene] = 2×10^{-2} *M* and [11-cis PRSB] = 1.8×10^{-5} *M*) assuring almost total light absorption by phenanthrene. Illumination leads to an initially linear increase in absorbance at 443 nm, characteristic of the 11-cis → all-trans transformation.[18] The photosensitized isomerization yield, $\phi^T{}_{ISO}$ (defined as the fraction of 11-cis PRSB molecules isomerized for each triplet produced by energy transfer from $^3P^*$), was calculated as described in detail for retinal[4] or RSB.[5] The value obtained was $\phi^T{}_{ISO} = 1.0 \pm 0.2$.[23] Identical experiments, carried out with the all-trans isomer, led to the value $\phi^T{}_{ISO} < 0.05$ for the photoconversion to 11-cis. For the 9-cis → all-trans and 13-cis → all-trans photosensitized isomerizations, we obtained $\phi^T{}_{ISO} = 0.5 \pm 0.2$ and 0.2 ± 0.1, respectively.

The above data unambiguously determine the role of the triplet state in the photosensitized cis-trans isomerization of the cis isomers of PRSB. As pointed out by Vander-Donkct and Porter,[24] it is extremely important to confirm that triplet-triplet energy transfer is the only mechanism responsible for any observed photosensitized changes. In the present work, we have ruled out other mechanisms by showing that dissolved O_2, which efficiently scavenges $^3P^*$ preventing the formation of $^3PRSB^*$, essentially eliminates the isomerization process.

b. Theoretical Estimates of the Cis-Trans Isomerization Barriers in the Lowest Triplet State. Calculations were carried out on the π-electron system of PRSB using the Pariser-Parr-Pople scheme, including configuration interaction (CI) between all singly excited states. An idealized molecular geometry (single and double bond lengths of 1.46 and 1.35 Å, respectively, and bond angles of 120°) was used. Values of the ionization potential (W_i) and one-center repulsion integrals (γ_{ii}) were taken from the scheme suggested by Dewar and Morita,[25] in which W_i is expressed as a function of the valence-shell electron density.

The expression for the resonance integrals was taken from Dewar and Klopman[26] with β_0 at 1.39 Å taken to be −4.462 eV for a C-C bond, −4.542 for C-N, and −4.616

Table I. Triplet-Sensitized Isomerization Yields (ϕ^T_{ISO}) of Molecules Related to the Visual Chromophore

Molecule	Solvent	Sensitizer	Isomer	ϕ^T_{ISO}
Retinal[a] (RCHO)	*n*-Hexane	Biphenyl	11-Cis	0.13 ± 0.002
			All-Trans	<0.002
N-Butylamine Schiff base, RSB[b] (RCH=NR′)	*n*-Hexane	Biphenyl	11-Cis	0.45
			9-Cis	0.06
			13-Cis	0.08
			All-Trans	0.02–0.05
N-Butylamine Protonated Schiff base, PRSB[c] (RCH=NHR′)+	Acidified ethanol	Phenanthrene	11-Cis	1.0 ± 0.2
			9-Cis	0.5 ± 0.2
			13-Cis	0.2 ± 0.1
			All-Trans	<0.05

[a] Values reported from ref 4d. [b] Values reported from ref 5. [c] This work.

for C–N+. These values were taken from the study of PRSB's of Kliger and Karplus.[27] The angular dependence of the resonance integral arises from the cos θ dependence of the overlap integral. The Ohno formula[28] which has been shown to be particularly appropriate for triplet energy levels[29] was used to calculate the two-center coulomb integrals (γ_{ij}) which were assumed to have no explicit angular dependence.

The energy of a particular conformation was obtained from eq 1, where E^π is the π-electron energy of the ground

$$E = E^\pi + E^c + E^T \qquad (1)$$

state, E^c is the repulsion energy of the positive core, and E^T is the vertical excitation energy to the lowest triplet. There is some question as to how the core repulsion should be treated. The repulsion between two positively charged nuclei is usually expressed in terms of coulomb integrals,[30] but a point-charge approximation[31] is also frequently used. We have adopted the former approach since it seems to have a firmer theoretical basis. It does, however, tend to minimize the conformation dependence of the total energy as expressed in eq 1, predicting, for example, a small (~.01 eV) preference for cis conformations in the ground state of most polyenes.[32] That trans conformations are more stable by ~0.1 eV is generally attributed to steric effects,[32] not included in eq 1. They are, however, considered in the discussion. It should be pointed out that, if the point-charge approximation is used to represent core repulsion, eq 1 alone can account for the greater stability of trans isomers.

As has been previously discussed,[2,16,27] the absorption maximum of the main $\pi\pi^*$ transition is very sensitive to the amount of charge placed on the nitrogen atom. For example, one can speak of a protonated Schiff base in which the anion is not completely dissociated so that the positive charge on the nitrogen will be effectively less than 1. With our set of parameters, it was found that a charge of ~0.5 reproduces the 443-nm absorption maximum of the main transition in all-trans PRSB.

In order to properly correlate theoretical values with our experimental results, we would have to obtain a reliable estimate of the barrier to cis–trans isomerization via the lowest triplet for all isomers. Calculations of potential energy surfaces have been reported previously for RSB's and PRSB's[16] but, because of the neglect of multiple excited configurations[33] in the CI scheme their accuracy must be questioned. The neglect of relaxation of the σ bonds upon twisting is also likely to produce significant errors.[34] For purposes of discussion, we have calculated barrier heights (E_b) for cis–trans isomerization with these same simplifying assumptions. The value for E_b appearing in Table II is obtained by subtracting the energy of the lowest triplet in the planar conformation from that at 90°, which in our calculations corresponds to the torsional angle at the barrier. An earlier study of torsional potentials in PRSB* also found the barrier in the lowest triplet to occur at 90°.[16] Calculations on related molecules have reported maxima at somewhat smaller angles for some excited states.[12,16] The qualitative discussion presented here does not depend on the exact value of the angle of the barrier. For each isomer, $E_b = E(\theta_d = 90°) - E(\theta_d = 0°)$, where θ_d is the dihedral angle of the 9–10, 11–12, or 13–14 double bond for the 9-cis, 11-cis, and 13-cis isomers, respectively. (θ = 180° corresponds to a planar trans conformation and 0° to a planar cis conformation.) Because of the approximation involved in the calculations, the numbers themselves are unreliable and should considerably overestimate the true barrier. Moreover, the barrier height changed by up to 0.5 eV when different approximations for the various integrals were tested.

The value for ΔE which appears in Table I is the increase in the π-electron energy of the lowest triplet due to twisting about single bonds in the polyene chain. Thus $\Delta E = E(\theta_d = 0°, \theta_s = \theta_s°) - E(\theta_d = 0°, \theta_s = 180°)$, where θ_s is the torsional angle about the particular single bond in question, and $\theta_s°$ is its equilibrium value. For the 9- and 13-cis isomers, the polyene chain is essentially planar so that θ_s = 180° for every single bond, and ΔE = 0. In the case of the 11-cis isomer, we took $\theta_{12\text{-}13}$ = 39° from the crystal structure of 11-cis retinal.[9] Thus, the value ΔE = 0.5 eV for 11-cis PRSB is the difference in the π-electron energy between the twisted 11-cis molecule and a hypothetical planar 11-cis molecule (with no steric hindrance).

The value for ΔE is probably far more reliable than that of E_b since both multiply excited configurations and σ-bond relaxation should be less important for single bonds than for double bonds. Moreover, ΔE relates to a relatively small torsional angle of 39°, whereas E_b requires a calculation at 90°. Reflecting the increased reliability of ΔE is the fact that its value was found to be totally insensitive to the parameters used in the π-electron calculation.

The value reported for ΔE neglects possible differences in the nonbonded energy between the twisted 11-cis conformers and the planar nonhindered cis isomers. However, it is likely that the nonbonded energy is similar in both cases since strain is easily removed by torsional motion about single bonds with relatively little loss of energy. Thus the energy minimum of the ground state of the twisted molecule corresponds to an essentially strain-free conformation.[35] Should there be extensive rearrangement of the σ structure accompanying relief of steric hindrance, this could significantly affect the π-electron energy of eq 1 which assumes constant bond lengths). As mentioned above, bond-length changes undoubtedly affect the value of E_b but are unlikely to introduce significant error into ΔE. For example, the bond lengths of all-trans retinal and 11-cis retinal are nearly identical in the crystal[9,10] despite the fact that the 11-cis isomer is highly twisted. On the other hand, steric hindrance in the latter is partially relieved by bond-angle opening;[9,10] the inclusion of this factor would be to increase ΔE.

Discussion

a. Isomerization Yields and Energy Barriers in the Triplet State. Table I summarizes the triplet-sensitized isomerization yields of PRSB, along with those previously reported by us for retinal[4d] and RSB.[5] Attention should be paid to the following general features. (a) The values of $\phi^T{}_{\rm ISO}$ for the all-trans isomers are very small, essentiallv on the limit of detectability. (b) The photosensitized isomerization yields for the cis isomers increase when passing from retinal to RSB and PRSB. (c) The highest values of $\phi^T{}_{\rm ISO}$ are observed for the 11-cis isomers. Of particular importance is the observation that in one case, i.e., 11-cis PRSB, $\phi^T{}_{\rm ISO}$ reaches the maximum value of unity.

In the case of 11-cis retinal for which substantial intersystem-crossing yields are observed, we have recently compared the triplet-sensitized isomerization yields with those obtained by direct excitation.[4d] An analysis of the oxygen effects on these processes led to the conclusion that the photosensitized 11-cis → all-trans isomerization precedes the formation of the fully thermalized triplet. Accordingly, we proposed a mechanism in which isomerization occurs from nonrelaxed vibronic triplet levels in competition with thermal deactivation. A similar analysis was not carried out for RSB[5] and PRSB (this work) for which there is no detectable intersystem crossing. (Oxygen effects on $\phi^T{}_{\rm ISO}$ due to selective quenching of ^{3}RSB* or ^{3}PRSB* cannot be easily obtained since O_2 competes with the acceptor for the triplet energy donor.) However, the observation that, for the cis RSB isomers, $\phi^T{}_{\rm ISO} < 1$ implies that, if isomerization proceeds from the thermalized triplet, the process is slow enough to be affected by the thermal (triplet) decay to the (cis) ground-state configuration which occurs in the microsecond range.

The situation is entirely different for 11-cis PRSB for which we observed $\phi^T{}_{\rm ISO} = 1$. It is of course possible to invoke once again the prethermalization mechanism arguing that, because of appropriate shapes of potential energy surfaces, thermalization leading to the all-trans trap is totally favored over that yielding the 11-cis configuration. Such an explanation would imply that the steepness of the potential-energy curve around the 11-cis potential well should exceed that around the all-trans minimum to an extent leading to 100% deactivation to the trans form. This situation is not in keeping with any of the available calculations of potential-energy surfaces. An alternative explanation for the unique value of $\phi^T{}_{\rm ISO} \simeq 1$ observed for 11-cis PRSB is that the potential barrier for the 11-cis → all-trans rotation of PRSB is considerably lower than that corresponding to all other cases (for which $\phi^T{}_{\rm ISO} < 1$) so that isomerization from the thermaized 11-cis triplet state is completed before deactivation to the 11-cis ground state. This implies that

$$k_{\rm ISO} = Ae^{-E_{\rm a}/RT} >> k_{\rm T} = 2 \times 10^4\ {\rm sec}^{-1}$$

where $k_{\rm ISO}$ is the isomerization rate constant of the thermalized 11-cis triplet, $E_{\rm a} = E_{\rm b} - \Delta E$ is the corresponding energy barrier, and $k_{\rm T}$ is the observed triplet decay rate constant. Taking[36] an upper limit of 10^{14} sec^{-1} for the frequency factor A, the above condition is fulfilled only if $E_{\rm a}$ is below ~0.6 eV. Assuming that the A values are of the same order of magnitude, the fact that, for the 9-cis, 13-cis, and all-trans isomers, $\phi^T{}_{\rm ISO} < 1$ implies that $E_{\rm a} \simeq 0.7$ eV for the planar cis isomers, and $E_{\rm a} \geq 0.8$ eV for all-trans.

Comparing these values with those reported in Table II, it is clear that, as expected, the calculations seriously overestimate $E_{\rm b}$ for all isomers. That the barrier height for trans PRSB must be at least 0.1 eV greater than those of the planar cis isomers is due to the greater stability of the trans isomer. As mentioned above, this result does not appear in the π-electron calculations but may be understood in terms of mild steric hindrance in cis molecules (the energy difference between cis and trans butadiene is about 0.1 eV which is the right order of magnitude).

Table II. Calculated Barrier Heights for Cis–Trans Isomerization of PRSB Isomers[a]

	$E_{\rm b}$	ΔE	$E_{\rm a}$
9-Cis	1.3 (30)	0 (0)	1.3 (30)
11-Cis	1.4 (32.3)	0.5 (11.5)	0.9 (20.7)
13-Cis	1.7 (38.1)	0 (0)	1.7 (38.1)

[a] Symbols defined in text. Values are given in electron volts. Values in parentheses are in kcal/mol.

The calculated values of $E_{\rm b}$ would predict that the ordering of the quantum yields for cis isomers be 9-cis > 11-cis > 13-cis, whereas consideration of twisting by using $E_{\rm a} = E_{\rm b} - \Delta E$ predicts the observed trend: 11 > 9 > 13.

It is of importance to consider what other factors might explain the anomalously high quantum yield of the 11-cis isomer. One possibility could be the calculated value of $E_{\rm b}$, that is, an inherently lower barrier height for the 11-cis isomer independent of steric hindrance. We believe this to be unlikely. Even though the value of $E_{\rm b}$ is clearly unreliable and is, for example, changed by as much as 0.5 eV when different parameters were used, the trend 13-cis > 11-cis > 9-cis was found to be parameter independent. This trend appears to involve greater electron densities in double bonds closer to the nitrogen, which draws electrons to its end of the polyene chain. It is of course possible that more extensive calculations of $E_{\rm b}$ might explain the anomaly, but we cannot think of any obvious reason why this should be the case. It is clear that a set of parallel experiments on a planar series of retinal analogs, for example, 13-des methyl retinal,[37] would be of great interest in this regard.

If in fact isomerization proceeds from the thermalized triplet, our calculations indicate that single-bond twisting provides a plausible explanation for the low barrier height of the 11-cis isomer. At $\theta_{11\text{-}12} = 90°$, steric hindrance is no longer a problem, and it is reasonable to assume that, at the barrier, $\theta_{12\text{-}13} = 180°$. Thus, the true torsional potential for the 11-cis isomer should be schematically represented by the dotted line in Figure 2. (To obtain the actual shape of this curve, it would be necessary to account for the change in $\theta_{12\text{-}13}$ from 39 to 180°, as $\theta_{11\text{-}12}$ increases from 0 to 90°.) The theoretical barrier obtained in these calculations is given by $E_{\rm a}$ in Table II. It can be seen that the value of $E_{\rm a} = 0.9$ for the 11-cis isomers is significantly lower than that of the 9-cis and 13-cis, but it is still too large to account for the previously suggested thermal isomerization mechanism which requires $E_{\rm a} < 0.6$ eV. However, as discussed above, the values of $E_{\rm b}$ are unreliable, being probably too large. Thus, in the case of PRSB, a value of $E_{\rm b} \sim 0.7$ eV for the 9-cis isomer (which is consistent with its quantum yield of 0.5, see above) would yield $E_{\rm a} \sim 0.2$ eV for the 11-cis isomer. Of particular importance is the actual size calculated for ΔE in the first triplet. It is a significant fraction of the calculated values of $E_{\rm b}$ (which are too large) and is about equal to the barrier heights obtained from experimental considerations for the other cis isomers. Thus, it is extremely likely that it plays a major role in determining the quantum yields for isomerization in the lowest triplet. The extent to which single-bond twisting is a factor in other states (i.e., isomerization via the lowest singlet) depends on the absolute and relative magnitudes of $E_{\rm b}$ and ΔE for those states and, of course, on the assumption that isomerization proceeds only following thermalization.

It is of interest (see Table I) that, in the cases of retinal and RSB, $\phi^{T}_{ISO} < 1$ even for the 11-cis isomer, indicating E_a values larger than that of (11-cis) PRSB. Since $E_a = E_b - \Delta E$, the effect may be due either to a decrease in E_b or to an increase in ΔE when going from retinal to RSB and PRSB. Although calculations[35c] do predict a small (~0.1 eV) increase in ΔE, for PRSB relative to RSB, the difficulty in obtaining reliable values for E_b precludes a quantitative interpretation of this effect. However, the decrease in ϕ^{T}_{ISO} for all cis isomers when going from PRSB to RSB and retinal (Table II) may be indicative of an increase of the corresponding rotational barriers E_b.

We should finally point out that we have not considered the effect of solvent on the cis–trans isomerization around double bonds.[38] For example, the values of ϕ^{T}_{ISO} (Table I) for retinal and RSB were obtained in nonpolar *n*-hexane, while those for PRSB are reported for acidified methanol. One possible solvent effect on ΔE may be to influence the relative population of 12 s-cis and 12 s-trans conformers which are probably in thermal equilibrium in 11-cis retinal.[8,13,39] (In this study, we have taken the crystal structure to represent 11-cis PRSB.) An additional solvent effect may be that of stabilizing either cis or trans isomers leading to a solvent-dependent E_b.

b. PRSB and the Photochemistry of Visual Pigments. Since there is now strong evidence that the chromophore is bound to the opsin via a protonated Schiff base linkage, it is of interest to consider the extent to which our results on PRSB's are related to the photochemistry of visual pigments. In fact there are certain analogies between the enhanced 11-cis photolability observed for the triplet sensitized isomerization of RSB and PRSB and data reported for visual pigment analogs. Thus, the study of Nelson et al.[37] has suggested that an inherently twisted chromophore increases the bleaching efficiency of the pigment. They observed that both 9-cis and 11-cis 13-des methyl rhodopsin which have planar chromophores have approximately the same quantum efficiencies as isorhodopsin whose 9-cis retinal chromophore is also planar. The reported yields are approximately half those of rhodopsin. Similar conclusions are suggested from studies on the pigments formed from 14-methyl retinal[39] (retinal with an additional methyl group attached to 14C). The planar 9-cis and 9,13 dicis isomers of this molecule form pigments with approximately the same quantum yields for bleaching as other pigments with planar chromophores. However, the highly twisted 11-cis isomer of 14-methyl retinal forms a pigment whose bleaching efficiency is 50% higher than that of rhodopsin itself.[39]

These analogies, if not circumstantial, might suggest that the photoisomerization of visual pigments proceeds via a triplet state intermediate. This would also be consistent with the high quantum yields of the triplet sensitized cis → trans isomerization of PRSB, which are comparable to those associated with the photobleaching of the pigments. The main difficulty with this interpretation is that there is no detectable intersystem crossing following direct excitation of PRSB's. Though it is possible that chromophore–opsin interactions (such as charge transfer) will enhance intersystem crossing in the pigments,[5] it should be recalled that fast excitation of rhodopsin, even with nanosecond time resolution techniques,[22] failed to detect a transient absorbance attributable to a triplet state. It therefore seems highly unlikely that a triplet is involved in the main isomerization pathway of visual pigments.

If the photoisomerization of rhodopsin takes place exclusively in the singlet manifold, the analogies discussed above could be rationalized by assuming that the reduction of barrier heights, suggested for the 11-cis PRSB triplet, may be a factor in singlet states as well. However, preliminary results[40] have indicated that the main photoisomerization patterns, following direct excitation of PRSB in ethanol, are substantially different from those of visual pigments. First, the quantum yields for cis → trans isomerization following direct excitation within the α band of 11-cis PRSB are at least an order of magnitude smaller than the quantum yield (0.6) for bleaching rhodopsin. Moreover, these quantum yields exhibit a wavelength dependence (~0.05 at 438 nm and ~0.005 at 405 nm), in contrast with the wavelength-independent photochemistry of visual pigments. The direct-excitation isomerization patterns observed for PRSB are plausible if one recalls that (at room temperature) the fluorescence quantum yield (ϕ_F) is below 0.001.[41] Assuming a fluorescence emission rate (k_F) of the order of 10^8 sec^{-1}, this implies [$\phi_F = k_F/(k_d + k_F)$] that in the lowest thermalized singlet state (S_1), isomerization has to compete with deactivation rates (k_d) of the order of at least 10^{11} sec^{-1} as compared with ~10^6 sec^{-1} in the lowest triplet state (T_1). Thus, if the values of E_a in S_1 and T_1 are of the same order of magnitude, then, since $k_{ISO} \ll k_d$, no detectable thermal isomerization in the relaxed S_1 state is expected to occur. This picture is consistent with the low yields and the vibronic effects observed in the direct-excitation photochemistry of PRSB. On the other hand, the wavelength-independent photochemistry of visual pigments which are also nonfluorescent must be interpreted in terms of very low rotational barriers (E_a) in the S_1 state. In such a case ($E_a \lesssim 0.1$ eV), isomerization rates may well be of the order of below 10^{11} sec^{-1}, efficiently competing with deactivation (to S_0) in vibrationally relaxed, nonfluorescent, S_1 states.

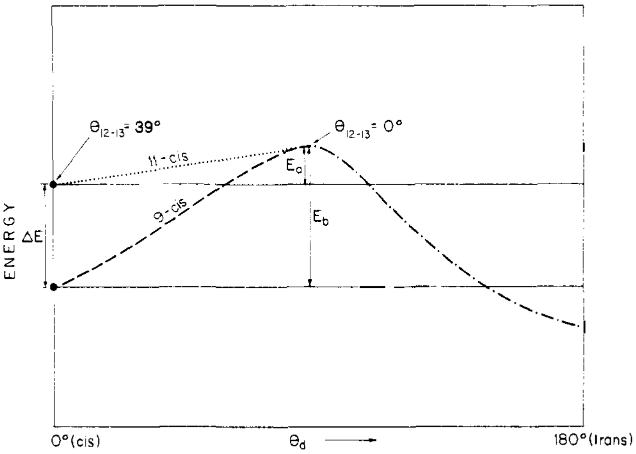

Figure 2. Schematic potential-energy diagram for the lowest triplet state of PRSB. The curve for the 13-cis isomer is similar to that of the 9-cis molecule.

When considering the degree to which PRSB in solution may be used as a model for visual pigments, it is necessary to understand the specific interactions which not only lead to the pigment's spectral shifts (relative to the 443-nm band of PRSB in ethanol), but also to a considerable lowering of the (S_1) rotational barrier around the 11–12 bond.

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Note Added in Proof. After this manuscript was accepted, a study reporting the detection of the PRSB triplet state and the occurrence of triplet-sensitized cis–trans isomerization has appeared [M. M. Fischer and K. Weiss, *Photochem. Photobiol.*, **20,** 423 (1974)].

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