# A Comprehensive Investigation of the Mechanism and Photophysics of Isomerization of a Protonated and Unprotonated Schiff Base of 11-*cis*-Retinal

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**Abstract:** A comprehensive study of the photoisomerization quantum yield ($\phi$) of 11-*cis*-retinal *n*-butylamine Schiff base and the protonated Schiff base as a function of the nature of the solvent as probes has been accomplished. Both laser-flash and steady-state techniques were employed. For the Schiff base, the yield varies from as low as $\leqslant 0.01$ in hexane to 0.34 in acetonitrile. A specifically H-bonded complex in hexane shows a high $\phi$ value of 0.31. In addition to photoisomerization, a non-excited-state transient was seen for which the quantum yield, $\phi_X$, and particularly the lifetime, $\tau_X$, vary as a function of the solvent. The values of $\phi$ and $\phi_X$ are not affected by the presence of oxygen. A fixed 12-13-*s*-trans conformer (of the 13-demethyl-11-cis Schiff base) exhibits $\phi$ values only equal to or less than those of the 11-cis Schiff base. The results are interpreted in terms of increased "$^1B_u^{+}$" mixing into "$^1A_g^{-}$" in the lowest excited state as a function of increased polarity of the solvent and a concomitant lowering of the activation energy for isomerization which originates in the Franck–Condon or equilibrium state. It is clear that there is nothing special regarding the facilitation of photoisomerization by the "$^1A_g^{-}$" state, and in fact the evidence is that it is the "$^1B_u^{+}$" state that facilitates isomerization. In the case of the protonated Schiff base, the $\phi$ is approximately constant ($\sim 0.20$) as a function of the nature of the solvent in marked contrast to the Schiff base, and no transient of any kind is observed. There is a notable increase of $\phi$ for the protonated case, compared to the unprotonated case, *only* in nonpolar solvents. However, in polar solvents, $\phi$ is not larger but in fact is smaller or equal to that of the Schiff base in a number of polar solvents. These results clearly indicate protonation is not a requirement for significant photoisomerization except in nonpolar solvents. We find no excitation wavelength dependence of $\phi$ for the protonated Schiff base in five solvents in marked contrast to a previous finding in one solvent. For both the Schiff base and protonated Schiff base, proof of isomerization to the all-trans isomer was made. Photophysical mechanistic proposals for photoisomerization are given and, importantly, these are extended into a consideration of the photophysical mechanism for isomerization in rhodopsin as well as the role of protonation (or H bonding) in the visual process.

The initial photochemical step in the visual sequence involving the pigment rhodopsin is believed to be the 11-cis to all-trans isomerization of the retinyl chromophore.[1,2] This photoisomerization step has been reported to be very efficient with a quantum yield as high as 0.67.[3] In attempts to understand the mechanism of this photoisomerization and it's high quantum efficiency, much work has been done on model linear polyenes and retinals, but relatively less on retinal Schiff bases. Because the Schiff base of retinal is the closest structural analog to the chromophore in rhodopsin we have focused our research efforts on it.

Spectroscopic studies on linear polyenes, retinals, and retinal Schiff bases have revealed that the lowest lying $(\pi,\pi^*)$ states are the spectroscopically allowed "$^1B_u^{+}$" state and the forbidden "$^1A_g^{-}$" state. The relative ordering of these $(\pi,\pi^*)$ levels has been shown to be dependent on temperature, solvent,[4] and even conformational changes.[5] It has been generally accepted that the "$^1A_g^{-}$" state is the lowest lying $(\pi,\pi^*)$ state in many linear polyenes and retinals (and their corresponding Schiff bases, see text).[4,6–8] In the case of the protonated Schiff base the "$^1B_u^{+}$" state is believed to be highly mixed into a lowest "$^1A_g^{-}$" state,[4] or possibly the lowest level.[4,9] Upon excitation, large changes in the dipole moment have been observed for the 11-cis and all-trans retinals[10–12] and the protonated and unprotonated all-trans Schiff bases.[10] Large changes in the polarizability of these systems have also been found.[11,12]

11-*cis*-Retinal is known to have two conformers, 12-13-*s*-cis and 12-13-*s*-trans, which coexist in solution. The relative populations of these conformers have been shown to be both solvent[13] and temperature dependent.[4,14] It has been proposed that in rhodopsin, the retinyl chromophore is in the 12-13-*s*-trans conformation.[15] For this reason, retinal analogs which are constrained to the 12-13-s-trans conformer (such as 13-demethyl-11-*cis*-retinal) have been synthesized and studied.[16] No attempts to study the Schiff bases of these compounds has yet been published.

The photochemistry of the protonated and unprotonated *n*-butylamine Schiff base of 11-*cis*-retinal (henceforth designated as 11-cis SB and H⁺-11-cis SB, respectively) have been studied in a limited way by both direct excitation and triplet sensitization.[17–19] In direct excitation studies by steady irradiation methods, the quantum yield for photoisomerization, assuming 11-cis to all-trans isomerization, was reported to be quite small, $\phi = 0.004$ for the 11-cis SB.[17] The H⁺-11-cis SB was also studied and it's quantum yield for photoisomerization was found to be wavelength dependent,[18] varying from 0.005 to 0.25, assuming cis to trans isomerization. In subsequent direct excitation studies employing a pulsed nitrogen laser, essentially no isomerization was detected for the unprotonated 11-cis SB in methylcyclohexane when monitoring at 360 nm (no changes in the optical density were seen).[19] For the H⁺-11-cis SB in the same solvent, positive absorption changes at 463 nm indicated a cis to trans isomerization, but again, no proof of the photoproduct as the all-trans isomer was made.[19]

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In our previous communication of a laser flash study, we reported the difference spectra (ΔOD spectra) and quantum yields for the 11-cis SB and H⁺-11-cis SB in hexane and three polar solvents.[20] Our results showed a significant solvent dependence of $\phi$ for the 11-cis SB. In polar solvents the value of $\phi$ ranged from 0.07 to 0.14. TLC analysis of the photoproducts after irradiation confirmed for the first time that cis to trans isomerization occurred. In hexane, however, the ΔOD spectra clearly revealed negative optical density changes in the region of 340–380 nm with slightly positive ΔOD's from 380 to 450 nm. The negative optical density change found in the region of 360 nm is in contrast to no optical density change reported previously[19] in methylcyclohexane at this wavelength and is inconsistent with any significant cis to trans isomerization.

In the case of the H⁺-11-cis SB, we[20] observed positive ΔOD spectra characteristic of an 11-cis to all-trans isomerization in both polar and nonpolar solvents. The cis to trans isomerization process was confirmed for the first time by TLC analysis of the photoproducts. Values of $\phi$ ranged from 0.07 to 0.17.

The observation of a significant isomerization of the unprotonated 11-cis SB and a notable solvent effect has prompted us to more thoroughly investigate the photophysics and mechanism of the cis to trans isomerization process. We have, therefore, done an extensive study on the effects of various solution environments on the absorption spectroscopy of the 11-cis SB and all-trans SB. We have also studied the photoisomerization of the 11-cis SB in many of these same solution environments, including the effects of solvent polarity, polarizability, hydrogen bonding, and protonation. Also, the effect of conformational changes about the $C_{12}$–$C_{13}$ single bond was investigated. Quantum yields of photoisomerization of the unprotonated 11-cis SB were determined both by steady state methods and from deconvolution of ΔOD spectra obtained from the laser-flash experiments.

The quantum yield of the H⁺-11-cis SB was studied as a function of the nature of the solvent as well as a function of the exciting wavelength (355, 417, and 532 nm) and determined directly from the laser flash data.

The photoisomerization yield of the protonated and unprotonated n-butylamine Schiff base of 13-demethyl-11-cis-retinal (13-DM-11-cis SB) was also briefly studied in a polar and nonpolar solvent and compared to the results for the unconstrained protonated and unprotonated 11-cis SB.

An improved TLC method was developed to analyze the photoproducts.

## Experimental Section

The 11-cis- and all-trans-retinals were obtained from Hoffman-LaRoche. TLC analysis of each sample revealed the presence of only one isomer. A sample of purified 13-demethyl-11-cis-retinal was obtained from Dr. W. Waddell. The n-butylamine Schiff bases were prepared as described before.[4] The n-butylamine obtained from fisher was purified by distillation over KOH pellets. Trichloroacetic acid (TCA) was used to protonate the Schiff bases in solution. Spectrograde or reagent grade solvents were dried and kept over 3 Å molecular sieves and used without further purification. Absorption spectra were recorded on a Cary 15 or Hewlett Packard 8450A spectrophotometer.

The laser-flash experiments were carried out with a Q-switched Nd:YAG laser (11 ns pulse width). For the unprotonated Schiff base experiments, the 355 nm third harmonic was used as the excitation source. In the case of the protonated Schiff base, both the second and third harmonics (532 and 355 nm) as well as the 417-nm line generated from a high-pressure hydrogen gas Raman shift cell (pumped by the 355-nm line) were all used as excitation sources. The kinetic absorption spectrometer used to detect optical density changes after excitation has been described previously.[21] The experiments were done on solutions in rectangular quartz cells with 5-mm path lengths along the monitoring light. Monitoring light levels were kept to a minimum to prevent photochemical process from occurring in the time period between the opening of the electrochemical shutter of the kinetic spectrometer and the excitation pulse. Degassing of the samples was performed by bubbling nitrogen gas through each solution; in addition, oxygen was bubbled in
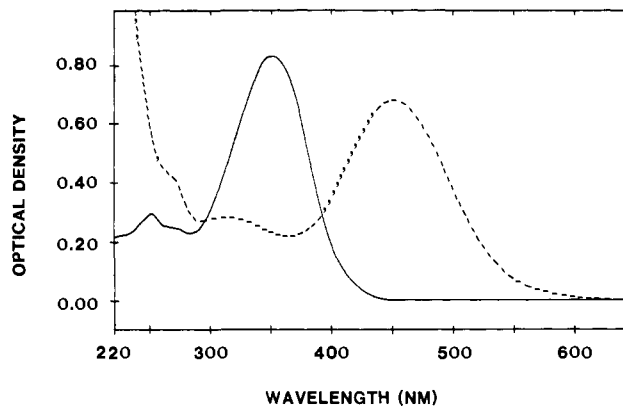


Figure 1. Room-temperature absorption spectra of the unprotonated (—) and protonated (- - -) 11-cis-n-butylamine Schiff base in acetonitrile.

separate experiments to examine its influence on isomerization.

Quantum yields of photoisomerization, $\phi$, for the H⁺-11-cis SB were determined by a comparison method.[22] For 355-nm excitation, benzophenone in benzene ($\Delta\epsilon_{TT}^{532} = 7630$, $\phi_T = 1.0$)[23] was used as a reference (actinometer). Ruthenium tris(bipyridinium) dichloride in water ($\Delta\epsilon_{TT}^{360} = 27300$, $\phi_T = 1.0$)[24] was used as the reference for 532- and 417-nm excitation. Quantum yields were calculated by utilizing data obtained from absorption spectra of 11-cis and all-trans SB isomers in the appropriate solvent ($\Delta\epsilon_{ct} = \epsilon_t - \epsilon_c$, where $\epsilon_t$ and $\epsilon_c$ are the extinction coefficients for the all-trans and 11-cis isomers respectively). The intensity of the laser radiation incident on the sample cell was controlled by the use of wire mesh screens. Intensities were chosen so that no more than 5–10% depletion of the ground state absorption occurred for either the actinometer or the Schiff base. Typical Schiff base concentrations employed were all in the range of $2 \times 10^{-5}$ M.

Quantum yields of photoisomerization for the unprotonated Schiff base were determined by two independent methods: (1) deconvolution of ΔOD spectra obtained from the 355-nm laser-flash experiments and (2) steady-state irradiation experiments. Deconvolution of each ΔOD spectrum obtained at times less than 400 μs after the laser flash was necessary due to the presence of a long-lived transient absorption which significantly overlapped with the expected absorption due to cis to trans isomerization. The steady-state experiments were also carried out at 355 nm. The excitation source for the steady-state experiments consisted of a 150W short-arc Xenon lamp and Aminco monochromator (dispersion of 6.5 nm/mm). Some qualitative experiments were done employing the light from a medium-pressure Hg lamp passed through a Corning 7-39 filter. Quantum yields were determined by a comparison method with a photochromic fulgide [(E)-α-(2,5-dimethyl-3-furylethylidene)(isopropylidene)succinic anhydride] as the chemical actinometer ($\phi_{PC} = 0.20$ and $\Delta\epsilon_{SS}^{494} = 8200$).[25] The quantum yield for formation of the colored form, $\phi_{PC}$, was also independently measured by a laser-flash experiment employing benzophenone in benzene as the reference. The result of this experiment yielded the same quantum yield (0.20) as reported earlier.[25] Typical concentrations for the 11-cis SB again were in the vicinity of $2 \times 10^{-5}$ M.

Analysis of the photoproducts after irradiation of the 11-cis SB and H⁺-11-cis SB was accomplished by an improved high performance thin layer chromatography (HPTLC) method. The Schiff bases were hydrolyzed back to their corresponding retinals and separated on HPTLC silica gel plates with 40% ether in hexane as the eluting solvent. The solutions after irradiation were applied to the HPTLC plates with a contact spotter developed specifically for the purpose of producing very tight spots of high concentration.[26] The plates were developed first in one direction and then in a second perpendicular direction in order to obtain better resolution of the photoproducts.

## Results

Absorption spectra of both the unprotonated and protonated 11-cis and all-trans SB were recorded at room temperature im-

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**Table I.** Absorption Spectral Data for the 11-cis and 13-Demethyl-11-cis Schiff Bases

| solvent system | unprotonated | | | protonated[a] | | |
|---|---|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | $\Delta\nu_{hex}{}^b$ (cm$^{-1}$) | $\epsilon_{max}{}^c/10^4$ | $\lambda_{max}$ (nm) | $\Delta\nu_{hex}{}^b$ (cm$^{-1}$) | $\epsilon_{max}{}^c/10^4$ |
| hexane | 347 | 0 | 3.23 | 458 | 0 | 2.72 |
| carbon tetrachloride | 359 | −960 | | 460 | −90 | |
| benzene | 357 | −810 | 3.23 | 455 | +140 | 3.16 |
| toluene | 357 | −810 | | 450 | +388 | |
| ethyl benzene | 358 | −890 | | 455 | +140 | |
| diethylamine | 351 | −320 | | | | |
| diethyl ether | 348 | −80 | | 430 | +1420 | |
| chloroform | 363 | −1270 | | 459 | −50 | |
| chlorobenzene | 361 | −1120 | | | | |
| ethyl acetate | 351 | −320 | 3.07 | 428 | +1530 | 2.43 |
| dichloromethane | 359 | −960 | 3.26 | 465 | −330 | 3.29 |
| acetone | 354 | −570 | | 446 | +590 | |
| methanol | 358 | −890 | 2.63 | 442 | +790 | 2.67 |
| acetonitrile | 356 | −730 | 3.63 | 445 | +640 | 3.17 |
| hexane/HFIP (3.6 mM) | 361 | −1120 | 2.93 | | | |
| hexane/CH$_3$CN (3.6 mM) | 347 | 0 | 3.23 | | | |
| 13-demethyl-11-cis SB | | | | | | |
| hexane | 350 | 0 | d | 441 | 0 | d |
| methanol | 357 | −560 | d | 433 | +420 | d |

[a] Schiff bases protonated in solution with excess TCA. [b] Shifts in maxima measured in wavenumbers with respect to maximum in hexane. [c] Units of L/(mol·cm). [d] Due to a limited quantity of the 13-demethyl-11-cis SB, $\epsilon$ was not measured but was assumed to be equal to that for the 11-cis SB in the respective solvent.

**Table II.** Absorption Spectral Data for the All-Trans Schiff Base

| solvent system | unprotonated | | | protonated | | |
|---|---|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | $\Delta\nu_{hex}{}^a$ (cm$^{-1}$) | $\epsilon_{max}{}^b/10^4$ | $\lambda_{max}$ (nm) | $\Delta\nu_{hex}{}^a$ (cm$^{-1}$) | $\epsilon_{max}{}^b/10^4$ |
| hexane | 355 | 0 | 4.96 | 458 | 0 | 4.69 |
| benzene | 363 | −621 | 5.16 | 455 | +144 | 4.43 |
| diethyl ether | 355 | 0 | | 435 | +1155 | |
| ethyl acetate | 358 | −236 | 4.78 | 430 | +1422 | 3.82 |
| dichloromethane | 364 | −696 | 5.08 | 465 | −329 | 4.36 |
| methanol | 364 | −696 | 5.23 | 445 | +638 | 4.98 |
| acetonitrile | 359 | −314 | 5.31 | 450 | +388 | 4.64 |
| hexane/HFIP (3.6 mM) | 366 | −847 | 4.37 | | | |

[a] Shifts in maxima measured in wavenumbers with respect to maximum in hexane. [b] Units of L/(mol·cm).
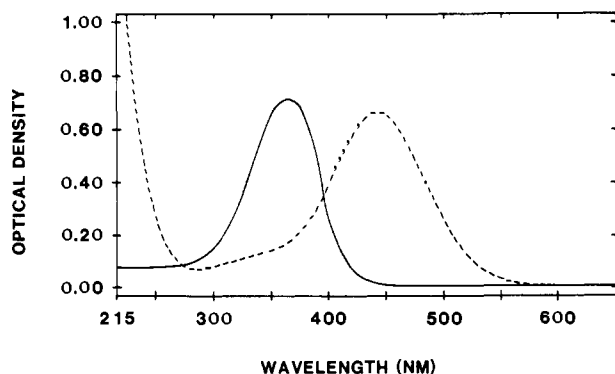


**Figure 2.** Room-temperature absorption spectra of the unprotonated (—) and protonated (- - -) *all-trans-n*-butylamine Schiff base in methanol.
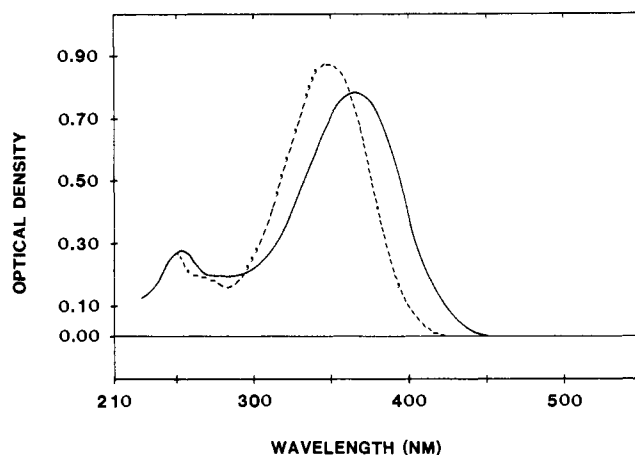


**Figure 3.** Room-temperature spectra of the 11-*cis-n*-butylamine Schiff base in hexane (- - -) and after the addition of hexafluoroisopropyl alcohol (—). The concentration of added HFIP corresponds to 3.6 mM.

mediately upon dissolving of the Schiff base. Typical spectra obtained are shown in Figures 1 and 2. Spectra of the unprotonated Schiff bases were not seen to undergo any changes over the period of 1 h at room temperature. In contrast, spectra of the protonated Schiff bases were seen to undergo small absorption changes indicative of double bond isomerization (with an isobestic point at ~360 nm) over a matter of 10 min. These observations are in agreement with previous work in which thermal isomerization rates of the 11-cis SB and H$^+$-11-cis SB were measured.[27] Spectra of the 11-cis SB and all-trans SB were recorded in a wide variety of solvents, and all absorption data obtained including wavelength maxima, shifts with respect to hexane, and molar absorptivity coefficients are shown in Tables I and II. Absorption data for 13-DM-11-cis SB were recorded

in hexane and methanol and are shown in Table I.

In the cases of the unprotonated Schiff bases, a general red shift was noted with increasing dielectric constant. However, substantial red shifts were also seen in solvents with high polarizabilities as was seen, for example, in the case of benzene with respect to hexane. Solvents with H-bonding capability were also seen to have red shifts larger than expected from their polarity nature alone.

A substantial red shift for the 11-cis and all-trans SB with respect to hexane ($\Delta\bar{\nu} \sim$ 1100 and 850 cm, respectively) was also noted upon doping hexafluoroisopropyl alcohol (HFIP, 3.6 mM) into a hexane solution of the Schiff bases (see Figure 3). The equilibrium constant for the 11-cis hydrogen-bonded complex was
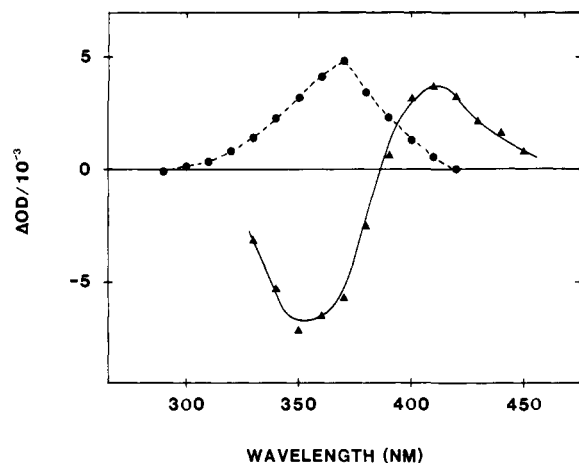
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**Figure 4.** Difference (ΔOD) spectra obtained for the 11-cis *n*-butylamine Schiff base in benzene: (▲) spectrum recorded 10 μs after the 355-nm laser flash; (●) spectrum recorded from steady-state experiments approximately 2 min after irradiation.
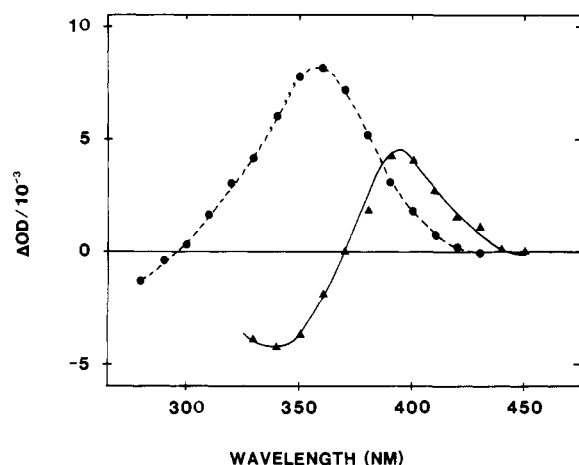


**Figure 5.** Difference (ΔOD) spectra obtained for the 11-cis SB in ethyl acetate: (▲) spectrum recorded 10 μs after the 355-nm laser flash; (●) spectrum recorded from steady-state experiments approximately 2 min after irradiation.

determined from Benasi–Hildebrand-type plots to be approximately 2.4 ± 0.6 × 10³ L/mol. In a parallel experiment, no changes were noted in the absorption spectrum of 11-cis SB in hexane upon doping with an equimolar amount of acetonitrile (same as HFIP). Reasons for doing this experiment are given later.

In the case of the protonated Schiff base, large red shifts were seen in all solvents with respect to the unprotonated species (Tables I and II). However, among solvents in which the protonated Schiff base was dissolved, both red and blue shifts were seen relative to hexane. An excess of TCA was added in each case to achieve complete protonation. In some polarizable solvents such as dichloromethane the absorption maximum of the protonated Schiff base was found to further red shift with relatively large amounts of added TCA. This result is in accordance with earlier results in which the absorption maxima of the H⁺-11-cis SB in dichloroethane was found to vary linearly as a function of the log of the TCA concentration.[28]

Laser flash experiments on the unprotonated 11-cis SB and 13-DM-11-cis SB were carried out with use of the 11-ns pulse at 355 nm from the Nd:YAG laser both with nitrogen and oxygen bubbling. No triplet transients were seen with nitrogen or oxygen, and there was no effect on photoisomerization yields or ΔOD spectra when oxygen replaced nitrogen. Difference (ΔOD) spectra
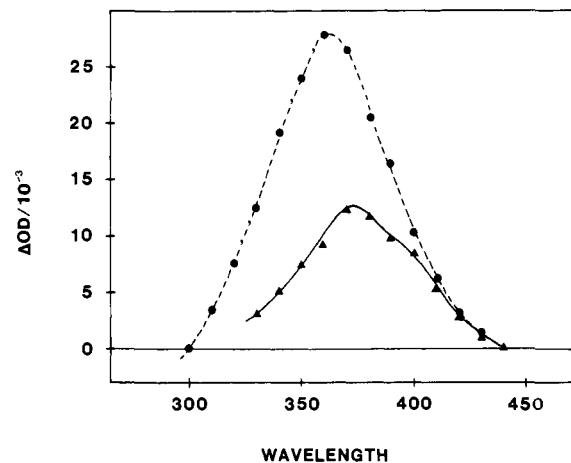
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**Figure 6.** Difference (ΔOD) spectrum obtained for the 11-cis SB in acetonitrile: (▲) spectrum recorded 10 μs after the 355-nm laser flash; (●) spectrum recorded from steady-state experiments approximately 2 min after irradiation.
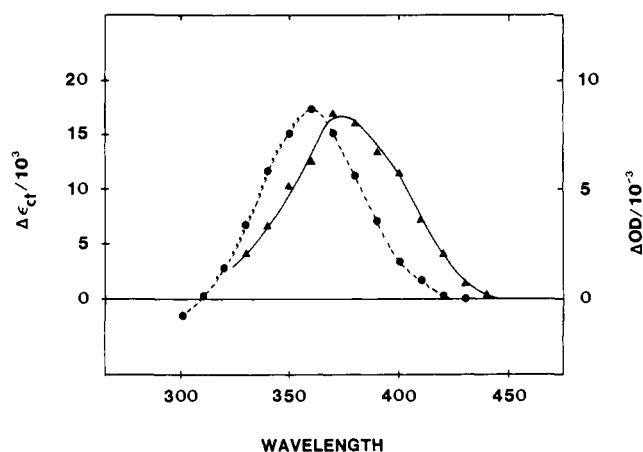


**Figure 7.** Comparison of the ΔOD spectrum (▲) obtained from laser-flash experiments on the 11-cis SB in acetonitrile with an ideal Δε_ct spectrum (●) expected for an 11-cis to all-trans isomerization. The Δε_ct spectrum was generated from absorption spectra of the 11-cis SB and all-trans SB in acetonitrile. The ΔOD spectrum was recorded 10 μs after the laser flash.

were obtained for the 11-cis SB in several pure solvents of varying polarity and in hexane with either HFIP or acetonitrile doped in at a concentration of 3.6 mM. Representative ΔOD spectra are shown in Figures 4–7. All ΔOD spectra obtained were not seen to change over the time domain of 1 to 400 μs.

In relatively nonpolar solvents such as hexane or benzene, ΔOD spectra with both a negative minimum and a positive maximum were obtained over the wavelength region studied (330–450 nm), as seen in Figure 4. The negative optical density changes seen in the region of 340–360 nm were in direct contrast to the results for a cis–trans isomerization for which positive ΔOD's are expected in this region. With increasing solvent polarity gradual changes in the ΔOD spectra occurred. These changes were characterized by an increase in the maximum relative to the minimum ΔOD and a blue shifting of the maximum. An example of these changes is seen in Figure 5 when ethyl acetate was used as solvent. In the cases of solvents of relatively high polarity (as measured by dielectric constant, *D*, and static dipole moment, *μ*), the minimum disappeared all together and a further blue shift in the maximum occurred, as seen in the case of acetonitrile (Figure 6). The ΔOD spectrum in acetonitrile more closely resembled the theoretically expected spectrum for the cis–trans isomerization, but some differences were still evident (as seen in Figure 7). This also was the case for the 11-cis in SB in methanol, acetone, and dichloromethane and for the hydrogen-bonded complex (with HFIP) in hexane. In the parallel experiment where acetonitrile was doped into the 11-cis SB hexane solution (instead of HFIP), a ΔOD

**Table III.** Photoisomerization and Transient Formation Quantum Yields for the 11-Cis Schiff Bases in Various Solvents

| sovlent system | $\mu^a$ | $D^b$ | $n^c$ | $\phi$ | $\phi_x$ | $\tau_x$ (s) |
|---|---|---|---|---|---|---|
| hexane | 0 | 1.89 | 1.375 | ≤0.01 | 0.04 | 120 |
| carbon tetrachloride | 0 | 2.24 | 1.594 | 0.03 ± 0.01 | 0.05 | 25 |
| benzene | 0 | 2.28 | 1.501 | 0.04 ± 0.01 | 0.07 | 8 |
| toluene[d] | 0.36 | 2.40 | 1.496 | ~0.05 | ~0.07 | |
| ethyl benzene[d] | 0.59 | 2.41 | 1.496 | ~0.05 | ~0.07 | |
| diethyl ether | 1.15 | 4.34 | 1.353 | 0.04 ± 0.01 | 0.03 | 75 |
| chloroform[d] | 1.01 | 4.81 | 1.483 | ~0.15 | ~0.07 | |
| chlorobenzene[d] | 1.69 | 5.7 | 1.524 | ~0.10 | ~0.07 | |
| ethyl acetate | 1.78 | 6.02 | 1.372 | 0.10 ± 0.02 | 0.07 | ~0.5 |
| dichloromethane | 1.60 | 9.08 | 1.424 | 0.23 ± 0.03 | 0.07 | $e$ |
| acetone | 2.88 | 20.7 | 1.359 | 0.19 ± 0.03 | 0.07 | $e$ |
| methanol | 1.70 | 32.6 | 1.329 | 0.20 ± 0.04 | 0.08 | $e$ |
| acetonitrile | 3.92 | 38.8 | 1.344 | 0.34 ± 0.03 | 0.09 | $e$ |
| hexane/HFIP | | | | 0.31 ± 0.03 | 0.08 | $e$ |
| hexane/acetonitrile | | | | ≤0.01 | 0.04 | |

[a] Static dipole moment of the solvent in Debye units. [b] Dielectric constant of the solvent (20–25 °C). [c] Index of refraction of the solvent. [d] Quantitative steady-state experiments were not performed on the 11-cis SB in these solvents. The values listed for $\phi$ are estimates based only on laser-flash work. [e] Lifetime between 0.4 ms and 1.0 s.

spectrum identical with the spectrum for pure the hexane case was obtained.

Steady-state experiments on the 11-cis SB revealed that the ΔOD spectra undergo substantial changes over the microsecond to second time regimes. In the cases of relatively nonpolar solvents, such as hexane, a ΔOD spectrum similar to laser-flash experiments was obtained with a minimum at about 350 nm and a positive maximum at 400 nm. Over time (seconds) the negative signal (~350 nm) was seen to recover with the same lifetime as the decay of the positive absorption at 400 nm. This decay (recovery) was found for the 11-cis SB in all solvents studied, but with variable lifetimes (see Table III). In hexane the lifetime for this recovery was 120 s. In more polar solvents, the decay was much more rapid, in some cases too fast to measure by these methods. In all solvents the final ΔOD spectrum after complete decay was found to parallel the theoretical $\Delta\epsilon_{ct}$ spectrum for an 11-cis to all-trans isomerization (see Figures 4–6). These facts led us to the conclusion that the ΔOD spectra obtained immediately (microseconds) after excitation were characteristic of two competing processes leading from the excited state: (1) 11-cis to all-trans isomerization and (2) formation of a long-lived (non-excited-state) transient species.

Steady-state experiments were also used to demonstrate that the transient decayed back to the 11-cis SB. In the case of the 11-cis SB in hexane, the ΔOD spectrum was observed to thermally decay essentially back to the base line with only very small positive ΔOD's remaining near 350 nm. This was also indicative that the transient species did not decay to the all-trans SB. If the transient did give rise to the all-trans isomer then the final net positive optical density observed after the decay would be substantially greater than is seen.[29] The residual all-positive ΔOD spectra seen in all solvents after the decay of the transient was due to 11-cis to all-trans isomerization which had already occurred on a time scale faster than microseconds.

Further verification of cis to trans isomerization was accomplished by the HPTLC method given earlier. The all-trans isomer was the only detectable (permanent) photoproduct from laser-flash and steady-state experiments.

Analysis of the ΔOD spectrum for the 11-cis SB in hexane yielded an absorption maximum for the transient of 390 nm with an extinction coefficient of approximately 14 000 L/(mol·cm). The lifetime for the transient in carbon tetrachloride was measured at 0 °C as well as room temperature. Assuming an Arrhenius dependence for the lifetime as a function of temperature, we estimated an activation energy of 8 ± 4 kcal/mol for the thermal

decay of the transient back to the 11-cis isomer.

Since it appears that 11-cis to all-trans isomerization and the formation of the long-lived transient are parallel reactions leading from the 11-cis SB after excitation, the ΔOD spectra obtained from the laser-flash experiments can then be seen as consisting of two parts. The change in the optical density at any wavelength can then be writted as

$$\Delta OD(t) = l\{(\epsilon_x - \epsilon_c)C_x(t) + (\epsilon_t - \epsilon_c)C_t(t)\}$$

where $C_x$ and $C_t$ are the concentrations of the transient and all-trans isomer formed after excitation, $\epsilon_x$, $\epsilon_c$, and $\epsilon_t$ are the extinction coefficients of the transient, 11-cis, and all-trans Schiff bases respectively, and $l$ is the monitoring path length. Over a relatively long time period (seconds) the concentration of the transient will decay away exponentially, leaving only the term describing the cis–trans isomerization. The relative magnitudes of these two contributions, of course, depends on the quantum yields for cis–trans isomerization, $\phi$, and for formation of the transient, $\phi_x$. Therefore, the calculation of the quantum yield of photoisomerization is not straightforward. In our previous communication,[20] the existence of the long-lived transient was not recognized in the laser-flash spectra obtained for the unprotonated 11-cis SB in the solvents studied. Thus the values for the quantum yields calculated in the polar solvents could only be taken as lower limits. In order to obtain the correct quantum yields for photoisomerization and transient formation we used both (1) steady-state experiments and (2) deconvolution of the laser-flash ΔOD spectra.

In the steady-state experiments, quantum yields of isomerization were calculated from optical density changes due soley to isomerization. These changes were measured after time periods long enough for complete decay of the transient absorption. A spectral deconvolution procedure was developed for analysis of the laser-flash spectra based on the observations above that isomerization and transient formation are parallel reactions. This deconvolution procedure yielded the same values for the isomerization yield as those from the steady-state experiments (within the error limits reported). The values for $\phi$ and $\phi_x$, as well as the lifetimes for the long-lived transient, $\tau_x$, in each solvent are shown in Table III. These results demonstrate a marked dependence of the photoisomerization quantum yield on solvent polarity.

Although it clearly appeared that increasing values of $\phi$ were due to increases in the solvent polarity, it is possible that the presence of significant amounts of water in more polar solvents could contribute to the increased isomerization yields. To investigate this possibility, $\phi$ was measured for the 11-cis SB in ethyl acetate with and without the addition of a relatively large concentration of water (up to 0.8 M). Ethyl acetate was chosen because of it's high miscibility with water and because of the relatively low value of $\phi$ found in that solvent compared to methanol or acetonitrile. There was essentially no change in $\phi$ (0.10 to 0.12) upon the addition of water, indicating that spurious

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(29) If the long-lived transient gave rise to the all-trans SB, then the final net ΔOD in the region of 350 nm would be given by

$$\Delta OD = \frac{(\epsilon_t - \epsilon_c)}{-\epsilon_c}\Delta OD(t = 0)$$

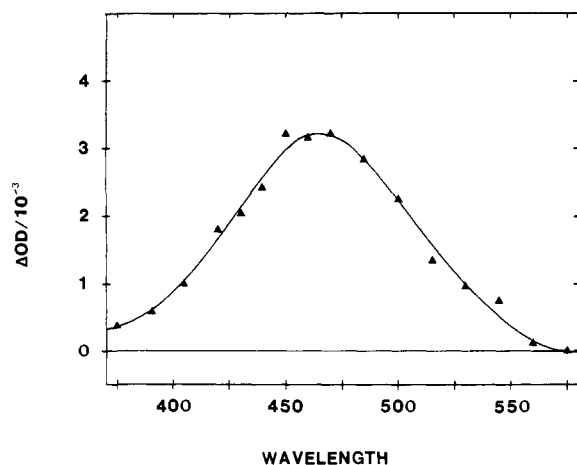where ΔOD($t$ = 0) is the initial optical density due to depletion of the 11-cis SB.

**WAVELENGTH**

**Figure 8.** Difference ($\Delta$OD) spectrum obtained from laser-flash experiments on the $H^+$-11-cis SB in benzene. Spectrum recorded 10 $\mu$s after the 355-nm laser flash.

water may not account for the high $\phi$ values in the more polar solvents.

Due to a limited quantity of the 13-demethyl-11-cis SB available to us, only laser-flash experiments could be done. Significant deviations from ideal cis–trans spectra were seen again. In hexane, the spectrum obtained had a negative minimum at $\sim$350 nm and a maximum at 400 nm. In methanol, the spectrum more closely resembled a cis–trans isomerization spectrum, but the maximum was red shifted ($\sim$375 nm) and there was significant broadening. Quantum yields were obtained from deconvolution of the spectra. A significant dependence of $\phi$ on solvent polarity for the unprotonated 13-demethyl Schiff base was again seen with a small isomerization yield in hexane ($\phi \lesssim 0.03$) and a significantly greater yield seen in methanol ($\phi = 0.13$). The quantum yields for transient formation in both hexane and methanol were 0.05 and 0.06, respectively.

In contrast to the unprotonated Schiff base studies, laser-flash experiments (at 355, 417, and 532 nm) on the protonated Schiff base revealed $\Delta$OD spectra characteristic of only a cis to trans isomerization. A $\Delta$OD spectrum obtained for the $H^+$-11-cis SB in benzene is shown in Figure 8. Spectra recorded as early as 1 $\mu$s after laser excitation were found to match the theoretical spectrum expected for an 11-cis to all-trans isomerization for the protonated Schiff base. No changes were noted in the laser-flash spectra up to 400 $\mu$s. These spectra were also not seen to change in the presence of pure oxygen or nitrogen when bubbled through the solution. Steady-state experiments also produced spectral changes matching those from the laser-flash experiments. No evolution in these spectra was seen over a period of several seconds. These results indicate the absence of any long-lived transient found for the unprotonated Schiff base. Final confirmation of 11-cis to all-trans isomerization was made by HPTLC analysis, vida supra, of the photoproducts in solution after laser-flash and steady-state experiments.

The values of $\phi$ obtained were essentially independent of solvent and excitation wavelength, see Table IV. It should be noted that the yields determined in hexane, methanol, dichloromethane and acetonitrile ($\lambda_{exc} = 355$ nm) all show increases from the values reported in our previous communication.[20] These changes are explained by a correction made in our laser excitation energies which previously were too high and lead to non-linear changes in the optical density.

Quantum yields of isomerization for the protonated 13-DM-11-cis SB in hexane and methanol were determined in the same manner as described above and are also shown in Table IV. It appears that $\phi$ is approximately constant as a function of solvent.

## Discussion

The data in Table III obtained by using 355-nm excitation clearly indicate that for the 11-cis SB, two major factors contribute to the existence and/or magnitude of the photoisomerization yield:

**Table IV.** Quantum Yields of Photoisomerization for the Protonated Schiff Bases

| system | solvent | $\phi$ (355 nm) | $\phi$ (417 nm) | $\phi$ (532 nm) |
|---|---|---|---|---|
| $H^+$-11-cis SB | hexane | $0.18 \pm 0.04$ | $0.28^a$ | $0.21 \pm 0.04$ |
| | benzene | $0.22 \pm 0.03$ | $0.20^a$ | |
| | diethyl ether | $0.20^a$ | | |
| | ethyl acetate | $0.20 \pm 0.01$ | | |
| | acetone | $0.16^a$ | | |
| | dichloromethane | $0.16 \pm 0.02$ | $0.21 \pm 0.04$ | $0.18 \pm 0.04$ |
| | methanol | $0.19 \pm 0.02$ | $0.20 \pm 0.02$ | $0.26 \pm 0.04$ |
| | acetonitrile | $0.23 \pm 0.02$ | $0.23^a$ | $0.20 \pm 0.07$ |
| $H^+$-13-DM-11-cis SB | hexane | $0.18 \pm 0.05$ | | |
| | methanol | $0.13 \pm 0.05$ | | |

$^a$ Done only once.

(a) polarity (dipole moment, dielectric constant, polarizability) and (b) H bonding.

In the three solvents of zero dipole moment and small dielectric constant (hexane, benzene, and carbon tetrachloride) little isomerization occurs but is increased in the polarizable solvents ($<0.01$ to $\sim 0.03$). The quantum yield of the transient, $\phi_x$, also increases in the more polarizable solvents (from 0.04 to $\sim 0.06$). The presence of local bond dipoles as in carbon tetrachloride does not enhance $\phi$ to a value greater than in benzene. Qualitative data in the low dipole moment, low dielectric constant solvents toluene and ethyl benzene show $\phi$ and $\phi_x$ to be nearly the same as for benzene.

The value of $\phi$ progressively increases going from ethyl acetate ($\sim 0.10$) to methanol, dichloromethane, and acetone ($\sim 0.20$), and finally to acetonitrile (0.34) while $\phi_x$ stays approximately constant ($\sim 0.08$) for this group of solvents. In this group of solvents, there is a progressive increase in the polarity of the solvents particularly reflected by the dielectric constant. In diethyl ether with a lower polarity than any of the solvents of the group, both $\phi$ and $\phi_x$ are small (0.04 and 0.03, respectively).

It is worth pointing out here that water present in the more polar solvents could also potentially hydrogen bond to the 11-cis SB, allowing for an enhanced isomerization yield. However, as discussed above, the addition of 0.8 M water to ethyl acetate demonstrated little effect on $\phi$ for the 11-cis SB in this solvent. If the reason for the relatively high values of $\phi$ (0.2–0.3) in still more polar solvents was the presence of water, and the value of $\phi$ in ethyl acetate (0.10) was lower because of its lower water content, then the addition of 0.8 M water should have certainly increased $\phi$ to that of the more polar solvents (such as acetone or dichloromethane). Therefore, although the influence of water on $\phi$ cannot be excluded, the foregoing suggests that the presence of water does not account in any significant way for the high values of $\phi$ observed in the highly polar solvents.

On the basis of the observations given in the section on Results, the transient thermally decays back to the 11-cis SB. If the upper limit of the activation energy determined for return of the transient to the 11-cis SB is correct, then isomerization of the terminal C=N would be a reasonable choice for the formation of the transient. On the other hand, the lower limit of the activation energy could be consistent with a single bond conformational change. Considering the low extinction coefficient obtained for the transient relative to the Schiff base, it would not be expected that isomerization of a terminal double bond would have such a dramatic lowering effect. Given these facts, the choice is not clear, but we tend to favor a single bond conformational change.

The effect of specific H bonding is clearly demonstrated for the case of HFIP doped in hexane. Here, $\phi$ (0.31) is, within error limits, identical with that of pure acetonitrile (0.34). Recall in hexane, $\phi \sim 0$. In order to be sure that a specific H-bonded complex exhibited this relatively high $\phi$ and that it was not due to a general solvent (shell) effect of HFIP, the experiment was repeated, doping in the same quantity of acetonitrile as HFIP (where the pure solvent $\phi$ for acetonitrile was essentially identical with that of HFIP in hexane). In this case, the $\phi$ was $\sim 0$, as in hexane, clearly demonstrating that a specific H-bonded complex

did exist with HFIP and did have a relatively high $\phi$.

We shall now consider the "protonated" 11-cis SB (H$^+$-11-cis SB). We wish to emphasize that in using the term protonated we mean a relatively short nitrogen–hydrogen distance associated with a main band absorption maximum at $\sim$450 nm vs. "nonprotonated" with a maximum near $\sim$355 nm (and $\sim$364 nm for a H-bonded complex). We do not intend necessarily the case of a formal proton attached to nitrogen. It can be seen from Table IV that there is a very significant change for $\phi$ compared to 11-cis SB in the solvents hexane and benzene (Table III). The values are approximately 20-fold greater than those for the SB. In the other solvents (see Table IV), which are polar, the $\phi$ values are essentially the same as those for the former solvents. Thus in the case of the H$^+$-11-cis SB, a leveling of $\phi$ occurs among all solvents (of a value of $\sim$0.19). These results are obviously in marked contrast to those of 11-cis SB and show that changes in the solvent dipole moment, dielectric constant, and polarizability are relatively inconsequential in affecting $\phi$ of H$^+$-11-cis SB.

It is important to point out that the $\phi$ of the 11-cis SB in acetonitrile, methanol, dichloromethane, and acetone, and for the H-bonded complex, are higher, or nearly the same, as that for the H$^+$-11-cis SB in any solvent studied (Table III). This establishes the salient fact that protonation is not a requirement to obtain significant photoisomerization of the 11-cis isomer and that either H bonding or even a polar medium can promote substantial photoisomerization, which is essentially the same or greater than that for the H$^+$-11-cis SB (in any solvent).

We shall shortly explore photophysical aspects that we believe explain these observations, but first, considerations regarding state assignments need to be made. In all following discussion, it is to be understood that reference to a pure state character as "$^1B_u^+$" or "$^1A_g^-$" means to a first-order approximation. Also, these terms will be abbreviated to "Bu" and "Ag". On the basis of the natural radiative lifetime for all-trans SB,[4] two-photon absorption spectroscopy of *all-trans*-retinal,[7] and existence of a lowest lying, "Ag" state in the Schiff base of 2,4,6,8,10-dodecapentaenal,[8] we assign the lowest excited singlet state of the 11-cis SB in an alkane solvent as principally "Ag" in nature.

For the H$^+$-11-cis SB, based on the natural radiative lifetime for the H$^+$-all-trans SB,[4] two-photon spectroscopy of the H$^+$-11-cis SB,[30] and molecular orbital calculations,[9,31] it appears that the "Bu" state is highly mixed into the lowest "Ag" state, and quite possibly "Bu" is the lowest excited state.
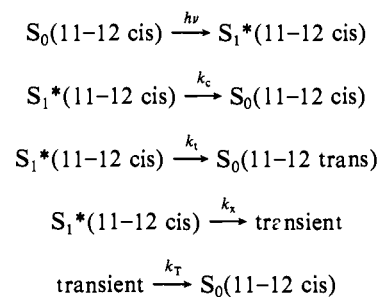
Recent data on the changes of dipole moment ($\Delta\mu$) of *all-trans*- and 11-*cis*-retinals as well as all-trans SB and H$^+$-all-trans SB in nonpolar systems indicate that the dipole moment is significantly greater, 10-16 D, in the lowest excited electronic state of Franck–Condon nature.[10,11] Also, *all-trans*-retinal shows a significant increase in polarizability ($\Delta\alpha$) of 600–1000 Å$^3$ in the lowest excited state.[11,12] Furthermore, on the basis of equations in ref 11, $\Delta\mu$ could be $\geq$ 20 D for retinal in polar solvents (vs. 13–15 D in nonpolar systems) used in the present study such as acetone. Parallel increases in $\Delta\mu$ for a Schiff base in a polar solvent should also occur. On the basis of large changes (increases) occurring in $\Delta\mu$, the "Ag" and "Bu" states will be variably mixed in the first electronic excited state of Franck–Condon nature, depending on the nature of the solvent.

On the basis of all of the foregoing facts, the "Bu–Ag" mixing in general will be least in nonpolar, low dielectric constant and low index of refraction (polarizable) solvents, greater in intermediate dipole moment and dielectric constant solvents, and greatest in the high dipole moment and dielectric constant solvents as well as in the hydrogen-bonded complex. *We propose* that the progressively increased mixing of "Bu" into "Ag" (or possibly even a final state order change to "Bu" slightly lower) that occurs permits isomerization to progressively become more efficient. It is clear that this progressive mixing and increase in efficiency of

isomerization infers a change in a salient parameter pertinent to the degree of presence of isomerization in the solute. We believe that this parameter is the activation energy for isomerization in the lowest excited electronic state. Therefore, we must come to the conclusion that the activation energy is variably lower in the Franck–Condon state as a function of the nature of the solvent. Thus, principally, as the dipole moment and dielectric constant of the solvent progressively increase, the state mixing progressively increases and the activation energy progressively decreases (the polarizability can also be important).

We have inferred the Franck–Condon state as the originating state for isomerization. This was done since the large changes in dipole moment were determined in the Franck–Condon state and mixing would be induced in this state. In the case of the nonpolar solvents, there is not expected to be any essential difference in the Franck–Condon and equilibrium states of the solute since solvent reorientation is essentially absent. However, for polar solvents, solvent relaxation (reorientation) will occur and this can be fast (for example, the dielectric relaxation of methanol and chloroform are 73 and 7 ps, respectively, at 293 K).[32] Given this fact, we conclude that isomerization could as well begin in the equilibrium state unless the rate of isomerization is clearly faster than the rate of solvent relaxation. It is also possible that the state mixing and activation energies are different in the equilibrium state. In any event, the principal feature promoting isomerization, whether in the Franck–Condon or equilibrium state, is the increased mixing of "Bu" into "Ag" and concomitant lowering of the activation energy for isomerization.

A proposed mechanism for isomerization would be

$$S_0(11\text{--}12 \text{ cis}) \xrightarrow{h\nu} S_1^*(11\text{--}12 \text{ cis})$$

$$S_1^*(11\text{--}12 \text{ cis}) \xrightarrow{k_c} S_0(11\text{--}12 \text{ cis})$$

$$S_1^*(11\text{--}12 \text{ cis}) \xrightarrow{k_t} S_0(11\text{--}12 \text{ trans})$$

$$S_1^*(11\text{--}12 \text{ cis}) \xrightarrow{k_x} \text{transient}$$

$$\text{transient} \xrightarrow{k_T} S_0(11\text{--}12 \text{ cis})$$

One other area of consideration relative to the results of $\phi$ as a function of the nature of the solvent and the photophysical explanation could involve differences in conformeric species concentration. In the case of the 11-cis SB, we have earlier concluded that 12-13 single-bond conformers exist and that the 12-13-s-cis predominates (but by an unknown amount) at room temperature in an alkane solvent (12-13-s-trans predominates at low temperature). It was believed[16,33] that the 12-13-s-trans nature of the 13-demethylretinal derivative[34] was responsible for the higher value of $\phi$ (11-cis to all-trans) compared to the 11-*cis*-retinal itself in an alkane solvent (where presumably, for the latter, a dominance of 12-13-s-cis over 12-13-s-trans existed). First, it is to be pointed out that for 11-*cis*-retinal, the $\phi$ was already high (0.25) in an alkane solvent and there was only a 2-fold increase in $\phi$ going to the 13-demethyl case. Second, for 11-*cis*-retinal, going from an alkane (12-s-cis dominant) to a methanol solvent (12-s-trans dominant), there was a 6-fold decrease in $\phi$ (instead of an increase, which could in part be caused by state order changes involving (n,$\pi^*$) and ($\pi,\pi^*$) states). This is in marked contradistinction to our results for the 11-cis SB where a 20-fold (or greater) increase in $\phi$ occurred between the same two solvents. Third, our $\phi$ data on the 13-demethyl-11-cis SB (12-13-s-trans) in an alkane solvent does not indicate any substantial difference from that of the 11-cis SB—both have small $\phi$ values, $\leq$0.03. In methanol, again there is no substantial difference between the

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11-cis SB and the 13-demethyl derivative (in fact, the 13-demethyl derivative could well have a lower $\phi$). In summary, the results found for the two retinals[16] plus our data on the corresponding Schiff bases do not indicate that the very substantial changes of $\phi$, >20-fold, found for the 11-cis SB as a function of the nature of the solvent are related in any significant way to a redistribution of conformeric species concentrations.

Further consideration of the above regarding state order as a function of the conformer and the expected photoisomerization results is important. In the case of the 11-cis-12-13-s-cis isomer, the "Bu" state has been assigned as the lowest excited state, while for 11-cis-12-13-s-trans, the "Ag" was assigned as the lowest excited state.[5] Presumably, the "Ag" state would then be lowest for the 13-demethyl-11-*cis*-retinal as well. Moreover, it has been stated that the "Ag" excited state would have a lower barrier to double bond isomerization than the "Bu" excited state.[35] From our earlier discussion above for the 11-cis SB, the "Ag" state is expected to be lowest in alkanes. However, we find insignificant isomerization (<0.01) of the 11-cis SB in this solvent. Also, we do not find that the Schiff base of 13-demethyl-11-*cis*-retinal has substantially different isomerization properties than the 11-cis SB. Thus it is clear, at least in the Schiff base, that there is not something special about the "Ag" excited state regarding its photochemical (isomerization) lability and that it does not have a lower barrier to double bond isomerization than the "Bu" excited state.

For the protonated 11-cis SB, very little variation is seen in $\phi$ as a function of the nature of the solvent (regarding any solvent parameter) and $\phi$ is ~0.20 in hexane. Recall, vida supra, that on the basis of the natural radiative lifetime data of H$^+$-all-trans SB in the Franck–Condon state, the "Bu" state is expected to be highly mixed into a lower, close-lying "Ag" state, or the "Bu" state is somewhat lower, even in an alkane solvent. These are to be contrasted with the unprotonated Schiff base where in an alkane solvent the mixing is clearly less, the "Ag" state is lowest, and $\phi$ is <0.01. Therefore, we must conclude that protonation causes (1) increased state mixing (or state-order change) in the lowest excited state of Franck–Condon nature and (2) lowering of the activation energy to permit significant isomerization even in an alkane (and benzene) solvent. Apparently, the activation energy is nominally minimal and $\phi$ is relatively insensitive to further solvent changes. Also, it is clear that protonation results in the absence of a non-excited transient. Here again as for the Schiff base, it is not clear whether isomerization originates in the Franck–Condon or equilibrium state. The analysis given earlier for the situation in the Schiff base is also equally applicable to the protonated Schiff base, vida supra.

It has been proposed[9] that in isolated protonated Schiff bases, mixing of a higher "Ag" into a considerably lower "Bu" state occurs upon twisting of the $C_{11}$–$C_{12}$ double bond and that this results in a negative barrier (barrierless) for the isomerization process ("Ag" lowest near 90°). It also has been proposed[9] that a solution environment could alter the barrier for cis–trans isomerization in the excited state such that it would no longer be negative and $\phi$ would decrease. Our data for the protonated 11-cis SB are not in agreement with this proposal. Moreover, the state mixing proposed here does not require any significant twisting to exist and is opposite in character, meaning "Bu" and "Ag". The data for the unprotonated Schiff base indicate a parallel situation exists for it regarding the foregoing points.

Despite our case where there appears to be a "Bu" into "Ag" mixing requirement, it certainly is possible in general that it is *the mixing process* that is important (mixing of "Ag" into "Bu" may work as well). We also have to allow for the fact that it is possible that having a "Bu" excited state lowest may be sufficient although we believe at least in the case of the 11-cis SB that the "Bu" and "Ag" states are probably significantly mixed.

Of important consideration is the significance of our findings relative to rhodopsin (Rh) and the primary step in the visual

sequence. It is clear that the role of "protonation" need be explored. First, based on our findings for the 11-cis Schiff base and the protonated 11-cis SB, and the fact that the isomerization of Rh occurs within a few ps,[36] we propose for rhodopsin that it is the protonation that (1) produces a mixed "Bu–Ag" (or "Bu") lowest excited state of Franck–Condon character at 0° twist and (2) produces a low "zero" activation energy (without twisting) for efficient isomerization from the Franck–Condon state.

It seems clear that protonation is not a requirement for photoisomerization in general (although it appears to be true specifically for nonpolar environments). Thus, if the Schiff base (of lysine) region of Rh is truely isolated in a nonpolar hexane-like environment, "protonation" would be a requirement for photoisomerization to occur. Of course, the protein itself could influence the isomerization yield, but it seems highly unlikely it could increase it from <0.01 found for the Schiff base itself (in hexane) to ~0.6 found in Rh without some H bonding or protonation of the nitrogen atom (of the Schiff base). We earlier observed[37] an increase of the p$K_a$ and negative charge density of nitrogen upon exciting a protonated SB with no twist and a substantial decrease in the charge density upon twisting to 90°. Others have noted a marked deuterium isotope effect on the kinetics of formation of bathorhodopsin.[38] On the basis of these observations and the results reported herein, we reemphasize our earlier proposal[37] that the primary photostep in vision involves the proton coming off in the excited state during the twisting to ~90° onto one site of the protein and returns from another site of the protein after relaxation to and twisting in the ground state from ~90° to whatever angle is appropriate to bathorhodopsin.

Another consideration which is important that bears on the validity of the H$^+$-11-cis SB as a model for rhodopsin is the wavelength dependence of $\phi$. It has been reported that the H$^+$-11-cis SB in methanol as well as the H$^+$-all-trans SB show a very substantial dependence of $\phi$ on the exciting wavelength[18] (for the 11-cis isomer, $\phi$ was 0.25 at 254 nm, 0.15 at 314 nm, 0.005 at 405 nm, 0.25 at 504 nm, and 0.07 at 577 nm). On the other hand, it has been reported that the quantum yield of bleaching of rhodopsin, $\phi_{Rh \rightarrow BRh}$, presumably the same as $\phi$, shows no wavelength dependence at room[39,40] or low temperature.[18] We have found (see Results section) that essentially no excitation wavelength dependence of $\phi$ of the protonated 11-cis Schiff base exists over the wavelengths 355, 417, and 532 nm in four different solvents including hexane, dichloromethane, methanol, and acetonitrile (nor for benzene at 355 and 417 nm). Our wavelengths correspond as closely as reasonably achievable to the short (313 nm), intermediate (405 nm), and long (540 nm) wavelength values previously used.[18] A variation of 5000% was seen between 405 and 540 nm in earlier data, whereas we see only ~30% variation over 417 and 532 nm (as well as 355 nm). We do not know the basis for the differences between ours and other work.[18] On the basis of our results for four to five solvents, we must conclude there is essentially no excitation wavelength dependence of $\phi$ of H$^+$-11-cis SB and that this model accurately reflects characteristics of rhodopsin regarding this issue.

## Summary

(1) The 11-cis Schiff base isomerizes only to all-trans on the basis of high-performance TLC analysis. Quantum yields of photoisomerization are small in nonpolar solvents (≤0.01–0.04), intermediate (0.1) in intermediate polar solvents, and relatively high in polar solvents (0.20–0.34). The quantum yield is also high in a H-bonded complex (0.31). A non-excited-state transient exists

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in all solvents which decays back to the 11-cis Schiff base (quantum yield varying from 0.03 to 0.09 and lifetime varying from ms to 120 s).

On the basis of photoisomerization results previously known for 11-*cis*-retinal and 13-demethylretinal as well as our data for the 13-demethyl-11-cis Schiff base, single bond conformer distribution cannot account in any significant manner for the variation of the efficiency of isomerization of the 11-cis SB as a function of the polarity of the solvent.

(2) For the 11-cis Schiff base, the increase in the efficiency of isomerization as a function of principally the polarity of the solvent is assigned to be the result of increased mixing of a "Bu" state into a lowest "Ag" state with a concomitant decrease in the energy of activation of isomerization. The mixed electronic state from which isomerization begins cannot be clearly assigned as Franck–Condon or equilibrium in nature. If the lifetime for isomerization is very fast, that is ~5 ps, then the originating state nature is Franck–Condon.

On the basis of the results given in item 1, it is clear that there is nothing special about the "Ag" state regarding its photochemical lability. In fact, the lowest excited state becomes less photoisomerization labile as the "Ag" character becomes more pure.

(3) The protonated 11-cis Schiff base exhibits only minor variation in the quantum yield of isomerization ($0.19 \pm 0.03$) as a function of the nature of the solvent in marked contrast to the Schiff base. Moreover, the isomerization efficiency of the Schiff base in polar solvents is as high as, or even higher than, that of the protonated Schiff base in any solvent.

Protonation of the Schiff base results in (a) increased state

mixing of "Bu" into "Ag" relative to that in the Schiff base (or "Bu" somewhat lowest), (b) lowering of the energy of activation to a nominally minimal value to permit significant isomerization which is relatively insensitive to the nature of the solvent, and (c) elimination of a side reaction transient present in the Schiff base.

(4) The mixing given above, "Bu" into "Ag", is the opposite of that proposed by theory to account for efficient isomerization of the protonated Schiff base. Moreover, twisting in the excited state as previously proposed is not a requirement for mixing.

(5) Previously reported large variations in the quantum yields of isomerization of the protonated 11-cis Schiff base with exciting wavelength in methanol were not found in this study for five solvents (including methanol).

(6) A proposal is made for the mechanism of the primary photoreaction of the vision process based on our earlier results and results reported herein.

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# Observation of a Common Intermediate in the Photocycloaddition and Photocycloreversion of Linked Anthracenes
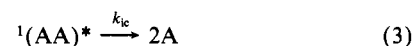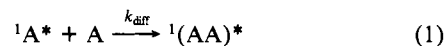
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**Abstract:** Picosecond dynamics of intramolecular photocyclization ($1 \rightarrow 2$) for a series of anthracenes linked at the 9 position (**1**) have been measured after 355-nm excitation by a 25-ps laser pulse. Picosecond dynamics for the corresponding photocycloreversion ($2 \rightarrow 1$) have also been measured after 266-nm excitation of **2** by a 25-ps laser pulse. It is shown that electronic excitation of either **1** or **2** creates a common intermediate (CI). The electronic structure of the CI is different in polar vs. nonpolar solvents and leads to differing absorption spectra for the CI in various solvents. The absorption spectrum of the CI in acetonitrile is consistent with a structure where electron transfer has occurred from one anthracene ring to the other. The absorption spectrum of CI in diethyl ether is less definitive. The rate of CI formation from the lowest excited singlet of **1**, $S_1$\* (**1**), is dependent on solvent and is consistent with charge transfer occurring in the transition state from $S_1$\* (**1**) to CI. Consideration is given to the coincidence of excimer and ion-pair structures for linked arenes in polar media.

Anthracene photodimerizations were first reported by Fritzche[1] and have since been the subject of intensive investigations.[2] The generally accepted mechanism involves interaction of singlet excited anthracene ($^1$A\*) with ground-state anthracene (A) to form an excimer ($^1$AA\*) which disproportionates between dimerization ($k_{dim}$, to give products linked at the 9 and 10 positions) and internal conversion ($k_{ic}$, to give two ground-state anthracenes), Scheme I.

**Scheme I**

$$^1A* + A \xrightarrow{k_{diff}} {}^1(AA)* \qquad (1)$$

$$^1(AA)* \xrightarrow{k_{dim}} A_2 \qquad (2)$$

$$^1(AA)* \xrightarrow{k_{ic}} 2A \qquad (3)$$

Recently, investigations have been carried out with linked systems in which intramolecular interactions (including dimerization) can occur between two anthracene moieties which are connected by an alkyl chain.[3-15] An advantage of using linked

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