J. Am. Chem. Soc. **1986**, *108*, 1245–1251

Comparative Investigation of the Photoisomerization of the Protonated and Unprotonated *n*-Butylamine Schiff Bases of 9-*cis*-, 11-*cis*-, 13-*cis*-, and *all-trans*-Retinals

Kenn A. Freedman† **and Ralph S. Becker***

Contribution from the Department of Chemistry, University of Houston, University Park, Houston, Texas 77004. *Received June 24, 1985*

Abstract: Laser-flash and steady-state excitation accompanied by high-pressure liquid chromatography (HPLC) have been used to evaluate the quantum yields of isomerization (ϕ) of the 9-*cis*-, 11-*cis*-, 13-*cis*-, and *all-trans-n*-butylamine Schiff bases of retinal, as well as their protonated analogues. In hexane, for all the Schiff bases, the ϕ were ≤ 0.01 and a ground-state transient species was formed after excitation and was seen to decay to the original isomer with lifetimes dependent on solvent polarity. In methanol, all Schiff bases, except the 13-cis isomer, showed relatively high values of ϕ. For the latter solvent and for the 9-cis and 11-cis isomers, the all-trans isomer was formed but the 13-cis isomer showed more complicated behavior, while for the all-trans isomer a mixture of cis isomers was produced. No triplet transients were observed. In the case of the protonated Schiff bases, 11-cis isomerized to the all-trans with high quantum yields in both solvents and no transients were seen. For the protonated all-trans, a mixture of cis isomers was produced where the relative proportions of each was solvent dependent and no transients were seen. For both protonated 9-cis and 13-cis isomers in both solvents, difference spectra did not correspond to the formation of the "normal" all-trans isomer, but analysis by HLPC showed retention times corresponding to "normal" all-trans. Explanations are offered of the results for the latter protonated cases, as well as for the unprotonated 13-cis Schiff base in methanol. The overall results are considered pertinent to the primary reactions in the visual process (rhodopsin and isorhodopsin) and *halobacterium halobium* (light-adapted and dark-adapted bacteriorhodopsin) from which meaningful comparisons and new insights arise.

Retinals are found in nature as the chromophores in some important biological systems including visual pigments and photosynthetic bacteria. These systems are involved in phototransduction. Each of these pigment systems includes an opsin apoprotein to which is bound an isomer of retinal via a Schiff base linkage to a lysine residue. Examples of these pigments and their corresponding retinal chromophores are rhodopsin (11-*cis*-retinal), isorhodopsin (9-*cis*-retinal), and light-adapted bacteriorhodopsin (*all-trans*-retinal). A few comprehensive reviews on these pigment systems have been published.[1-3] Several studies on these pigments employing ultrafast laser spectroscopy have provided evidence that cis to all-trans isomerization[4] (or all-trans to 13-cis isomerization in the case of bacteriorhodopsin)[5] is one of the first steps after the absorption of light.

The photoisomerization of various retinal isomers has been well-studied.[6] However, much less work has been done on the Schiff bases which are closer structural analogues of the chromophores in the pigments. The *n*-butylamine Schiff bases of 11-*cis*- and *all-trans*-retinal Schiff bases have been studied in the past by both direct excitation and triplet sensitization.[7-13] No work yet has been done on the photochemistry of a Schiff base of 9-*cis*- or 13-*cis*-retinal.

Recently, we have published a comprehensive investigation into the photophysics and mechanism of the photoisomerization of the *n*-butylamine Schiff base of 11-*cis*-retinal.[12] From laser-flash and steady-state experiments, we observed that the quantum yield of photoisomerization, ϕ, was markedly dependent on solvent polarity, hydrogen-bonding, and protonation. In addition to 11-cis to all-trans isomerization, the competitive formation of a non-excited-state transient was observed after excitation of the unprotonated Schiff bases. The quantum yield was found to increase dramatically with solvent polarity as demonstrated by a very low ϕ in hexane (≤ 0.01) as compared to much higher values in methanol (~ 0.2) and acetonitrile (~ 0.3). A hydrogen-bonding environment was also found to promote efficient isomerization ($\phi \sim 0.3$). Protonation of the Schiff bases served to eliminate both the transient side reaction and the dependence of ϕ on solvent, yielding an average value of ~ 0.2. These results were interpreted in terms of increased mixing of the ${}^1B_u^{*+}$ and ${}^1A_g^{*-}$ excited states as a function of solvent polarity or protonation, which results in a lowering of the activation energy for 11–12 double-bond isomerization.

With the insight gained from unraveling the rather complex problem for the 11-*cis*-retinal Schiff base (11-cis SB),[12] we have extended our study to the photochemistry of the 9-*cis*-, 13-*cis*-, and *all-trans-n*-butylamine retinal Schiff bases, which will henceforth be referred to as 9-cis SB, 13-cis SB, and all-trans SB, respectively. Using laser-flash and steady-state methods, we have again observed dramatic solvent effects and the competitive formation of a long-lived transient which served to complicate the observed difference spectra. We have studied the photoisomerization of these isomers in a nonpolar solvent and in polar solvents and evaluated the effects of protonation on the process. The protonated Schiff bases will be designated as H^+-9-cis SB, H^+-11-cis SB, H^+-13-cis SB, and H^+-all-trans SB. High-pressure liquid chromatography (HPLC) was done to determine the Schiff base isomers present in solution both *after synthesis and after irradiation* for all the systems studied.

We shall consider the significance of the results relative to the primary photoprocess in vision as well as that for *halobacterium halobium.*

---

† Current address: Texas Tech University Health Sciences Center, Medical School, Lubbock, TX 79430.

(1) Ottolenghi, M. "Advances in Photochemistry"; Ditts, J., Ed.; Wiley: New York, 1980; Vol. 12, pp 97–200 and references therein.

(2) Birge, R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315 and references therein.

(3) Callender, R. "Biological Events Probed by Ultrafast Laser Spectroscopy"; Alfano, R., Ed.; Academic Press: New York, 1982; pp 239–258 and references therein.

(4) Buchert, T.; Stefanic, V.; Doukas, A.; Alfano, R.; Callender, R.; Pande, J.; Akita, H.; Balough-Nair, V.; Nakanishi, K. *Biophys. J.* **1983**, *43*, 279.

(5) Chang, C.; Govindjee, R.; Ebrey, T.; Bagley, K.; Dollinger, G.; Eisenstein, L.; Marque, J.; Roder, H.; Vittitow, J.; Fang, J. M.; Nakanishi, K. *Biophys. J.* **1985**, *47*, 509.

(6) Liu, R.; Asato, A. *Tetrahedron* **1984**, *40*, 1931.

(7) Rosenfield, T.; Alchalel, A.; Ottolenghi, M. *Photochem. Photobiol.* **1974**, *20*, 121.

(8) Rosenfield, T.; Honig, B.; Ottolenghi, M. *Pure. Appl. Chem.* **1977**, *49*, 341.

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

(10) Huppert, D.; Rentzepis, P.; Kliger, D. *Photochem. Photobiol.* **1977**, *25*, 193.

(11) Becker, R. S.; Freedman, K.; Causey, G. *J. Am. Chem. Soc.* **1982**, *104*, 5797.

(12) Becker, R. S.; Freedman, K. *J. Am. Chem. Soc.* **1985**, *107*, 1477 and references therein.

(13) Donahue, J.; Waddell, W. *Photochem. Photobiol.* **1984**, *40*, 399.

0002-7863/86/1508-1245$01.50/0

Table I. HPLC Analysis of the Retinal Schiff Bases after Synthesis/Hydrolysis

	% isomers detected				
compd synthesized	all-trans	13-cis	11-cis	9-cis	9-cis, 13-cis
all-trans SB	89	5	4	2	0
H^+-all-trans SB	93	7	t	t	0
13-cis SB	12	88	0	0	0
H^+-13-cis SB	15	85	0	0	0
11-cis SB	15	2	83	t[a]	0
H^+-11-cis SB	12	3	85	t	0
9-cis SB	5	0	0	92	3
H^+-9-cis SB	4	0	0	92	4

[a] t = trace amount was detected.

Experimental Section

The *9-cis-*, *11-cis-*, *13-cis-*, and *all-trans*-retinals were all obtained from Hoffman-La Roche (also 13-cis and all-trans from Sigma). The *n*-butylamine Schiff bases were prepared as described before. Carefully dried trichloroacetic acid (TCA) always in the presence of 3-Å molecular sieves was used to protonate the Schiff bases in solution. Spectrograde 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 employed a Q-switched Nd:YAG laser using the 355-nm third harmonic (11-ns pulse width) as the excitation source. Descriptions of the laser-flash system have been given elsewhere.[12-15] The excitation source in the steady-state experiments consisted of a 1000-W xenon lamp plus a GCA McPherson monochromator (2 nm/mm dispersion). Sample solutions were irradiated (λ_{exc} = 355 nm) in 1 cm path length quartz cells and absorption changes recorded on the Cary 15.

Quantum yields of isomerization were determined from optical density changes measured in the laser-flash and steady-state experiments on the unprotonated and protonated Schiff bases. Details on the comparison method used in the determinations and actinometry, are given in our previous paper.[12]

Analysis of the photoproducts after steady-state irradiation was accomplished by a HPLC method. HPLC analyses were performed by using a Beckman gradient liquid chromatograph (Model 334), equipped with a Hewlett-Packard 3390A reporting integrator, a μ-Porasil column, and a flow-cell detector (λ = 360 nm). The eluting solvent used was 6% anhydrous ether in hexane. Prior to HPLC analysis, both irradiated samples and nonirradiated control solutions of Schiff bases were hydrolyzed back to corresponding retinals by the addition of water to the organic solvent (50% H_2O/50% organic solvent). After hydrolysis at room temperature, the organic solvent was removed by vacuum evaporation and then the retinals were extracted in anhydrous ether, followed by evaporation to dryness and redissolving the retinals in the HPLC solvent. The total time for the hydrolysis, extraction, and evaporation was 60–90 min. In the cases of the TCA-protonated Schiff bases, triethylamine was added to neutralize the solutions prior to the addition of water, and two hexane extractions, instead of one ether extraction, were employed to remove excess TCA from the sample before HPLC.

Results and Discussion

Before reporting results on the photochemistry of the retinal Schiff bases, we should consider the isomeric purity of each Schiff base after preparation from the corresponding retinal. The isomeric purity of each of the initial retinals was determined by HPLC to be greater than 96% for all four isomers. After synthesis, each Schiff base or protonated SB was hydrolyzed according to previously described procedures, and the resulting retinal(s) was separated by HPLC. The results of this for all the unprotonated and protonated Schiff bases are shown in Table I. Isomerization appears to have occurred during the synthesis and/or hydrolysis steps, but in all cases, no more than 7–17% of the undesired isomers were formed. On the basis of observations of others,[13] hydrolysis and extraction procedures appear not to result in any significant isomerization of the Schiff bases (or retinals). As can be seen from Table I, even for the protonated Schiff bases, the production of undesired isomers is still within the above 7–17% range. This is in marked contrast to results obtained earlier for the 9-cis, 11-cis, and 13-cis Schiff bases where very substantial isomerization, ~50%, was observed *using HCl* as the protonating agent.[13] It is known that HCl is a powerful catalyst for the isomerization of the *n*-butylamine Schiff base of 11-*cis*-retinal.[16] Because there exists no successful technique to separate and recover the Schiff base isomers in pure form, the principal isomer containing the minor ones was employed for the photochemical study. *However*, based on the above point regarding the effect of hydrolysis, the impurity of each isomer will be considered in the interpretation of spectra and in the calculation of quantum yields.

Table II. Absorption Data for the Protonated and Unprotonated Schiff Bases

		unprotonated		protonated	
isomer	solvent	λ_{max}, nm	$10^4\epsilon$, M^{-1} cm^{-1}	λ_{max}, nm	$10^4\epsilon$, M^{-1} cm^{-1}
9-cis[a]	hexane	346	4.83	441	3.85
	methanol	353	4.81	432	3.94
11-cis[b]	hexane	347	2.87	458	2.43
	methanol	358	2.10	442	2.33
13-cis[a]	hexane	347	3.58	443	2.41
	methanol	355	3.36	434	2.39
all-trans[b]	hexane	355	5.17	458	4.86
	methanol	364	5.52	445	5.18

[a] Values for extinction coefficients given are corrected for isomeric impurity. [b] Values were taken from ref 12 and were corrected for isomeric impurity.

Absorption spectra of the unprotonated and protonated Schiff bases were recorded at room temperature in hexane and methanol immediately after synthesis. Absorption data for all isomers are shown in Table II. The extinction coefficient data shown have been corrected for the presence of impurities in each isomer after synthesis. This corrected extinction coefficient data were necessary for the calculation of the quantum yields of isomerization.

To aid in the interpretation of ΔOD spectra obtained from laser-flash and steady-state experiments on each isomer, spectra for cis–trans (or trans–cis) isomerization were calculated from the ground-state absorption data for the protonated and unprotonated Schiff bases. The quantity, $\Delta\epsilon$, is defined as the difference in the molar extinction coefficients between the initial isomer and the final isomer(s) produced (or expected) after irradiation. For example, for the 9-cis SB, the all-trans isomer is the expected photoproduct and $\Delta\epsilon = \epsilon_t - \epsilon_9$, where ϵ_9 and ϵ_t are the molar extinction coefficients for the 9-cis isomer and the all-trans isomer, respectively. Quantum yields of isomerization, ϕ, were calculated by using these $\Delta\epsilon$ data as well. In those cases, where the ΔOD spectrum obtained did not match the expected $\Delta\epsilon$ spectrum for solely a cis $\leftrightarrow$ trans isomerization, a value for ϵ of the photoproduct was estimated from a deconvolution of the OD spectrum and used in the calculation of ϕ.[12] In the cases of the 9-cis, 11-cis, and 13-cis isomers, ϕ is the yield for cis to all-trans isomerization, whereas for the all-trans isomer ϕ represents the sum of yields for isomerization of the all-trans to each of the monocis isomers.

Laser-flash experiments on the *unprotonated* 9-cis, 13-cis, and all-trans Schiff bases revealed no triplet transients or other comparably short-lived transients in N_2- or O_2-saturated solvents. Optical density changes were noted, however, between 300 and 450 nm, and these ΔOD spectra were not observed to change over the time domain of 1–400 μs. A typical spectrum for the 13-cis SB in hexane is shown in Figure 1.

From steady-state experiments, we observed that for all the unprotonated Schiff bases, in hexane and methanol, these spectra showed significant changes over the microsecond to second time regimes. An example of this is also shown in Figure 1. These results were indicative of the formation of a long-lived transient species similar to that seen earlier for the 11-cis SB after irradiation.[12] Experimental evidence suggested that the formation of this transient is competitive with cis–trans (or trans–cis)

---

(14) Das, P. K.; Kogan, G.; Becker, R. S. *Photochem. Photobiol.* **1979**, *30*, 689.

(15) Das, P. K.; Becker, R. S. *J. Am. Chem. Soc.* **1979**, *101*, 6348.

(16) Lukton, D.; Rando, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 258.

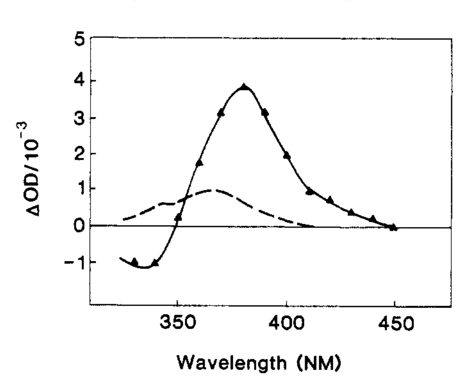


Figure 1. Difference (ΔOD) spectra obtained for the unprotonated 13-cis Schiff base in hexane: (▲) spectrum recorded 10 μs after the 355-nm laser flash; (---) spectrum recorded from a steady-state experiment approximately 5 min after irradiation.

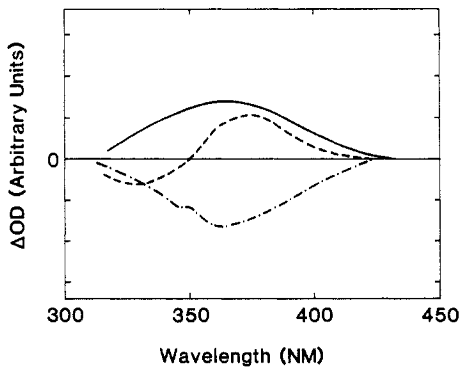


Figure 2. Difference (ΔOD) spectra obtained for the 9-cis (---), 11-cis (—), and all-trans (–•–•–) unprotonated Schiff bases in hexane from steady-state experiments. These spectra were recorded after the complete decay of any transient species; see text.

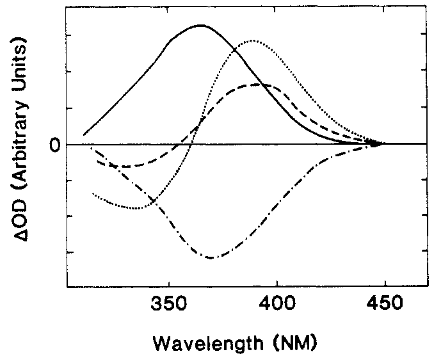


Figure 3. Difference (ΔOD) spectra obtained for the 9-cis (---), 11-cis (—), 13-cis (•••), and all-trans (–•–•–) unprotonated Schiff bases in methanol. These spectra were recorded approximately 1 min after steady-state irradiation.

Table III. Quantum Yields of Isomerization and Photoproducts Detected by HPLC for the Unprotonated Retinal Schiff Bases

isomer	solvent	ϕ	photoproducts (from HPLC)
9-cis	hexane	≤0.01	all-trans
	methanol	0.14 ± 0.02	all-trans
11-cis[a]	hexane	≤0.01	all-trans
	methanol	0.24 ± 0.03	all-trans
13-cis	hexane	≤0.01	all-trans
	methanol	~0.03[b]	all-trans
all-trans	hexane	≤0.01	13-cis
	methanol	0.12 ± 0.02	13-cis, 11-cis, 9-cis, 7-cis (1.9:3.7:1.0:trace)

[a] Quantum yields from ref 12 for the 11-cis isomer have been corrected for the presence of other isomers in the solution prior to irradiation. [b] ΔOD spectrum was not indicative of 13-cis to all-trans isomerization; see Discussion section on the 13-cis isomer.

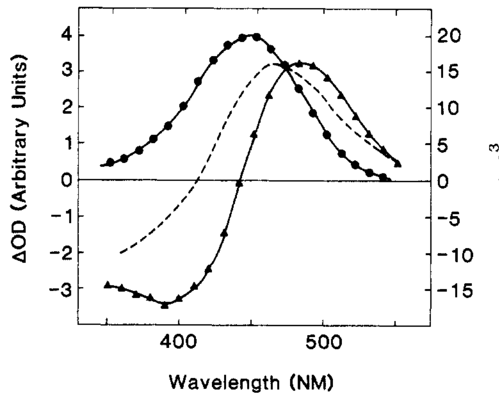


Figure 4. Difference (ΔOD) spectra obtained for the 11-cis (●) and 9-cis (▲) protonated Schiff bases in methanol. These spectra were recorded approximately 1 min after steady-state irradiation. Also shown is the Δε spectrum (---) calculated for a 9-cis to all-trans isomerization.

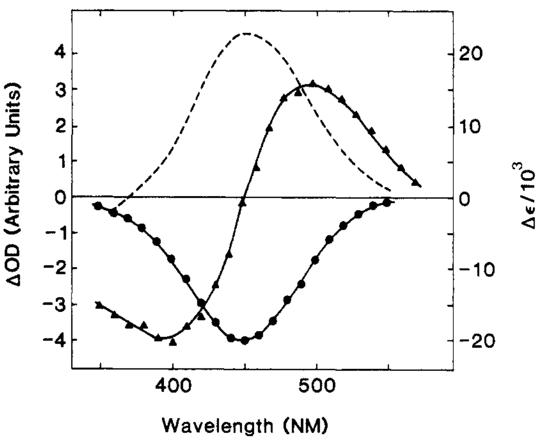


Figure 5. Difference (ΔOD) spectra obtained for the 13-cis (▲) and all-trans (●) protonated Schiff bases in methanol. These spectra were recorded approximately 1 min after steady-state irradiation. Also shown is the Δε spectrum (---) calculated for a 13-cis to all-trans isomerization.

isomerization. The single-exponential decay of this transient back to the original isomer (that was in solution before irradiation) was found to be dependent on solvent polarity. The lifetimes for decay of the transient in hexane were 120 s for the 11-cis SB, 90 s for the 13-cis SB, 450 s for the 9-cis SB, and 720 s for the all-trans SB. In methanol, these decay times were all much faster (≤1 s) and could not be measured accurately with the methods available to us. Final OD spectra, after the complete decay of the transient are shown in Figures 1, 2, and 3. HPLC analysis of the photoproducts after irradiation of these Schiff bases is shown in Table III along with the quantum yields of isomerization.

Laser-flash experiments on the *protonated* 9-cis, 13-cis, and all-trans Schiff bases again revealed no triplet transients in N_2- or O_2-saturated solvents as was the case for the unprotonated Schiff bases. Typical OD spectra obtained for the H^+-9-cis SB, H^+-11-cis SB, H^+-13-cis SB, and H^+-all-trans SB are shown in Figures 4 and 5. In contrast to the unprotonated Schiff bases, these spectra were not observed to undergo any changes over the microsecond to second time domains: i.e., the OD spectra obtained in both the laser-flash and steady-state experiments were the same. The quantum yields of isomerization and HPLC analysis of the photoproducts after irradiation of these protonated Schiff bases are shown in Table IV.

Results from the HPLC experiments confirmed that double-bond isomerization does occur for all the protonated and unprotonated Schiff bases of the 9-*cis*-, 11-*cis*-, 13-*cis*-, and *all-trans*-retinals after irradiation at 355 nm (see tables III and IV). However, ΔOD spectra from laser-flash and steady-state experiments were not always characteristic of cis–trans (or trans–cis) isomerization, based on predictions from calculated Δε spectra. These results varied as a function of solvent, protonation, and the particular isomer studied. Therefore, we will discuss these results one isomer at a time. Henceforth, all reference to difference

Table IV. Quantum Yields of Isomerization and Photoproducts Detected by HPLC for the Protonated Retinal Schiff Bases

isomer	solvent	ϕ	photoproducts (from HPLC)
9-cis	hexane	$\leq 0.05^a$	all-trans
	methanol	$\leq 0.05^a$	all-trans
11-cis	hexane	0.23 ± 0.04^b	all-trans
	methanol	0.25 ± 0.03^b	all-trans
13-cis	hexane	$\leq 0.05^c$	all-trans
	methanol	$\leq 0.05^c$	all-trans
all-trans	hexane	0.14 ± 0.02	13-cis, 11-cis, 9-cis, 7-cis (1.7:6.4:1.0:trace)
	methanol	0.13 ± 0.02	13-cis, 11-cis, 9-cis, 7-cis[d] (1.8:14:3.0:1.0)

[a] Difference spectra did not match $\Delta\epsilon$ spectra calculated for a 9-cis to all-trans isomerization; see Discussion section on the 9-cis isomer. [b] Quantum yields from ref 12 for the 11-cis isomer have been corrected for the isomeric impurity of the samples prior to irradiation. [c] Difference spectra did not match $\Delta\epsilon$ spectra calculated for a 13-cis to all-trans isomerization; see Discussion section on the 13-cis isomer. [d] Absorption data for 7-*cis*-retinal obtained from: DeGrip, W.; Liu, R.; Ramamurthy, V.; Asato, A. *Nature* (*London*) **1976**, *262*, 416.

(ΔOD) spectra will refer to those spectra recorded from *steady-state* experiments after the decay of any transient species. Note that the nominal values for the H^+-11-cis SB (Table IV) are 20–35% greater than we previously reported because of the correction for the presence of other isomers found after synthesis and hydrolysis procedures (Table I) which were not known to us earlier. The same is true for the 11-cis SB in methanol, Table III.

11-Cis Isomer. Spectral results for the *unprotonated* 11-cis SB from our previous study[12] are given briefly here for the purpose of comparison with the results for the other isomers. We found difference (ΔOD) spectra that were measured minutes after irradiation to be characteristic of 11-cis to all-trans isomerization in both polar and nonpolar solvents. We also found that ϕ for isomerization was notably dependent on the solvent (Table III). HPLC results from the present study reconfirmed our earlier observations that the all-trans SB is the only permanent photoproduct formed after irradiation of the 11-cis SB.

Upon *protonation* of the 11-cis SB with TCA, OD spectra characteristic of 11-cis to all-trans isomerization were observed in both hexane and methanol (Figure 4), and HPLC results again confirmed the all-trans isomer as the only photoproduct. In addition, protonation also served to eliminate the effect of solvent on the isomerization quantum yield compared to the 11-cis SB, with an average value of $\phi \sim 0.2$–0.25 seen in several polar and nonpolar solvents including methanol and hexane.

9-Cis Isomer. Difference spectra obtained for the *unprotonated* 9-cis SB in both hexane and methanol paralleled the expected spectra and therefore were indicative of a 9-cis to all-trans isomerization. HPLC analysis confirmed the all-trans isomer as the only photoproduct. As was the case for the 11-cis SB, ϕ was highly dependent on the solvent polarity with $\phi \leq 0.01$ in hexane and $\phi = 0.14$ in methanol. Steady-state experiments on the 9-cis SB in acetonitrile yielded a ΔOD spectrum characteristic of 9-cis to all-trans isomerization, and ϕ had a value of approximately 0.1–0.2. An explanation for this solvent dependence will be offered in the following section.

In contrast to the unprotonated 9-cis SB, ΔOD spectra obtained for the *protonated* 9-cis SB in both hexane and methanol did not match spectra calculated for a 9-cis to all-trans isomerization (see Figure 4). The presence of small amounts (3–5%) of the all-trans and 9,13-dicis isomers in solution could account for small changes in the ΔOD spectrum but not for the significant difference between the observed difference spectrum and the predicted $\Delta\epsilon$ spectrum. No changes were noted in this spectrum over a period of 1 h after irradiation. Analysis of the ΔOD spectrum revealed the formation of a product with an absorption maximum of $\sim$460 nm in methanol and $\sim$470 nm in hexane. This is in contrast to what would be expected for a "normal" H^+-all-trans SB, which was the expected photoproduct and which has an absorption maximum in the vicinity of 440–450 nm (in hexane or methanol) immediately after synthesis. In both solvents, the quantum yield for the formation of this new photoproduct was quite low ($\leq$0.05).

HPLC analysis of the photoproducts of H^+-9-cis SB revealed only two peaks, one corresponding to the 9-cis isomer and the other corresponding to the all-trans isomer. The apparent discrepency between this result and the results from the ΔOD spectra can be explained by the formation of some intermediate species after irradiation. This intermediate could be, for example, some specific conformer of the H^+-all-trans SB, which has different spectral properties from the "normal" H^+-all-trans SB found in solution immediately after synthesis. This intermediate may then be converted to "normal" all-trans during the hydrolysis step and/or after dissolving the retinal(s) in the HPLC solvent (6% ether in hexane) and/or during HPLC analysis. It is also possible that this all-trans-like intermediate has essentially the same retention time as the "normal" all-trans isomer using our HPLC conditions and cannot be distinguished.

These results for the H^+-9-cis SB isomer are in sharp contrast to those obtained for the H^+-11-cis SB. Difference spectra obtained for the H^+-11-cis SB in both hexane and methanol matched calculated $\Delta\epsilon$ spectra expected for the usual all-trans isomer; moreover, the quantum yields of isomerization were significantly larger ($\sim$0.2) than for the H^+-9-cis SB ($\leq$0.05, estimated from the deconvolution technique described earlier).

13-Cis Isomer. The ΔOD spectrum obtained for the *unprotonated* 13-cis SB in hexane minutes after irradiation was indicative of 13-cis to all-trans isomerization (Figure 1). HPLC results confirmed the all-trans isomer as the only photoproduct. The quantum yield in hexane was quite low ($\phi \leq 0.01$) as was also the case for the 11-cis SB and 9-cis SB in the same nonpolar solvent. In contrast to these results in hexane, the 13-cis SB in methanol revealed a ΔOD spectrum (Figure 3) which did *not* parallel the $\Delta\epsilon$ spectrum expected for an 13-cis to all-trans isomerization. Analysis of the spectrum revealed the formation of a product with an absorption maximum at $\sim$380 nm, substantially red-shifted from the maximum expected for the all-trans SB ($\sim$362 nm). A similar spectrum was observed for the 13-cis SB in acetonitrile. The quantum yield for the formation of this species was estimated to be $\sim$0.03 in both methanol and acetonitrile, employing the deconvolution technique described earlier. HPLC analysis of the photoproduct in methanol yielded only one product with a retention time equal to that of the all-trans isomer.

An explanation for these observations may lie in the formation of a different conformer of the all-trans SB in a polar solvent than the conformer formed in hexane. For example, the 13-cis SB could exist as one of two conformers about a single bond as C_{14}–C_{15}. The relative ratio of these conformers in solution may vary in polar and nonpolar solvents as was observed earlier for the 12–13 single-bond conformers of 11-*cis*-retinal.[17] Possibly one of the conformers of the 13-cis SB may be dominant in a polar solvent methanol, whereas the other may be dominant in hexane. Therefore, if the all-trans photoproduct observed after irradiation retains the conformation of the initial species, then different conformers can be formed in different solvents.

It is also possible that the conformer of the all-trans SB formed in methanol is converted to the other conformer during the hydrolysis step and/or after dissolving the retinal mixture in the HPLC solvent and/or during the chromatographic separation. Also, both conformers of the all-trans isomer may well have essentially the same retention times when our HPLC conditions are used and cannot be distinguished.

Nevertheless, *the most important conclusion we can draw from these observations is that a polar solvent* (*for example, methanol*) *does not promote efficient isomerization of the 13-cis SB to the all-trans isomer* (*$\sim$0.03*) *as was the case for the 11-cis SB* (ϕ = 0.23) as well as for the 9-cis SB (ϕ = 0.14).

Results obtained for the *protonated* 13-cis SB were parallel to those for the H^+-9-cis SB. Difference spectra again significantly deviated from the calculated $\Delta\epsilon$ spectra for a 13-cis to all-trans isomerization in hexane and methanol. These spectra were not

---

(17) Birge, R.; Sullivan, M.; Kohler, B. *J. Am. Chem. Soc.* **1976**, *98*, 358.

seen to undergo any changes for up to 1 h after irradiation. The presence of H^+-all-trans SB as an impurity in solution (15%) prior to irradiation (see Table I) could not account for the significant deviation from the ideal 13-cis to all-trans spectrum. Analysis of these spectra revealed a product with an absorption maximum of ~470 nm in methanol and ~480 nm in hexane. This is again in contrast to that expected for "normal" H^+-all-trans SB which has a maximum in the vicinity of 440–450 nm in solution immediately after synthesis. In both solvents, the quantum yield for the formation of this new species was again quite low (≤0.05), as it was for the H^+-9-cis SB.

As was the case for the H^+-9-cis SB, HPLC analysis of the irradiated H^+-13-cis SB identified the photoproduct as having the same retention time as the all-trans isomer. Since the photoproduct detected from the ΔOD spectrum had an absorption maximum significantly shifted from that of "normal" H^+-all-trans SB, we again propose that an intermediate species of the all-trans isomer is formed after irradiation. This intermediate could be a specific single-bond conformer of the H^+-all-trans SB which apparently cannot be distinguished from the "normal" all-trans isomer in HPLC under our conditions or is converted to the "normal" all-trans isomer during the procedures prior to and/or during HPLC.

All-Trans Isomer. The *unprotonated* all-trans SB yielded OD spectra consistent with trans to cis isomerization, and HPLC analysis confirmed the formation of one or more monocis isomers. In hexane, very little isomerization occurred and only the 13-cis isomer was detected. In contrast, in methanol, the 13-cis, 11-cis, 9-cis, and 7-cis isomers were all seen in varying amounts, Table III.

It should be again noted that the quantum yield of isomerization we report for the all-trans isomer is the sum of the yields of formation of the cis isomers. Parallel to the 9-cis SB and the 11-cis SB, the all-trans SB showed very little isomerization in hexane (≤0.01) and significantly more in methanol ($\phi = 0.12$). However, it should also be noted that steady-state experiments on the all-trans SB in acetonitrile showed a quantum yield of only ~0.03, unlike the 9-cis and 11-cis isomers which demonstrated efficient isomerization in acetonitrile ($\phi \sim 0.1$–0.3). On the basis of the foregoing, trans to cis isomerization of the all-trans SB is apparently not dependent on the solvent polarity in the same way as is the cis to trans isomerization of the 9-cis- and 11-cis-unprotonated Schiff bases.

Additional experiments on the all-trans SB in a hexane solution doped with a small amount of hexafluoroisopropyl alcohol (HFIP, 3.6 mM) provided further insight. Previously, we observed the formation of a hydrogen-bonded complex between the all-trans SB and HFIP. The quantum yield of trans to cis isomerization for this hydrogen-bonded complex was estimated to be 0.1–0.2 and was found to be comparable to that for the all-trans SB in methanol (only an estimation for the HFIP complex could be made since the protoproduct ratio could not be determined). These results suggest that efficient isomerization (trans to cis) is facilitated by a hydrogen-bonding environment, as it is for the 11-cis SB.[12]

Protonation of the all-trans SB with TCA served to promote efficient trans to cis isomerization. For the H^+-all-trans SB in hexane or methanol, ΔOD spectra were characterized by negative OD changes, with a minimum in the region of 440–450 nm (Figure 5), suggesting isomerization of the all-trans isomer to a cis isomer(s). HPLC analysis of the photoproducts revealed that in fact the 13-cis, 11-cis, 9-cis, and 7-cis isomers were all formed to some extent. Unlike the *unprotonated* all-trans SB, the quantum yield of isomerization of the *protonated* all-trans SB was relatively independent of solvent, with $\phi = 0.13$–0.14 in methanol and hexane. Although the overall quantum yield of trans to cis isomerization did not vary signficantly with solvent, *there was a significant change in the relative ratios of the cis isomers formed*, Table IV. Large increases in the production of the 9-cis and 11-cis isomers were seen in going from hexane to methanol, Table IV.

Results from a previous study[13] of the H^+-all-trans SB suggested that the relative ratios of the monocis photoproducts varied as a function of solvent and the excitation wavelength. Our results show some agreement with this previous study. At excitation wavelengths of 405 and 436 nm, it was reported that the 13-cis, 11-cis, 9-cis, and 7-cis isomers were all formed in methanol, whereas with 468-nm excitation, only the 13-cis and 9-cis isomers were formed.[13] The quantum yield of trans to cis isomerization in methanol ranged from 0.2 (405 nm) to 0.14 (436 nm) in fair agreement with our yield (0.14) at 355 nm. With 436-nm excitation, others[13] noted that the 11-cis isomer was the predominant photoproduct in methanol as was seen in our study; however, our product ratios disagree to some extent. Besides the fact that we used a different excitation wavelength (355 nm), one possible explanation for the difference between our results and those mentioned above is in the method of protonation. In the previous study,[13] HCl was used to protonate the all-trans Schiff bases, whereas we used TCA.

Others[18] have discussed a mechanism for geometric isomerization that is accompanied by a conformational change of an adjacent bond. They believed the resultant photoproducts would be conformational unstable and entropically; a double-twist motion would not likely be competitive with a single-twist associated with geometric isomerization for conformationally homogeneous polyenes. In our case, whether this idea applies is uncertain since, for example, some of our photoproducts produced by geometric isomerization and believed to involve conformational changes are stable for up to a number of minutes (depending on the solvent).

Conclusions

The electronic states of linear polyenes, retinals, and their Schiff bases are based on those for an idealized all-trans polyene with C_{2h} symmetry. The two lowest lying π,π^* states are the spectroscopically allowed ${}^1B_u^{*+}$ state and the forbidden ${}^1A_g^{*-}$ state, the latter of which is believed to be the lower of the two in long-chain polyenes, and retinal Schiff bases.[12] This state ordering is assumed to exist for all four of the isomers of the retinal Schiff bases studied here. Due to the large changes in the dipole moment and polarizability seen for the 11-*cis*- and *all-trans*-retinals as well as the protonated and unprotonated all-trans SB[19–21] and the dependence of ϕ of the 11-cis Schiff base on solvent,[12] we have discussed the fact that significant mixing can occur between the foregoing two excited states when these systems are in polar environments.[12] Also, spectroscopic studies have suggested that upon protonation of these Schiff bases, the ${}^1B_u^{*-}$ state is significantly mixed into the lowest lying ${}^1A_g^{*-}$ state or is somewhat lower.[12]

Based on the present work and our previous findings[12,22] regarding the essential absence of any observable triplet state and any quenching effect of oxygen on the ϕ values, we assign the originating state for isomerization as a singlet.

In comparing the results for the cis isomers, 9-cis, 11-cis, and 13-cis Schiff base, it is clear that a polar solvent as methanol relative to a nonpolar one as hexane promotes efficient isomerization of the 9-cis and 11-cis Schiff bases but not for the 13-cis SB. As was proposed earlier,[12] it appears that a more polar environment causes an increased mixing of the ${}^1B_u^{*-}$ state into the ${}^1A_g^{*-}$ state which results in the lowering of the activation energy of double-bond isomerization in both the 9-cis and 11-cis systems but apparently not as much for the 13-cis SB, Table III. The reason for this difference for the 13-cis isomer is not clear and further theoretical and experimental investigations into the electronic structure and states of the 9-cis, 11-cis, and 13-cis Schiff bases may prove illuminating.

Of the H^+-9-cis SB, H^+-11-cis SB, and H^+-13-cis SB, only the 11-cis isomer demonstrated efficient isomerization. In the case of the H^+-11-cis SB, we have proposed that protonation served

---

(18) Liu, R. S. H.; Asato, A. E. *Proc. Natl. Acad. Sci.* **1985**, *82*, 259.

(19) Mathies, R.; Stryer, L. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2169.

(20) Ponder, M.; Mathies, R. *J. Phys. Chem.* **1983**, *87*, 5090.

(21) Davidsson, A.; Johansson, L. *J. Phys. Chem.* **1984**, *88*, 1094.

(22) Becker, R. S.; Freedman, K.; Hutchinson, J. A.; Noe, L. *J. Am. Chem. Soc.* **1985**, *107*, 3942.

to mix the ${}^1B_u^{*+}$ and ${}^1A_g^{*+}$ excited states sufficiently (or cause the ${}^1B_u^{*+}$ state to be lowest) so as to lower the activation energy for cis to trans isomerization and permit efficient isomerization in any solvent.[12] In the cases of the 9-cis and 13-cis isomers, protonation again appears to have eliminated a dependence of ϕ on the solvent polarity, but protonation apparently does not lower the activation energy sufficiently to permit significant double isomerization around the respective 9–10 or 13–14 double bonds. It is presumed that protonation would serve to mix the ${}^1B_u^{*-}$ and ${}^1A_g^{*-}$ states significantly, but until more information is gathered on the photophysical properties of the 9-cis and 13-cis protonated Schiff bases, there may be no clear explanation for this difference.

Efficient trans to cis isomerization was seen for the all-trans SB when either protonated or in a hydrogen bonding environment (but not in a nonpolar environment). It is possible that a polar but not hydrogen-bonding environment does assist isomerization to some extent, as evidenced by the increase in ϕ noted going from hexane ($\leq$0.01) to acetonitrile ($\sim$0.03), and also by the relatively larger yield in methanol (0.12) which is significantly polar (but more weakly hydrogen-bonding than HFIP). Even though relatively efficient isomerization is noted for the all-trans SB when protonated or in a hydrogen-bonding environment, it must be emphasized that the 13-cis, 11-cis, 9-cis, and 7-cis isomers are all formed to varying extents in different solvents.

Results from our recent fluorescence lifetime experiments[23] provide evidence that the rate of isomerization in these protonated and unprotonated Schiff bases is very fast. For the 11-cis-, 13-cis-, and all-trans-protonated and -unprotonated Schiff bases in methanol, fluorescence lifetimes of $\sim$30 ps or less have been observed. Recall that we have measured the isomerization lifetime of H^+-11-cis SB to be <8 ps in methanol.[22]

There are some interesting as well as illuminating observations that can be made when comparing the double-bond isomers 11-cis-, 13-cis-, and all-trans-protonated Schiff base data with that of rhodopsin (Rh) and light-adapated (568 nm, all-trans double bonds) bacteriorhodopsin (BR^{LA}). First of all, the quantum yield of formation[3] of bathorhodopsin (BRh, "all-trans") from Rh (11-cis) is 2 times that of the yield of the batho isomer K_{610} from BR^{LA} (all-trans double bonds). By comparison, the yield of the all-trans isomer (BRh analogue) from the H^+-11-cis SB (Rh analogue) is 2 times that of the yield for the formation of the cis isomer mixture (K_{610} analogue) from the H^+-all-trans SB (BR analogue), whether in hexane or methanol. Note the same comparative ratio of ϕ values. It is obvious that we obtain a mixture of double bond cis isomers from the H^+-all-trans SB, whereas it is believed/indicated that only "the 13-cis" bathoisomer (K_{610}) arises from BR^{LA}.[3] If it is indeed true that only one double bond isomerizes to give a "13-cis" isomer for BR^{LA}, then the protein environment is inducing a powerful stereodirecting control (for isomerization). It would perhaps be worthwhile to more closely examine the photoproducts from BR^{LA} to ascertain if *only* the 13-cis BR (K_{610}) is formed. If this is so, then the point on the stereodirecting power of the protein is notable.

On the basis of the H^+-11-cis SB data, where only an all-trans isomer is produced, and also the fact that the chromophore in BRh is all-trans, albeit distorted/twisted, the protein in Rh apparently need not, or does not, exercise such stereodirecting control as it may in BR^{LA}. In addition to the fact that 11-cis to all-trans isomerization occurs in both Rh and the H^+-11-cis SB, other similarities between the pigment Rh and the model system exist. The quantum yield of isomerization for both the H^+-11-cis SB and Rh is significant,[12,24] the rates of isomerization for both are very rapid (<8-ps lifetime),[4,22] and the fluorescence lifetimes for both are short and comparable ($\leq$10–30 ps).[23,25]

In dark-adapted bacteriorhodopsin (BR^{DA}), there is a 1:1 mixture of the protonated all-trans and 13-cis isomers. The 13-cis isomer is characterized by a light cycle different from that of the all-trans isomer and does not result in proton pumping across the cell membrane (see, for example, ref 1, 26). Recall that irradiation of the H^+-13-cis SB isomer (in hexane or methanol) led to a new species that could *not* be identified as the all-trans isomer based on its absorption spectrum (altough with workup and HPLC, a product with the same retention time or all-trans appeared). We believe it is of at least passing interest that these observations correlate with the fact that the *primary* bathoproduct (C_{610}, C^x or K^c) from 13-cis BR^{DA} is also *not* all-trans BR.[1]

Some additional points are worthwhile. It has been proposed in recent work that the chromophore in BR^{LA} (trans double bonds) can only be in a 12-s-cis confirmation,[27] while earlier work proposed a 14-s-trans conformation, and that the K_{610} was 13-cis,14-s-cis.[28] K_{610} shows a large increase (nearly 50%) in its molar absorption coefficient, as well as a red shift, going from 298[29] to 77 K.[30] We[31,32] earlier have shown that a parallel change in the molar absorption coefficient occurs on cooling the 11-cis SB and H^+-11 cis SB and that the latter effect is associated with a change from principally a 12-s-cis conformation at room temperature to a 12-s-trans at low temperature. Based on this and the most recent assignment of 12-s-cis for BR^{LA}, we would propose that the K_{610} is still 12-s-cis ("13-cis") at room temperature. Also, it has been noted that the *photocycle* of BR^{LA} should be associated with a conformational change involving at least one other polyene bond (besides $C_{13}{=}C_{14}$) and that on the basis of other evidence, this should be in the C_9—N section of the polyene.[33] Further, on the basis of the most recent data that the upper limit of the barrier height for K_{610} to L_{550} is only 3.8 kcal/mol, this is well within expectation for a 12-s-cis (K_{610}) to 12-s-trans (L_{550}) change and would be accompanied by a blue shifting of the maximum;[31] however, this does not imply that some other concomitant change could not be occurring as well (such as for the protein).

It has been observed[35] that an electrical signal apparently corresponding to a charge separation occurs with a time constant of 30 ± 10 ps (and the charge separation could actually be faster) which could correlate to the formation (time) of K_{610} or any state preceding formation of K_{610}. We have previously discussed changes, increases, in the pK_a and nitrogen charge density upon excitation of protonated Schiff bases and the fact that upon twisting by 90° in the excited state, the nitrogen charge density decreases.[12] We have also noted the significance of this in terms of the proton coming off during the lifetime of the excited state (or isomerization lifetime) and proton translocation pertinent to the visual process (Rh).[12] We believe this analysis is equally valid for the BR^{LA} system. Based on all of the above considerations, but particularly regarding the quantum yields and lifetimes of isomerization, it appears that (1) the roles previously considered for the proton,[12] (2) the fact that the protein very likely plays a more important role in steps after the primary one,[22] and (3) the fact that the protonated chromophore portion acts as a mediator for proton transfer[12] are all valid for both the Rh and BR^{LA} systems (albeit the protein is possible more stereocontrolling in BR^{LA}).

Finally, our results also have some implications on the rhodopsin and isorhodopsin systems. It is generally believed that rhodopsin (opsin + 11-*cis*-retinal) and isorhodopsin (opsin + 9-*cis*-retinal) form the same bathorhodopsin intermediate (opsin + "nearly" *all-trans*-retinal) after the absorption of light,[36] although there is some disagreement.[37] Our results show a similarity in the

---

(23) Becker, R. S.; Freedman, K., unpublished results.

(24) Dartnall, H. *Vision Res.* **1968**, *8*, 339.

(25) Doukas, A.; Lu, P.; Alfano, R. *Biophys. J.* **1981**, *35*, 547.

(26) Dencher, N. *Photochem. Photobiol.* **1983**, *38*, 753.

(27) Muradin-Syweykowska, M.; Droek, A.; Lugtenburg, J.; van der Bend, R.; van Dijck, P. *Recl.: J. R. Neth. Chem. Soc.* **1983**, *102*, 46.

(28) Schulten, K.; Tavan, P. *Nature (London)* **1978**, *272*, 85.

(29) Goldschmidt, C.; Kalisky, O.; Rosenfield, T.; Ottolenghi, M. *Biophys. J.* **1977**, *17*, 179.

(30) Becher, B.; Tokunaga, F.; Ebrey, T. *Biochemistry* **1978**, *17*, 2293.

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

(32) Waddell, W.; Schaffer, A.; Becker, R. S. *J. Am. Chem. Soc.* **1977**, *99*, 8456.

(33) Umadevi, P.; Sheves, M.; Rosenbach, V.; Ottolenghi, M. *Photochem. Photobiol.* **1983**, *38*, 197.

(34) Mao, B.; *Photochem. Photobiol.* **1981**, *33*, 407.

(35) Groma, G.; Szabo, G.; Varo, Gy. *Nature (London)* **1984**, *308*, 557.

(36) Rudzki, J.; Peters, K. *Biochemistry* **1984**, *23*, 3843.

photochemistry of the *unprotonated* 9-cis and 11-cis Schiff bases, indicating that either a polar or hydrogen-bonding environment must be provided for the chromophore for efficient isomerization. A good deal of experimental evidence suggests, however, that the chromophore in rhodopsin is protonated.[3] Importantly, our results for the *protonated* 9-cis, 11-cis, or 13-cis Schiff bases demonstrate that only the 11-cis isomer efficiently isomerizes, ≥5 times more efficiently than the 9-cis (or 13-cis) isomer. For parallel natural occurring systems, experimental evidence for rhodopsin (11-cis) shows that the quantum yield of conversion to bathorhodopsin (all-trans) is ~7 times greater than of isorhodopsin (9-cis) to bathorhodopsin for vertebrate pigments and 4 times greater for invertebrate pigments.[38] Note that our ratio of 5 times greater for the models is within this latter range of 4–7. Thus, our results provide insight into why the 11-cis isomer is the one naturally found in the visual pigment.

Recent results on poly(ethylene glycol) oligopeptide Schiff bases of 11-*cis*-retinal show a ϕ value as high as 0.37.[39]

Acknowledgment. The laser-flash experiments were performed at the Center for Fast Kinetics Research at the University of Texas at Austin, which is supported by NIH Grant RR-00886, the Biotechnology Branch of Research Resources, and the University of Texas. We thank Hoffman-LaRoche for their gifts of some of the retinal isomers.

---

(37) Spalink, J.; Reynolds, A.; Rentzepis, P.; Sperling, W.; Applebury, M. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 1887.

(38) Suzuki, T.; Callender, R. *Biophys. J.* **1981**, *34*, 261.

(39) Freedman, K.; Becker, R. S.; Hannak, D.; Bayer, E. *Photochem. Photobiol.*, in press.

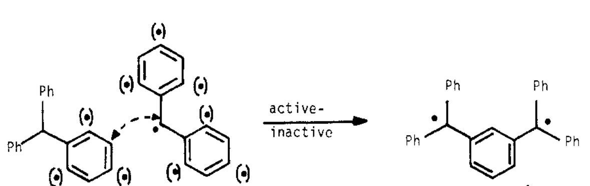
Synthesis of Two Bis-*m*-quinomethanes. An Experimental Study of Connectivity Effects on the Equal-Parity Criterion for Low-Spin Ground States in Alternant Non-Kekulé Molecules

David E. Seeger,[1a] Paul M. Lahti,[1a] Angelo R. Rossi,[1b] and Jerome A. Berson*[1a]

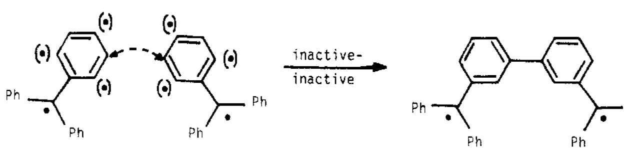
Contribution from the Department of Chemistry, Yale University, New Haven, Connecticut 06511, and the Department of Chemistry, University of Connecticut, Storrs, Connecticut 06268. *Received August 1, 1985*

Abstract: As tests of quantum mechanical theories that predict the spin of the ground state of non-Kekulé molecules, precursors to two such species were synthesized: the "C_{2v}" series, initiated by photolysis of 1*H*,1a*H*,2*H*,4*H*,4a*H*,5*H*,5a*H*,6b*H*-1,5-dimethylenedicycloprop[*a*,*h*]-*s*-indacene-2,4-dione (**13**) to a primary triplet photolysis product, which upon selective further irradiation is converted to 3,6-dimethyleneanthracenediyl-1,8-dioxy (**7**), and the "C_{2h}" series, initiated by photolysis of 1*H*,1a*H*,2*H*,3b*H*,4*H*,4a*H*,5*H*,6b*H*-1,4-dimethylenedicycloprop[*a*,*g*]-*s*-indacene-2,5-dione (**12**), which is converted by a similar two-stage photolysis to 2,6-dimethyleneanthracenediyl-4,8-dioxy (**6**). The photoreactions are monitored by EPR and UV–vis spectroscopy. The "C_{2v}" series secondary product (**7**) is a quintet tetraradical in its ground state, whereas the "C_{2h}" series secondary product (**6**) is most probably a triplet biradical in its ground state. In the latter case, the observed adherence to Hund's rule is unexpected, since the substance is an equal-parity alternant system. Connectivity features of **6** that may restore some of the exchange coupling and perturb the inactive–inactive connectivity of an idealized equal-parity case are discussed.

Hückel[2] was the first to point out that making a union[3a] between inactive[3b] sites of two hydrocarbon radicals could lead to a biradical with a low-spin ground state, in violation of Hund's rule.[4] An illustrative case was provided by the pair of hydrocarbons **1** and **2**, synthesized in 1915 by Schlenk and Brauns.[5] Compound **1** is derived conceptually by an active–inactive union, whereas **2** can be constructed only by an inactive–inactive union. Since the simple HMO π-electron coefficients at inactive sites are zero, the exchange interaction of the two uniting moieties forming **2** is ex-



1



2

---

(1) (a) Yale University. (b) University of Connecticut.

(2) Hückel, E. *Z. Phys. Chem., Abt. B* **1936**, *34*, 339.

(3) (a) See: Dewar, M. J. S. "The Molecular Orbital Theory of Organic Chemistry"; McGraw-Hill: New York, 1969; p 194. (b) An inactive site in an odd alternant hydrocarbon is one at which the NBMO coefficient vanishes (see ref 3a, p 216 and references cited therein).

(4) (a) Although Hund's rule originally was promulgated for atoms,[4b] its application to molecules followed soon afterward.[4c–e] The first two applications to organic biradicals were to **1** and **2**.[4f,g] Given the paucity of related experimental data and the primitive state of molecular quantum theory at the time, the immediate recognition[2] that **1** and **2**, although superficially similar in structure, were likely to be fundamentally disparate in magnetic properties can only be regarded as remarkable. (b) Hund, F. "Linienspektren und periodisches System der Elemente"; Springer-Verlag: Berlin, 1927; p 124ff. (c) Hund, F. *Z. Phys.* **1928**, *51*, 759. (d) Mulliken, R. S. *Phys. Rev.* **1928**, *32*, 186. (e) We thank Professor F. Hund for directing our attention to ref 4c and 4d. (f) Hund, F., as cited in ref 4g. (g) Müller, E.; Bunge, W. *Ber.* **1936**, *69*, 2168. (h) For later relevant application of Hund's rule to π-conjugated biradicals, see inter alia: Longuet-Higgins, H. C. *J. Chem. Phys.* **1950**, *18*, 265.

(5) Schlenk, W.; Brauns, M. *Ber.* **1915**, *48*, 661, 716.

0002-7863/86/1508-1251$01.50/0