Visual Pigments. IV. Experimental and Theoretical Investigations of the Absorption Spectra of Retinal Schiff Bases and Retinals

Arnold M. Schaffer, Walter H. Waddell, and Ralph S. Becker*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77004. Received April 27, 1973

Abstract: The n-butylamine Schiff bases of all-trans-, 9-cis-, 11-cis-, and 13-cis-retinal and the methylamine Schiff base of all-trans-retinal have been prepared from the corresponding retinals. Detailed absorption spectral studies to ~48,000 cm^{-1} at 77°K and room temperature indicate that the absorption maxima and oscillator strengths of retinals and their Schiff bases are very similar. Four absorption bands are observed in the Schiff bases at approximately 28,000, 33,000 to 39,000, 40,000, and 45,000 cm^{-1}. SCF–MO calculations of the Pariser–Parr–Pople type with single-excited configurations are used to determine transition energies and oscillator strengths as a function of the conformation of each isomer. We are able to make certain predictions about the most stable isomer conformation by comparing our calculated and experimental values. For example, we conclude that the 11-cis isomer of retinal and Schiff base exists as an equilibrium mixture of a distorted 12-s-cis conformer (which predominates at room temperature) and a distorted 12-s-trans (which predominates at 77°K). In light of recent evidence that the lowest electronic state of polyenes may not be the strongly allowed ^1B_u, the relationship between the experimental bands and the calculated electronic transitions is carefully reevaluated for both retinals and their Schiff bases.

It has been established that in rhodopsin a Schiff base (SB) linkage exists[1] between the retinal chromophore and the protein opsin via an ε-amino group of lysine.[2,3] Therefore, Schiff bases rather than retinals may represent a more reasonable model for rhodopsin. Unlike retinals, however, the theoretical and spectral properties of the retinal Schiff bases have not been extensively studied. Absorption studies on the Schiff bases of retinal[4–7] have generally only been concerned with the location of the first transition. Except for a brief account of the absorption spectra of the 11-cis-retinal SB,[4] only absorption spectra of the all-trans SB have been reported. Similarly, previous molecular orbital calculations on the Schiff bases[8] have only been concerned with the first transition of the all-trans isomer.

We have previously reported[9,10] on a theoretical and experimental investigation of the absorption and emission properties of the all-trans and the three mono-cis isomers (9-, 11-, 13-) of retinal. In addition, we have recently reported on the determination and interpretation of the fluorescence quantum yields of retinals, their Schiff bases, and all-trans protonated SB.[11] Our present study includes an experimental examination of the absorption maxima and oscillator strengths of all-trans-, 9-cis-, 11-cis-, and 13-cis-n-butylamine Schiff bases and the all-trans-methylamine SB of retinal for all transitions to ~220 nm at both room (298°K) and low (77°K) temperatures. These results are compared with SCF–MO calculations where the effects of parameter and geometry changes are considered.

Experimental Section

all-trans-, 9-cis-, and 13-cis-retinal (Sigma Chemical Co.) and 11-cis-retinal (Hoffmann-La Roche Co.) were stored in the dark under vacuum at 0°. These were not purified prior to use. n-Butylamine was distilled and stored over a 4A molecular sieve at ambient temperatures prior to use. Anhydrous monomethylamine gas (Matheson Gas Co.) was used directly. Preparation of the various imines was similar to that previously reported.[5,11]

Absorption spectra were recorded on a Cary 15 spectrophotometer. All spectra were corrected for background absorption due to solvent and surface reflection of radiation. Unless otherwise indicated, the solvent for all experiments was 3-methylpentane (3MP) which was refluxed and distilled from dri-Na then passed through an 18-in. column of silver nitrate–alumina.[12] Flat-faced 1- and 2-mm cells were used in conjunction with flat-faced liquid nitrogen dewars. All optical quartz was of Suprasil II.

When making a comparison between the calculated and experimental values, the absorption maximum in the gas phase should be used. The results in perfluoro hydrocarbons, which have a low refractive index, come closest to those in the gas phase. We therefore made some room-temperature absorption measurements using perfluorodimethylcyclobutane (E. I. du Pont) as a solvent.

Molar extinction coefficients, ε, were determined using cells calibrated to within ±0.01 mm. Concentrations of the SB in 3MP ranged from 0.73×10^{-4} to 5.24×10^{-4} M. Low-temperature (77°K) ε were calculated including a correction for the concentration change resulting from a 25% solvent contraction.

Oscillator strengths (f numbers) were determined by replotting the spectrum on an abscissa linear in wave number and an ordinate linear in ε, then empirically dividing the spectrum into individual transitions assuming Gaussian band shapes. The f numbers are expected to be accurate to within ±0.05. The experimental oscillator strengths do not include any correction due to the refractive index of the medium.

Theoretical Calculations

All calculations were carried out within the frame-

(1) G. A. Pitt, F. D. Collins, R. A. Morton, and P. Stok, *Biochem. J.*, **59**, 122 (1952).

(2) D. Bownds, *Nature (London)*, **216**, 1178 (1967).

(3) W. J. DeGrip, S. L. Bonting, and F. J. M. Daemen, *Biochem. Biophys. Acta*, **303**, 189 (1973).

(4) J. O. Erickson and P. E. Blatz, *Vision Res.*, **8**, 1367 (1968).

(5) C. S. Irving and P. A. Leermakers, *Photochem. Photobiol.*, **7**, 665 (1968).

(6) M. E. Heyde, D. Gill, R. G. Kilponen, and L. Rimai, *J. Amer. Chem. Soc.*, **93**, 6776 (1971).

(7) W. H. Waddell and R. S. Becker, *J. Amer. Chem. Soc.*, **93**, 3788 (1971).

(8) C. M. Lang, J. Harbour, and A. V. Guzzo, *J. Phys. Chem.*, **75**, 2861 (1971); N. E. Sharpless, J. R. Weisenfield, and E. W. Abrahamson, *Photochem. Photobiol.*, **8**, 487 (1968); H. Suzuki, N. Takizawa, and T. Komatsu, *Progr. Theor. Phys., Suppl.*, **46**, 16 (1970).

(9) Paper I: R. S. Becker, K. Inuzuka, and D. E. Balke, *J. Amer. Chem. Soc.*, **93**, 38 (1971).

(10) Paper II: R. S. Becker, K. Inuzuka, J. King, and D. E. Balke, *J. Amer. Chem. Soc.*, **93**, 43 (1971).

(11) Paper III: W. H. Waddell, A. M. Schaffer, and R. S. Becker, *J. Amer. Chem. Soc.*, **95**, 8223 (1973).

(12) E. C. Murray and R. N. Keller, *J. Org. Chem.*, **34**, 2234 (1969).
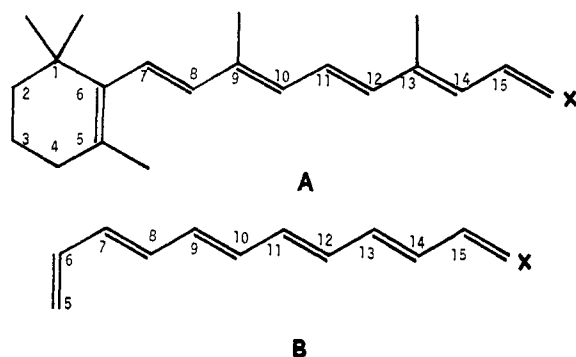
Figure 1. (A) Structure of *all-trans*-retinal (X = O) and its methyl (X = NCH$_3$) and *n*-butyl (X = NCH$_2$CH$_2$CH$_2$CH$_3$) Schiff bases. (B) Model used for theoretical calculations of retinals (X = O) and its Schiff base (X = NH).

Geometry

Recently, the crystal structures of *all-trans*-retinal[21] and 11-*cis*-retinal[22] have been determined. In the *all-trans*-isomer, atoms C$_6$ to oxygen lie in an approximate molecular plane while the C$_6$-C$_7$ single bond is twisted 62° from the planar s-cis configuration (Figure 1). The crystal structure of 11-*cis*-retinal differs from the all-trans not only by a rotation of 180° around the C$_{11}$-C$_{12}$ double bond but by a rotation of ~140° around the C$_{12}$-C$_{13}$ single bond and a rotation of ~40° (compared to 62° in the all-trans) around the C$_6$-C$_7$ single bond.

To study the effect of twisting around single bonds on calculated transition energies, we have considered a variety of conformations (Table I). Although we have

Table I. Torsional Angles[a] of Retinal and Schiff Base Conformations That Are Calculated in Table IV

Con-formation	θ_{6-7}	θ_{10-11}	θ_{12-13}	θ_{14-15}
A	180	0	0	0
B	120	0	0	0
C	140	0	140	0
D	140	0	40	0
E	120	20	0	0
F	120	0	0	20

[a] The s-cis conformation is defined to have a torsional angle of 180° and s-trans to have a torsional angle of 0°.

work of the usual SCF–MO–CI method as previously described,[9] in which all singly excited configurations up to 15 eV were included. Our results were obtained using the following procedure. The ground state energy was successively modified until self-consistency was obtained in the bond order. The usual Pariser formula[13] for the one-center Coulomb repulsion integral was modified using the relationship of Brown and Heffernan.[14] The two-center Coulomb repulsion integrals were calculated from the formula of Nishimoto and Mataga.[15] The core resonance integrals for carbon bonds were determined from a relationship suggested by Koutecky[16] while the corresponding integrals for the C=O and C=N bonds were calculated using Kon's formula.[17] The resonance integral for twisted single bonds, $\beta_{CC}(\theta)$, was calculated from the following equation

$$\beta_{CC}(\theta) = \beta_{CC}°|\cos\theta|$$

where θ is the torsional angle of twisting and $\beta_{CC}°$ is the resonance integral for the planar structure (0° twist).

We also investigated the sensitivity of the results to our chosen parametrization scheme. Calculations were performed without bond order self-consistency by adjusting the resonance integrals of the carbon bonds to match the first observed transition of *all-trans*-retinal. We also examined the effect of using the Pariser–Parr formula for the two-center repulsion integral[18] and Ohno's formula[19] for the core resonance integrals. Furthermore, we examined the applicability of the uniform parametrization scheme for π-electron PPP calculations recently suggested by Beveridge and Hinze.[20] For both retinals and Schiff bases, the maximum calculated shift of any transition is less than 20 nm over a wide range of parameters. More importantly, the relative change in calculated band maxima and *f* number upon going from a retinal to its SB is essentially insensitive to the choice of semiempirical parameters.

previously reported the results of PPP calculations on retinals, calculations were again made on these molecules using geometries more consistent with recent X-ray studies.[21,22] The calculated singlet transition energies and oscillator strengths were obtained using the average $R(C=C)$, $R(C—C)$, and $R(C=O)$ distances of 1.336, 1.460, and 1.200 Å, respectively.[21,22] The $R(C=N)$ bond distance was set at 1.280 Å (*vide infra*). Calculations were also made using the crystal coordinates directly, and the results of these will be discussed in a later section.

Although no X-ray crystal structures of retinal Schiff bases have been published, we expect the geometry of retinals and Schiff bases, except for the C=O and C=N bond distances, to be almost identical. This is supported by preliminary X-ray studies[23] and the fact that resonance enhanced Raman spectra indicate that the stretching frequency of the C=C and C—C bonds are essentially unchanged when a conversion from a retinal to a SB occurs.[6]

Results and Discussion

Before discussing the results of our calculations, we should compare the absorption spectra of retinals[9] with their Schiff bases. The room- and low-temperature absorption spectra of the *n*-butyl Schiff bases of *all-trans*-, 9-*cis*-, 11-*cis*-, and 13-*cis*-retinal are given in Figures 2–5, respectively. Table II presents the experimentally determined band maxima and oscillator strengths of the four isomers of retinal, methyl all-trans SB, and the four isomers of *n*-butyl SB.

(13) R. Pariser, *J. Chem. Phys.*, **21**, 568 (1953).
(14) R. D. Brown and M. L. Heffernan, *Trans. Faraday Soc.*, **54**, 757 (1958).
(15) K. Nishimoto and N. Mataga, *Z. Phys. Chem.*, **13**, 140 (1957).
(16) J. Koutecky, *Z. Phys. Chem. (Frankfurt am Main)*, **52**, 8 (1967).
(17) H. Kon, *Bull. Chem. Soc. Jap.*, **28**, 285 (1955).
(18) R. Pariser and R. G. Parr, *J. Chem. Phys.*, **21**, 466, 767 (1953).
(19) K. Ohno, *Theor. Chim. Acta*, **2**, 219 (1964).
(20) D. L. Beveridge and J. Hinze, *J. Amer. Chem. Soc.*, **93**, 3107 (1971).

(21) T. Hamanaka, T. Mitsui, T. Ashida, and M. Kakudo, *Acta Crystallogr., Sect. B*, **28**, 214 (1972).
(22) R. D. Gilardi, I. L. Karle, and J. Karle, *Acta Crystallogr., Sect. B*, **28**, 2605 (1972).
(23) T. Hamanaka, private communication.

2065

Table II. Experimental Band Maxima and Oscillator Strengths of the Retinal Isomers and Their Schiff Bases in 3MP[a,b]

Retinals[c]		ϵ_{max}[d]	I	II	III	IV
all-trans-	RT	50,000	368.5 (1.24)	260–310 (0.26)	247 (0.16 ?)	*e*
	77°K	54,000	385 (1.14)	260–310 (0.26)	251 (0.04)	~225 (0.01)
9-cis-	RT	39,000	361.5 (1.04)	280 (0.19)	247 (0.11)	~222 (0.04)
	77°K	43,400	379.5 (0.97)	296 (0.36)	~252 (0.10)	~225 (0.07)
11-cis-	RT	26,400	364.5 (0.71)	260–310 (0.21)	255 (0.32)	~224 (0.07)
	77°K	37,300	386 (0.88)	300 (0.30)	254 (0.20)	~225 (0.02)
13-cis-	RT	34,200	364 (0.86)	260–310 (0.13)	251 (0.11)	*e*
	77°K	48,000	380 (1.01)	311 (0.31)	~253 (0.12)	*e*
Schiff bases						
Methyl-*all-trans-*	RT	55,400	353 (1.37)	260–290 (0.15)	247 (0.12 ?)	~220 (0.06)
	77°K	51,200	360 (1.25)	260–290 (0.22)	250 (0.07)	~220 (0.04)
n-Butyl-*all-trans-*	RT	52,100	356 (1.28)	260–290 (0.11)	245 (0.02)	~225 (0.04)
	77°K	49,600	364 (1.13)	260–290 (0.09)	250 (0.02)	~225 (0.02)
n-Butyl-9-*cis-*	RT	47,300	350.5 (1.18)	260–290 (0.13)	250 (0.14)	~220 (0.08)
	77°K	47,100	364.5 (1.05)	265–300 (0.15)	252 (0.11)	~225 (0.05)
n-Butyl-11-*cis-*	RT	33,900	350 (0.81)	260–280 (0.11)	250 (0.31)	~235 (0.11)
	77°K	42,800[f]	367.5 (1.01)	270–290 (0.15)	256 (0.24)	~230 (0.06)
n-Butyl-13-*cis-*	RT	37,700	352 (0.85)	265–280 (0.04)	250 (0.17)	~225 (0.07)
	77°K	35,400	361.5 (0.92)	260–285 (0.07)	257 (0.22)	~230 (0.07)

[a] Numbers in parentheses are experimental oscillator strengths; all wavelengths are in nm for transition maxima of observed bands I, II, III, and IV. [b] The maxima of the first observed band (I) of *all-trans*-retinal, 9-*cis*-retinal, 11-*cis*-retinal, 13-*cis*-retinal, and all-trans SB in perfluorodimethylcyclobutane at 298°K are 354.5, 349, 352, 352, and 346 nm, respectively. [c] Results for retinals differ only slightly from those given in ref 9. [d] Error in the room-temperature extinction coefficient is ~5% while error in the 77°K extinction coefficient is ~10%. [e] Transition not observable. [f] This value for ϵ_{max} is an average of several measurements while Figure 4 gives the results of a single sample.
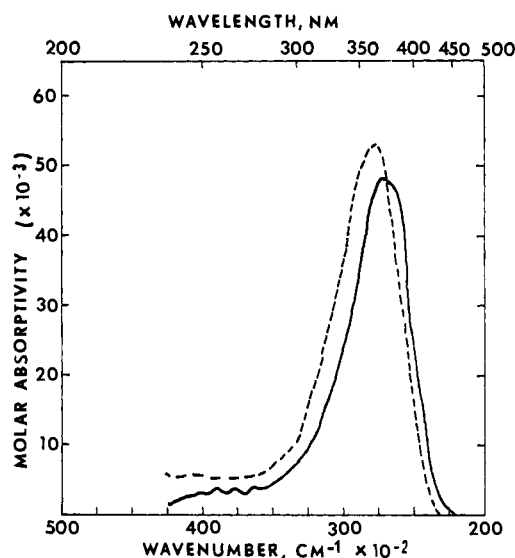
Figure 2. *n*-Butylamine Schiff base of *all-trans*-retinal at 298°K (- - -) and 77°K (—) in 3MP.
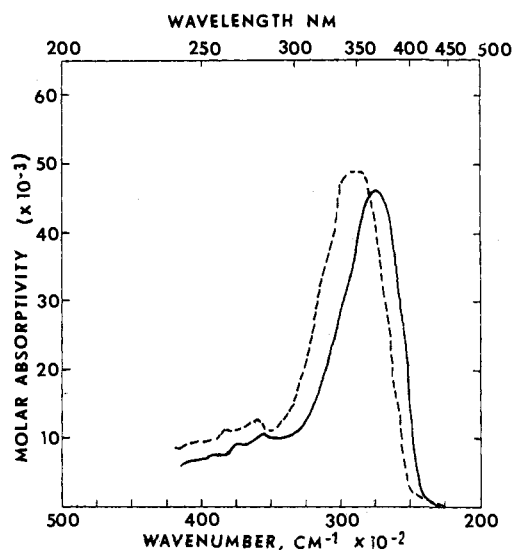
Figure 3. *n*-Butylamine Schiff base of 9-*cis*-retinal at 298°K (- - -) and 77°K (—) in 3MP.

Table III lists the position of the fine structure in the second observed band (II) of retinals and Schiff bases. The band maxima given for the retinals (Table II) differ slightly from that previously reported[9] since our present values represent an average of several more measurements.

In both retinals and Schiff bases a strong diffuse first band (I) is observed with hints of fine structure at low temperature. This band is blue shifted about 20 nm (~1500 cm⁻¹) at 77°K upon going from a retinal to the *n*-butyl SB. A comparable blue shift of the first observed band has been found to also occur in imines prepared from several polyene aldehydes.[24] Except for the 13-cis isomer, this band is slightly more intense in the SB than the corresponding retinal (Table II). In both retinals and Schiff bases (although somewhat

greater for retinals), the first band is red shifted upon going from room to low temperatures. In addition, for both 11-*cis*-retinal and its SB, the oscillator strength of this band (I) increases substantially with a decrease in temperature while the oscillator strength for the three other isomers shows only a small temperature dependence.

The half-band width of the first observed transition in both retinals and Schiff bases is ~5000 to 5500 cm⁻¹. Christensen and Kohler[25] have suggested that the non-bonded interactions between the β-ionylidene ring and the polyene side chain are a sufficient cause for the spectral broadness. However, why fine structure is still observed in the second observed band is not at all clear (see below).

Although there is some uncertainty in relating the observed bands in the 260–300-nm region (Table III)

(24) E. M. Kosower and T. S. Sorensen, *J. Org. Chem.*, **28**, 692 (1963).

(25) R. L. Christensen and B. E. Kohler, *Photochem. Photobiol.*, **18**, 293 (1973).

Schaffer, Waddell, Becker | Retinal Schiff Bases and Retinals

Table III. Experimental Maxima (cm⁻¹) in the Second Observed Band of Retinals and Their Schiff Bases

Compd	Band maxima, cm⁻¹					
all-trans-Retinal	32,050 (sh)	33,500 (sh)	34,900[a]	36,630[a]	38,100 (sh)	39,250 (sh)
all-trans SB	33,170 (sh)	34,540 (sh)	36,400[a]	37,740[a]	38,990	
9-*cis*-Retinal	31,500	32,390	33,780[a]	35,060	36,230 (sh)	37,740
9-*cis* SB	33,330 (sh)	34,500 (?)	35,970[a]	37,380[a]	38,830	
11-*cis*-Retinal	31,750	33,280	34,510	36,200	37,880 (?)	
11-*cis* SB		34,600 (?)	36,170[a]	37,520[a]	38,760[a,b]	
13-*cis*-Retinal	32,150	33,330	34,750[a]	36,520 (sh)		
13-*cis* SB		34,700	36,360[a]	37,590 (sh)		

[a] The band (or bands) with the greatest intensity. [b] This component may result from a merging of third and second observed bands.
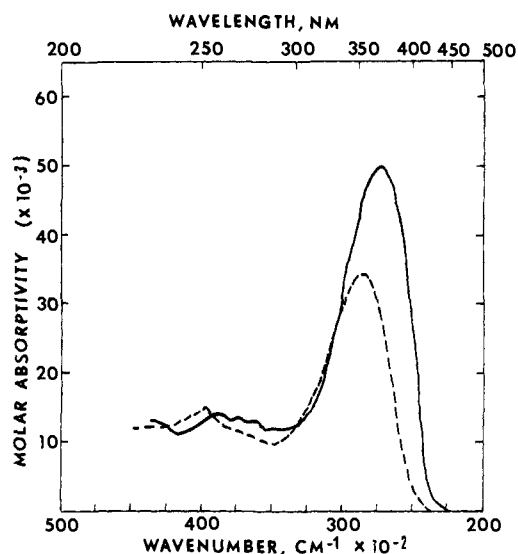

Figure 4. *n*-Butylamine Schiff base of 11-*cis*-retinal at 298°K (- - -) and 77°K (—) in 3MP.
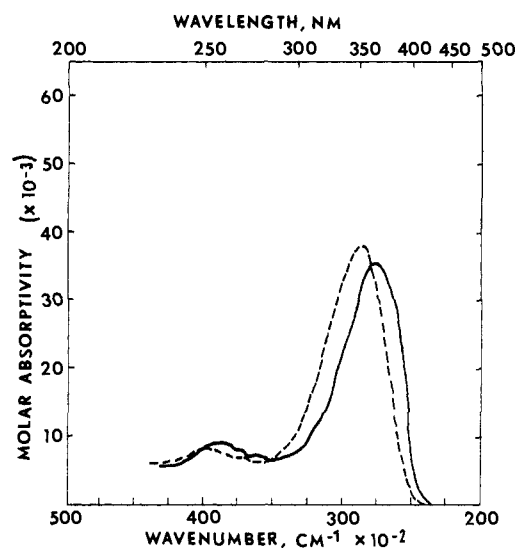

Figure 5. *n*-Butylamine Schiff base of 13-*cis*-retinal at 298°K (- - -) and 77°K (—) in 3MP.

to our calculated transition energies (*vide infra*), we do assign these bands as being vibrational components of a particular electronic transition. The position and intensity of these vibrational components do vary among the isomers (they are most clearly observed in 9-*cis*-retinal at 77°K) and upon going from a retinal to its SB (the retinal bands are red shifted and more intense than those of its SB). It should be noted that fine structure also appears in the second observed band of *all-trans*- and 13-*cis*-retinol.[26]

In the low-temperature spectra of *all-trans*-retinal and 11-*cis*-retinal in EPA, Sperling[27,28] also observed distinct fine structure in this region. He found an interband distance of 1450 cm⁻¹ between the bands in the 11-cis isomer and an interband distance of approximately 1500 cm⁻¹ in the all-trans isomer. Although the uncertainty in our measurements of the interband distance may be fairly large (∼150 cm⁻¹), instead of a constant energy separation, we find it to vary from 1200 to 1700 cm⁻¹. A comparable range was also found when we studied *all-trans*-retinal in EPA at 77°K. Although the interband separation of 1200 to 1700 cm⁻¹ indicates the possibility that carbon–carbon stretching vibrations are involved,[29] the nonconstant

separation indicates that we are not observing a simple vibrational sequence.

The third observed band at ∼250 nm is only slightly shifted from its position in the corresponding retinal. The relative intensities (11-cis > 13-cis > 9-cis > all-trans) are also the same as in the retinals (Table II). Lowering the temperature decreases the intensity of this band in 11-*cis*-retinal and 11-cis SB but has little effect on the same band of the other isomers.

There is only a slight difference between the absorption spectra of the *n*-butylamine and methylamine all-trans Schiff bases (Table II). This is not a surprising result since the position of the first transition of retinal Schiff bases is fairly insensitive to the substituent (except for conjugated rings) attached to the imine nitrogen.[4,5,30] Calculations within the framework of the PPP method also predict that SB spectra should be fairly insensitive (±3 nm) to the nature of the alkyl substituents.[31]

Table IV presents the calculated singlet transition energies and oscillator strengths for some representative conformers of the four isomers of retinals and the corresponding Schiff bases. We will use the results of our MO calculations to aid us in assigning the observed absorption bands and to supply, where possible, new information about the structure of these

(26) T. Yamaoka and R. S. Becker, unpublished.

(27) W. S. Sperling and C. N. Rafferty, *Nature (London)*, **224**, 591 (1969).

(28) W. S. Sperling in "Biochemistry and Physiology of Visual Pigments," H. Langer, Ed., Springer-Verlag, New York, N. Y., 1973, p 19.

(29) R. M. Gavin, Jr., and S. A. Rice, *J. Chem. Phys.*, **55**, 2675 (1971).

(30) P. A. Plack and D. J. Pritchard, *Biochem. J.*, **115**, 927 (1969).

(31) The effect of alkyl groups was determined using both the inductive and conjugative models. In the inductive model the assumed valence state ionization potential of nitrogen was changed from 14.12 to 12.64 eV, while for the conjugative model we used the method outlined by T. Morita, *Bull. Chem. Soc. Jap.*, **33**, 1486 (1960).

Table IV. Calculated Band Maxima and Oscillator Strengths of the Retinal Isomers and Their Schiff Bases[a,b]

Isomer	Con-formation[c]	I		II		III		IV	
		Retinal	SB	Retinal	SB	Retinal	SB	Retinal	SB
All-trans	A	375 (1.88)	366 (1.97)	280 (0.08)	278 (0.05)	265 (0.08)	260 (0.09)	230 (0.05)	231 (0.03)
	B	351 (1.85)	340 (1.95)	263 (0.13)	262 (0.04)	251 (0.01)	245 (0.02)	222 (0.01)	223 (0.01)
9-Cis	B	355 (1.60)	343 (1.69)	267 (0.13)	266 (0.04)	253 (0.22)	246 (0.26)	225 (0.03)	225 (0.02)
	E	348 (1.52)	337 (1.60)	264 (0.15)	264 (0.04)	251 (0.24)	245 (0.27)	223 (0.02)	223 (0.02)
11-Cis	B	356 (1.54)	344 (1.67)	268 (0.17)	267 (0.05)	251 (0.27)	245 (0.28)	224 (0.02)	225 (0.02)
	C	359 (0.66)	344 (0.78)	278 (0.24)	273 (0.17)	258 (0.46)	255 (0.61)	229 (0.10)	231 (0.04)
	D	346 (1.28)	336 (1.42)	271 (0.28)	265 (0.09)	252 (0.22)	250 (0.28)	227 (0.08)	228 (0.03)
13-Cis	B	353 (1.68)	342 (1.83)	265 (0.17)	263 (0.05)	253 (0.09)	246 (0.08)	224 (0.02)	224 (0.01)
	F	350 (1.67)	339 (1.81)	264 (0.17)	262 (0.05)	252 (0.08)	246 (0.08)	223 (0.01)	224 (0.01)

[a] Method of calculation given in text. [b] Numbers in parentheses are calculated oscillator strengths; calculated transition energies given in nm for electronic transitions I to IV. [c] Geometry of each conformation is given in Table I.

Table V. Dependence of Calculated Transition Energies and Oscillator Strengths of 11-*cis*-Retinals and 11-*Cis* SB on Rotation Around C_{12}–C_{13} Single Bond[a,b]

$\theta(12$–$13)$[c]	I		II		III	
	Retinal	SB	Retinal	SB	Retinal	SB
30	352 (1.39)	340 (1.52)	273 (0.22)	269 (0.07)	256 (0.28)	252 (0.33)
40	346 (1.28)	336 (1.42)	271 (0.28)	265 (0.09)	252 (0.22)	250 (0.28)
50	336 (1.18)	327 (1.35)	272 (0.32)	263 (0.11)	248 (0.16)	250 (0.19)
130	346 (0.67)	332 (0.84)	278 (0.32)	270 (0.25)	253 (0.37)	252 (0.42)
140	359 (0.66)	344 (0.78)	278 (0.24)	273 (0.17)	258 (0.46)	255 (0.61)
150	370 (0.66)	355 (0.75)	281 (0.22)	278 (0.10)	262 (0.65)	258 (0.80)

[a] Calculated transition energies given in nm for electronic transitions I, II, and III while calculated oscillator strengths given in parentheses. [b] Method of calculation given in text. [c] Geometry same as conformation C (Table I) except for rotation around the 12–13 bond.

compounds. We will then evaluate the validity of our conclusions in light of recent experimental and theoretical information about polyenes.

If we assume the all-trans isomer to have approximately C_{2h} symmetry, the calculated order of excited states I to IV is $1\,^1B_u < {}^1A_g^- < {}^1A_g^+ < 2\,^1B_u$. Although $1\,^1B_u$ may not be the lowest singlet (*vide infra*), it is clear that the strong first observed band in these compounds is associated with this state. There is good agreement between its experimental and calculated values, especially when the results obtained with perfluorodimethylcyclobutane as solvent are considered. The calculations predict that the intensity of the first transition should be greater in the SB than in its retinal. This prediction is confirmed experimentally except for the 13-cis isomer; however, steric interaction between the *n*-butyl group and the polyene chain may change the geometry of the 13-cis SB relative to the retinal. Our calculations predict the second observed band to be associated with $^1A_g^-$ and correctly indicate this band to be more intense in retinals. Our calculations indicate that the third observed band is derived from $^1A_g^+$, which becomes formally allowed in cis polyenes. The relative intensity of this band in retinals and Schiff bases (11-cis > 13-cis > 9-cis > all-trans) is sensitive to the position of the cis linkage, although we predict the intensity of 9-cis to be greater than 13-cis.

All calculations listed in Tables IV and V were made with a C=N bond distance of 1.28 Å. We find that as the C=N bond distance is decreased from 1.32 to 1.28 Å, the calculated blue shift of the first transition upon going from retinal to the SB approaches the experimental value. This result is independent of both the geometry of the carbon skeleton and the choice of semiempirical parameters. In a preliminary investigation of the crystal structure of *n*-butyl-*all-trans*-

retinal SB, Hamanaka[23] found a C=N bond distance of 1.272 ± 0.007 Å.

Calculations on *all-trans*-retinal and 11-*cis*-retinal with a geometry directly derived from X-ray crystal data[21,22] lead to very similar values for the transition energies. Moreover, such a geometry leads to calculated values of the oscillator strength of $1\,^1B_u$ (1.24 for *all-trans*-retinal and 0.80 for 11-*cis*-retinal) closer to the experimental values of Table II.

The experimental absorption maximum for the first observed band of the 9-*cis*- and 13-*cis*-retinals and Schiff bases is blue shifted relative to the all-trans compounds. Calculations using conformation B (Table I), which is planar except for a rotation of 60° around the 6–7 bond, give a value of $1\,^1B_u$ to the red of all-trans. In this conformation for 9-cis and 13-cis, the H–H distance is 1.80 Å, which is less than twice the van der Waals radius of hydrogen.[32] Relief of this H–H interaction can occur by 10–11 single-bond twisting in 9-cis (conformation E) and 14–15 single-bond twisting in 13-cis (conformation F). In both cases, a 20° single-bond rotation leads to a value of $1\,^1B_u$ more consistent with the experimental results.

The absorption spectra for retinals and their Schiff bases show a quite large temperature dependence.[33] This dependence is especially marked for the 11-cis isomer where a large change occurs in the oscillator strength as well as the location of the maximum of the first absorption band. Wald[34] suggested that the temperature dependence of the spectrum of 11-*cis*-

(32) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 260.
(33) That part of the shift and change in oscillator strength that may be ascribed to a pure solvent effect and that part which may be ascribed to temperature dependent geometry changes is discussed in greater detail elsewhere: A. M. Schaffer, W. H. Waddell, and R. S. Becker, to be submitted for publication.
(34) G. Wald, *Nature* (*London*), **184**, 620 (1959).

retinal reflected the increasing planarity of the molecule as the temperature decreased. However, recent calculations[35,36] and recent temperature dependent proton nmr studies[36] indicate that 11-*cis*-retinal consists of an equilibrium mixture of two conformers which differ only by rotation around the 12–13 single bond. One conformer has a distorted s-cis configuration (the structure found in the crystal[22]) while the other has a distorted s-trans configuration. Our experimental and theoretical results supply a strong foundation for this interpretation. From 298 to 77°K the oscillator strength of the first band in 11-*cis*-retinal and its SB increases while the oscillator strength of the third observed band (the cis peak) decreases. Our calculations on the 11-cis isomer (Table V) indicate that this result can be explained if at room temperature a distorted s-cis conformer (130 to 150°) predominates while at low temperature a distorted s-trans conformer (30 to 50°) predominates. Although there is some dependence of the specific values of the calculated transition energies and oscillator strengths on the choice of parameters, all parameter sets lead to the same explanation for the temperature dependence of the absorption of the 11-cis isomers. A more detailed description of this subject is given elsewhere.[33]

Calculations on polyenes (C_{2h} symmetry) that only include single-excited configuration interaction (SECI) give the three lowest singlets in the order $^1B_u < {}^1A_g^+ < {}^1A_g^-$. Until very recently, the first ^1B_u state, which gives rise to the strong first band in polyenes, was considered also to be the lowest electronic state.[37] However, detailed spectra studies on several polyenes[25,38–40] indicate a weak transition, which can be assigned as $^1A_g^-$, to the red of the strongly allowed ^1B_u. In support of these experimental studies, Schulten and Karplus[41] have recently investigated the effect of including double-excited configuration interaction (DECI), as well as SECI, in PPP calculations on butadiene, hexatriene, and octatetraene. They find the effect of including DECI to be relatively small on the state energies of ^1B_u and $^1A_g^+$; however, $^1A_g^-$ is sufficiently lowered so that it appears slightly below ^1B_u.

Including only single-excited configurations, the calculated state ordering for the retinals and Schiff bases is $^1B_u < {}^1A_g^- < {}^1A_g^+$. (The C_{2h} symmetry labels

(35) B. Honig and M. Karplus, *Nature (London)*, **229**, 558 (1971).

(36) R. Rowan, III, A. Warshel, B. D. Sykes, and M. Karplus, *Biochemistry*, in press.

(37) See, for example, J. N. Murrell, "The Theory of the Electronic Spectra of Conjugated Molecules," Wiley, New York, N. Y., 1963, Chapter 5.

(38) B. S. Hudson and B. E. Kohler, *Chem. Phys. Lett.*, **14**, 299 (1972).

(39) B. S. Hudson and B. E. Kohler, *J. Chem. Phys.*, **59**, 4984 (1973).

(40) It should be noted that since the polarization of the fluorescent state is nearly parallel to that of ^1B_u for retinol, diphenyloctatetraene, and *all-trans*-retinal, T. A. Moore and P. S. Song, *Chem. Phys. Lett.*, **19**, 128 (1973); *Nature (London)*, *New Biol.*, **243**, 30 (1973), conclude that the lowest excited state is ^1B_u. B. S. Hudson and B. E. Kohler, *Chem. Phys. Lett.*, **23**, 139 (1973), while agreeing with the experimental results, disagree with this interpretation.

(41) K. Schulten and M. Karplus, *Chem. Phys. Lett.*, **14**, 305 (1972).

for these molecules are now only approximate.) The inclusion of partial DECI as well as complete SECI in a PPP calculation on *all-trans*-retinal[10] has a small effect on ^1B_u and $^2A_g^+$, but it does lower $^1A_g^-$ to 0.2 eV above ^1B_u. The accuracy of all of these semiempirical calculations is such that even with DECI it is impossible to unambiguously determine which state is lower in each of the great number of polyenes and substituted polyenes (such as retinals and Schiff bases). However, from the experimental and theoretical evidence it seems clear that $^1A_g^-$ and ^1B_u are fairly close in energy, and we must therefore reevaluate the relationship between the experimental bands and the calculated electronic transitions.

Although there is no ambiguity about the nature of the first strong band in retinals and Schiff bases and little ambiguity about the assignment of the third observed band, our assignment of the second band may be incorrect (*vide supra*). Sperling and Rafferty[27] find absorption in this region (270–320 nm) to have a polarization different from that of the main maximum (I). This experimental evidence plus our calculations indicate that specific assignments of this band may not be possible although it is probably derived from an electronic transition other than the first $^1A_g^+$ or the first $^1A_g^-$. Schulten and Karplus[41] found that including DECI in PPP calculations on octatetraene did lead to the appearance of a second ^1B_u state below $^1A_g^+$.

Summary

(1) Except for the first two bands of the Schiff bases being blue shifted 1000 to 1500 cm^{-1}, the absorption spectra of the four isomeric Schiff bases are very similar to the corresponding retinals.

(2) SCF–MO calculations on retinals and Schiff bases with single CI are able to adequately describe the position and intensity of the first and third observed bands, although single CI calculations may be unable to characterize the second observed band.

(3) The calculations predict that the 9-*cis*- and 13-*cis*-retinals and Schiff bases are rotated $\sim 20°$ around their 10–11 and 14–15 single bonds, respectively.

(4) The 11-cis isomer very likely contains an equilibrium mixture of two conformers which differ only by their rotation around the 12–13 single bond. The experimental and theoretical results for both 11-*cis*-retinal and SB indicate that a distorted 12-s-cis (130–150°) conformer predominates at 298°K while a distorted 12-s-trans conformer (30–50°) predominates at 77°K.