

Figure 4. Calculated (see note 10) charges at C-14 for 11-*cis*-retinal (7), its Schiff base 8, the protonated Schiff base 9, and rhodopsin model 10.

chromophores as well as rhodopsin requires a model such as the one that we have proposed above. The model as shown was developed in order to account for the wavelength shifts in bovine rhodopsin. Wavelength shifts in other rod and cone visual pigments could be produced from the positioning of one or more external charges in other orientations with respect to the chromophore (see also note 9).

Figure 4 provides an explanation of the C-14 ¹³C NMR chemical shift reported by Shriver et al.¹⁶ They found that the ¹³C NMR peaks of C-14 in 11-*cis*-retinal and its propylamine Schiff base were around 130 ppm, whereas in the protonated Schiff base it is upfield shifted to 120.14 ppm. Since the ¹³C NMR peak in rhodopsin generated from [14-¹³C]retinal is at 130.8 ppm, it was concluded that the Schiff base in rhodopsin is unprotonated, a result which is in conflict with the resonance Raman data.¹⁷ This apparent discrepancy can be resolved since a negative charge near C-14 would be expected to reduce the π -electron density at this position through coulombic repulsion and, as a result, cause a large deshielding effect. To estimate the magnitude of the effect we calculated π -electron charge densities at C-14 for 11-*cis*-retinal, its Schiff base, the protonated Schiff base, and the rhodopsin model shown in Figure 3. As is clear from Figure 4, the effect of an external charge near C-14 (as in 10) is to reduce the charge density at this position from that of the protonated Schiff base 9. Thus, taking into consideration the Raman evidence which favors protonation, the important NMR study of Shriver et al.¹⁶ provides further support for positioning an external charge close to that shown in Figure 1.¹⁸

A further conclusion is suggested from our model. Rhodopsin, which has an 11-*cis* chromophore, is red shifted with respect to isorhodopsin (λ_{\max} 485 nm), which has a 9-*cis* chromophore, and the artificial pigment (λ_{\max} 450 nm) formed from 7-*cis*-retinal.¹⁹ If we assume that the ring is approximately fixed in the same position in each pigment, then the presence of a *cis* linkage close to the ring will move the polyene chain from the immediate vicinity of the external charges. The effect will be particularly strong for the 7-*cis* isomer and weaker in isorhodopsin, thus accounting for the respective shifts to shorter wavelengths.

We have assumed through this paper that external charges positioned around the chromophore are responsible for wavelength regulation in visual pigments. That such effects are possible and of the proper magnitude is supported by absorption data of simple synthetic models.²⁰ We note that an alternative model²¹ for the wavelength shift in rhodopsin involving twisting about the 11-ene is not supported by the 11,12-dihydrorhodopsin data since large red shifts are encountered in the absence of this double bond.

Finally, we emphasize that the presence of a charged group so close to the 11,12 double bond is likely to have a strong effect on the torsional potential for *cis*-*trans* isomerization at this position. The high quantum yield (0.67) for photoisomerization of rhodopsin relative to protonated Schiff bases in solution²² (0.05-0.2) may be due in part to the same electrostatic interactions that we have shown here to account for the absorption maximum of this pigment.²³

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- (10) In contrast, in bacteriorhodopsin the largest shifts are obtained for the full chromophore rather than for the dihydropyridone chromophores. These results suggest the existence of a negatively charged group in the vicinity of the β -ionone ring in that pigment (unpublished work).
- (11) See ref 6 for a detailed description of the calculations: $\beta_{ij} = \{\beta_{ij}^0 + \delta(U_i + U_j - 21.24)\exp[-\epsilon(r_{ij} - 1.397)]\}$. A distance dependent dielectric constant given by $r - 2$ was used in the calculation of the coulombic interaction with the external charge Q_k . This function is similar to that normally used in calculations on proteins: cf. A. Hopfinger, "Conformational Properties of Macromolecules", Academic Press, New York, 1973, pp 59-63. The magnitude of the shifts induced by the external charges in various positions was found to be independent of the parameterization scheme employed. CNDO/S calculations involving extensive configuration interaction were also carried out (unpublished results) but had no significant effect on the results given in Figure 2.
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- (23) The studies were supported by National Institutes of Health Grant EY 01253 and a grant by the Kultusministerium des Landes Niedersachsen for a Jerusalem-Göttingen exchange program.

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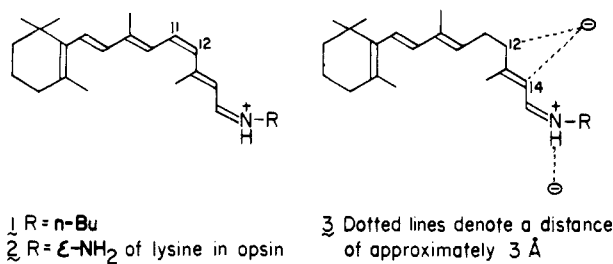
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Through-Space Electrostatic Effects in Electronic Spectra. Experimental Evidence for the External Point-Charge Model of Visual Pigments

Sir:

The protonated Schiff base (SBH⁺) formed between *n*-butylamine and 11-*cis*-retinal (1) absorbs at 440 nm in methanol;¹ however, in cattle rhodopsin, in which the retinal moiety is linked to the terminal amino function of a lysine residue 2, the λ_{\max} is shifted to 500 nm.² This red shift upon

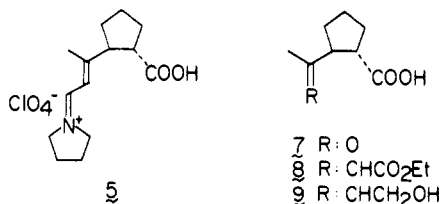


binding with the apoprotein is encountered in all other model retinals, including 11,12-dihydroretinal (SBH⁺, 275 nm; pigment, 315 nm^{3,4}) and the series of other dihydroretinals.⁵ An understanding of the mechanism through which the protein regulates the λ_{\max} of the chromophore is one of the most intriguing and important problems in vision chemistry. The effect of the protein obviously varies from pigment to pigment.⁶ For example, despite the fact that the three rhodopsins contained in human cone cells absorb at 440, 535, and 575 nm,² and thus constitute the basis for our color recognition, the chromophore is the same 11-*cis*-retinal SBH⁺ (2) for all three pigments.

On the basis of the data on dihydrorhodopsins, in particular those for the 11,12-dihydro pigment, we have proposed an external point-charge model.⁵ According to this model 3, a counteranion is placed 3 Å apart from the N⁺ of the protonated Schiff base; to account for the experimental value of 315 nm,^{3,4} it was necessary to place a second point charge ~ 3 Å from C-12 and C-14 (see 3). This model accounts for the λ_{\max} of other dihydro pigments and of bovine rhodopsin itself. In this communication we present experimental evidence which supports the external point-charge model.

The models we chose to synthesize were those related to 11,12-dihydroretinal (3); unlike longer systems, this short enal-derived chromophore has the least conformational ambiguity. The model should have a quaternary nitrogen and a second counteranion within the same molecule at a suitable distance. Furthermore, the ammonium nitrogen should be nonprotonated to prevent its deprotonation upon formation of the negative charge. Molecules such as 4 (Scheme I) and 5 would serve as models to check whether creation of a second negative charge, e.g., 6 in Scheme I, would indeed induce a red shift in the absorption maximum. Molecular models show that the distance between the center of the C=C bond and the closest of the two oxygens in the carboxylate group of the trans-substituted cyclopentane (see 6)⁷ is ~ 3.2 Å in both the syn (4) and anti (5) compounds.

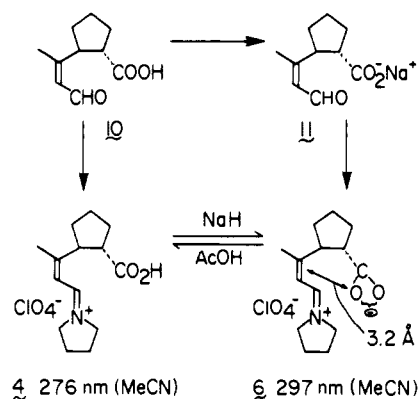
Reaction of diethyl phosphonoacetate with *trans*-1-acetyl-2-carboxycyclopentane (7)⁸ gave a mixture of syn and anti esters 8 in a ratio of $\sim 7:3$ (by ¹H NMR). Reaction of 8 with Dibal gave alcohols 9 which were separated by flash chroma-



tography;⁹ MnO₂ oxidation of the separated alcohols yielded the corresponding syn and anti aldehydes. The syn aldehyde 10 (Scheme I) was condensed with pyrrolidine perchlorate in ethanol¹⁰ to give, after solvent evaporation and trituration with ether and hexane, the carboxyl Schiff base 4 as a colorless oil.¹¹

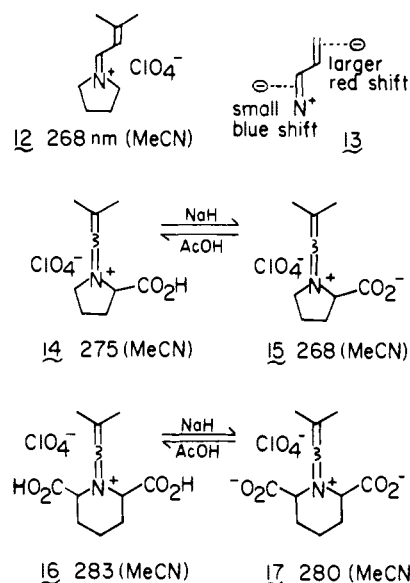
Deprotonation of the acid group was achieved by adding excess NaH to a solution of 4 in dry CH₃CN.¹² As shown in Scheme I, a 21-nm red shift from 276 (4) to 297 nm (6) was caused by the nonconjugated carboxylate. The shift was reversible and, by addition of CH₃COOH,¹³ the UV shifted back

Scheme I. Formation of Acidic and Basic Species of 4



to 276 nm. The *anti*-carboxyl Schiff base 5, prepared likewise from the anti alcohol 9, also gave a similar shift of 21 nm (from 276 to 297 nm). To ascertain that we were dealing with the correct species, aldehyde 10 was converted into carboxylate 6 by an alternate route (Scheme I). Thus, a solution of 10 in dry CH₃CN¹⁴ was treated with excess NaH suspended in CH₃CN to give 11. Filtration of NaH and removal of solvent gave an oil, which, after trituration with ether, was condensed with pyrrolidine perchlorate in ethanol to yield the carboxylate Schiff base identical with 6 prepared previously.¹⁵

Further data to support the through-space carboxylate effect was secured from the data on the enal Schiff base 12 which was prepared from acetone by a route similar to that for compounds 4 and 5. The λ_{\max} of this colorless oil at 268 nm (in CH₃CN) did not shift upon basification or acidification. It should be noted that, in contrast to the 268-nm peak of 12, the carboxyl



Schiff base 4 absorbs at 276 nm. This difference of 8 nm is caused by the presence of the carboxyl group, which presumably acts as an electron source.

The location of the external negative charge is crucial for determining the absorption maxima in visual pigments as well as in the model retinals. According to calculations a negative charge causes a small blue shift and larger red shift, respectively, when it is located ~ 3 Å from the C=N⁺ bond and C=C bond (see 13). To test this theory we synthesized compounds 14 (as a mixture of syn and anti) and 16 by condensation of 3,3-dimethylacrolein with the perchlorate salts of proline and piperidine-2,6-dicarboxylic acid, respectively.¹⁵ In these compounds the negatively charged carboxylate is located near the C=N⁺, and as expected a small *blue shift* is induced by addition of NaH.

The results described above provide experimental support for the external point-charge model which accounts for the bathochromic shifts of the dihydorhodopsins as well as natural rhodopsins. We have shown that diene models produce wavelength shifts of comparable magnitude (268 nm for **12** vs. 297 nm for **6**) with that seen upon formation of 11,12-dihydrorhodopsin (275 to 315 nm; see above). Moreover, when measured in reciprocal centimeters, the shifts that we have obtained, e.g., for the models **12** vs. **6** (3600 cm^{-1}), are even larger than the shifts between the SBH⁺ of 11-*cis*-retinal (440 nm) and rhodopsin (500 nm), i.e., 2700 cm^{-1} . Models **14–17** also show that the shifts induced by external negative charges are, in agreement with theoretical calculations, sensitive to their locations relative to the conjugated system. Most probably it is this spatial distribution of charges relative to the retinal SBH⁺ moiety that leads to the variation in λ_{max} of the various pigments and of the intermediates formed during the bleaching process.¹⁶

References and Notes

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- The compounds were characterized by IR and ¹H NMR data.
- Owing to the instability of the salt prepared in this manner, it was not possible to obtain the ¹H NMR and IR data.
- The carboxylate **6** could not be acidified with HCl because the acid degraded it into UV transparent species.
- Acetonitrile was the solvent of choice because it dissolved both **10** and **11** but not NaH.
- Despite the unstable properties, the salts thus prepared were sufficiently stable for ¹H NMR (CD₃CN) and IR (neat) measurements.
- Supported by National Institutes of Health Grant EY 01253.
- Supported by Dr. Chaim Weizmann postdoctoral fellowships for scientific research.

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Received May 11, 1979

Anti, Longitudinal Conformational Isomerism in Metacyclophanes

Sir:

The stereochemical aspects of cyclophanes have been of particular synthetic and theoretical interest for the past two

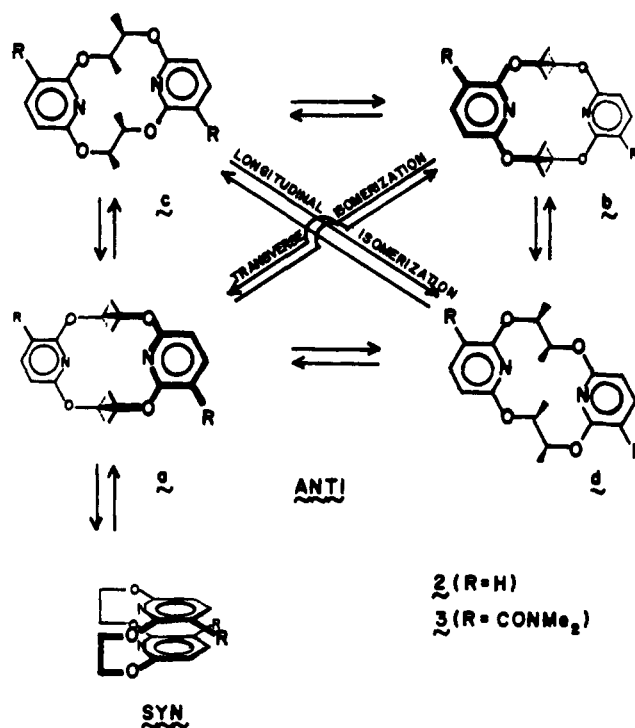
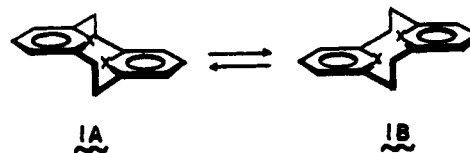


Figure 1.

decades.¹ The molecular geometry of [2.2]metacyclophanes has been exhaustively analyzed and summarized in an excellent review by Vögtle and Neumann.^{1f} From crystallographic² and NMR³ studies, [2.2]metacyclophanes possess a "stepped" conformation. The activation parameters for the inversion process (e.g., **1A** \rightleftharpoons **1B**) have been determined on the basis of



VT NMR studies. The free energy of activation (ΔG^\ddagger) and Arrhenius activation energy (E_A) for **1** ($X = N$) were found to be 14.8 and 15.3 kcal/mol, respectively.⁴ Related [2.2]-cyclophanes have been shown to exhibit a similar isomerization process.⁵ Syn-anti isomerization has also been reported in metacyclophanes possessing larger bridges;⁶ such isomerism is suggested from NMR studies in that the aromatic protons in the syn isomer experience a distinct upfield shift owing to the anisotropy of the juxtaposed ring.

In 1977, we proposed that **2** underwent a syn-anti isomerization (Figure 1) based on 100-MHz VT NMR spectral data. The free energy of activation (ΔG^\ddagger) was calculated to be 13.5 ± 0.3 kcal/mol from the coalescence temperature ($T_c = 288$ K) for the methylene hydrogens and chemical shift difference ($\Delta\delta = 137$ Hz). The nearly equal syn-anti isomer distribution, suggested by the equal intensity of the two resolved doublets ($J = 8.0$ Hz) at δ 6.28 and 6.32 for the 3,5-pyridine hydrogens at 223 K, was a major concern, since this distribution would not be expected to be equal. The chemical-shift differences for the pyridine protons should also have been larger for such syn-anti isomers. A single triplet ($J = 8$ Hz) at δ 7.50 for the 4-pyridine hydrogen was observed intact even at 223 K! The alternate mode isomerization (**2a** \rightleftharpoons **2b**), for which we suggest the term "anti, transverse", would have afforded only a single doublet for the 3,5-pyridine hydrogens over the entire temperature range, since these are equivalent by symmetry.

In view of recent X-ray crystal data for related macrocycles⁸