was the axial ligand in the aqueous oxygenation experiments.[20,21]

The EPR spectrum of **5** in 0.01 M 1-PVIm-glycerin[22] (4:1) at −160 °C is shown under $N_2$ (Figure 3a) and after exposure to 100 kPa of $O_2$ at room temperature for 10 min (Figure 3b). Although the resolution is very poor in $H_2O$ compared with the glasses obtained from 2-MeTHF, the important features of the spectra are clear. We assign the absorption at $g \approx 2$ to the oxy adduct of PVIm·Co{$\alpha^4$-P($C_6H_4NHCOCH_2C_2B_9H_{11}$·pipH)$_4$}. Since little oxygenation would be expected to take place after the solution is frozen (4:1 $H_2O$–glycerin mp −6 °C which would be depressed further by the ~5% acetone present), the EPR at −160 °C should reflect the *approximate* relative amounts of oxy:deoxy present[23] at room temperature assuming that the $K_{eq}$ for oxygenation is not too strongly dependent on $T$. The EPR samples can be thawed and refrozen to give the same spectrum showing that the oxy adduct does not rapidly oxidize (to Co(III)) upon warming to room temperature. Removing the $O_2$ from the system regenerates the deoxy Co(II) EPR spectrum. After several hours at room temperature the solutions are EPR silent suggesting oxidation to diamagnetic Co(III) species. Again the room temperature visible spectrum shows no change upon exposure to $O_2$;[24] thus there is no direct evidence of oxygenation at room temperature.

The apparent oxygenation of **5** under these conditions in aqueous solutions casts some doubt on the premise[25] that metalloporphyrinates must have a hydrophobic environment in order to undergo oxygenation. Note that the cobalt atom in **5** binds $O_2$ in preference to $H_2O$[26] even though $[O_2] \approx 10^{-5}[H_2O]$ in these solutions and the hydrophobic environment is at best very local. This situation may not be true for a similar iron(II) porphyrinate; iron porphyrinates have a much greater affinity than cobalt porphyrinates for a sixth ligand in the axial position. A previous report of reversible oxygen binding by a water-soluble, polymer-bound iron porphyrinate[27] has been questioned by other workers.[28]

The apparently reduced $O_2$ affinity of **5** in solution might be related to a lower local solubility in the polar binding pocket of the cage-degraded porphyrinate than the nonpolar pocket of the undegraded porphyrinate. Dioxygen is more soluble in nonpolar than polar solvents.[29] The fact that the EPR shows complete oxygenation at low temperature is consistent with the expected increased solubility at lower temperatures.

As shown here carboranylporphyrins are versatile and useful ligands for the investigation of metalloporphyrinate oxygenation reactions. Starting from the basic ligand, such properties as solubility and extent of oxygenation can be altered by suitable modification of the carboranyl cages while keeping the immediate environment of the porphyrinate core approximately constant.

**Acknowledgment** is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

## References and Notes

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(8) Abbreviations used are TPP = tetraphenylporphyrinate, Tpiv = tetrapivalimidophenylporphyrinate, MeIm = 1-methylimidazole, TrIm = 1-triphenylmethylimidazole, ar = atropisomerically random, $\alpha^4 = \alpha\alpha\alpha\alpha$ atropisomer (~$C_{4v}$) of a porphyrin, P = tetra-meso-substituted porphyrinate nucleus, THF = tetrahydrofuran, 2-MeTHF = 2-methyltetrahydrofuran, pipH = piperidinium cation.
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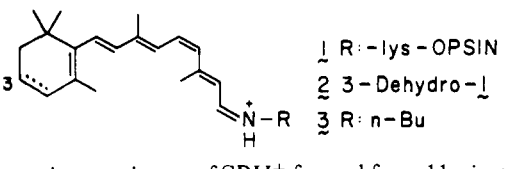
(10) Calcd for $C_{36}H_{68}N_4B_{40}Co$: C, 41.26; H, 6.49; N, 5.34; B, 41.30. Found: C, 41.24; H, 6.59; N, 5.22; B, 41.17. Visible (THF): 530 nm (note that the B band appeared in all $Co^{II}P$'s reported here at ~415 nm). For EPR see Figure 1.
(11) Calcd for $C_{60}H_{80}N_8O_4B_{40}Co$·THF: C, 49.90; H, 5.72; N, 7.28; B, 28.09. Found: C, 49.49; H, 6.33; N, 6.91; B, 27.97. Visible (THF): 530 nm. EPR: for **1**·MeIm (solid), $g = 2.3$.
(12) Visible (acetone): 530 nm. EPR: for **3**·MeIm (solid), $g = 2.3$.
(13) (a) Visible (acetone): for **4** and **5**, 530 nm. (b) See R. C. Haushalter and R. W. Rudolph, *J. Am. Chem. Soc.*, **100**, 4628 (1978).
(14) See ref 9 for a discussion of this metalation technique.
(15) Spang Microanalytical Laboratory, Eagle Harbor, Mich.
(16) Calcd for $C_{204}H_{196}N_8O_4B_{36}P_8Rh_4Co$: C, 62.28; H, 5.08; N, 2.85; B, 9.89; Rh, 10.46; Co, 1.50. Found: C, 61.24; H, 5.27; N, 2.36; B, 9.48; Rh, 9.99; Co, 1.67. Visible (THF): 530 nm. IR (KBr): $\nu_{BH}$ 2520, $\nu_{NH}$(amide) 3370, $\nu_{CO}$ 1681, $\nu_{CH}$(PhH) 3052. $^1H$ NMR: although the porphyrin signals were broadened and shifted by the Co(II) center making assignment difficult, the $Ph_3P$ signals were observed at $\delta$ ~7.3 and the hydride at −6.5.
(17) T. E. Paxson and M. F. Hawthorne, *J. Am. Chem. Soc.*, **96**, 4674 (1974).
(18) (a) The measured $g$ values are typical for $Co^{II}P$ complexes; see ref 7 for example. (b) See ref 1, p 149, and references therein for a discussion of diffusion of $O_2$ into solid Co(II)–Schiff base complexes.
(19) These visible spectrum changes are similar to those reported in ref 9.
(20) In these oxygenation studies, smaller 1-R-imidazoles such as 1-MeIm did not prevent oxidation, presumably owing to coordination on the hindered side of the porphyrin thus allowing coordination of $O_2$ on the unprotected porphyrin face and subsequent oxidation.
(21) The concentration of the polymer was calculated as moles of imidazole present. This polymer was a gift from Professor Overberger of this department.
(22) If pure water solutions are frozen, often no EPR signal can be observed, presumably owing to aggregation of the paramagnetic species with the resultant dipolar coupling greatly broadening the signals. Addition of glycerin minimizes this affect. Even with glycerin present, no signals are observed if the sample is frozen too slowly.
(23) The fact that the EPR of **9** shows both the deoxy and oxy species while TrIm·**5**·$O_2$ shows only the oxy species may be due to one or more of the following: (a) the solution was not as cold when it froze (thus, the extent of oxygenation was not as great); (b) $H_2O$ is competing with $O_2$ for the axial site on the cobalt; (c) 1-PVIm is less effective as an axial ligand than a monomeric imidazole.
(24) J. P. Collman et al., *J. Am. Chem. Soc.*, **96**, 6800 (1974), reported manometric $O_2$ uptakes corresponding to oxygenation of 50% of the Co(II) sites in their porphyrin accompanied by only minor visible absorption spectrum changes.
(25) Reference 1, p 149; ref 1, p 160.
(26) EPR measurements show the odd electron in the deoxy cobalt $d_{z^2}$ orbitals is delocalized into the $\pi^*$-type orbital of $O_2$ when the oxy adduct forms. $H_2O$ has no similar MO available for delocalization. The $^{59}Co$ superhyperfine splittings observed here for the deoxy and oxy forms of either TrIm·**3** or TrIm·**5** are ~83 and 15 G, respectively. These are typical values (ref 1, p 151).
(27) E. Bayer and G. Holzbach, *Angew. Chem.*, **89**, 120 (1977).
(28) H. R. Allcock, P. P. Greigger, J. E. Gardner, and J. L. Schmutz, *J. Am. Chem. Soc.*, **101**, 606 (1979).
(29) Solubilities of $O_2$ in solvents of decreasing polarity follow (millimolar at 25 °C): $H_2O$, 1.23; EtOH, 8.32; acetone, 12.12; *n*-perfluoroheptane, 23.7. From "Matheson Gas Data Book", Matheson Gas Products, Hasbrouck Heights, N.J. 07604.

**Robert C. Haushalter, Ralph W. Rudolph***
*Department of Chemistry, University of Michigan*
*Ann Arbor, Michigan 48109*
*Received June 28, 1979*

## Hydroretinals and Hydrorhodopsins[1]

*Sir:*

In the visual pigments rhodopsins,[2,3] the chromophore 11-*cis*-retinal is bound to the $\epsilon$-amino of a lysine moiety of the apoprotein opsin through a protonated Schiff base ($SBH^+$) **1**.[4]




The absorption maximum of $SBH^+$ formed from 11-*cis*-retinal and *n*-$BuNH_2$ (**3**) is at 440 nm in the leveling solvent metha-

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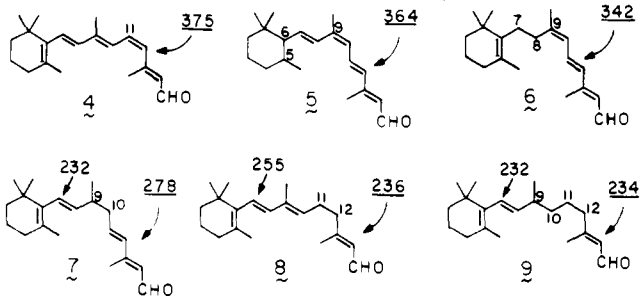
**Table I.** Absorption Data of Retinals and Rhodopsins[a]




| | 4 "B" | 5 "B" | 6 "B" | 7 "B" | 8 "NB" | 9 "NB" |
|---|---|---|---|---|---|---|
| aldehyde[b,c] | 375 | 364 | 342 | 278 | 236 | 234 |
| SBH* [b] | 440 | 425 | 392 | 322 | 270 | 275 |
| bovine rhodopsin[d] | 500 | 460 | 420 | 345 | 315 | 310 |
| $\Delta\nu$ ($cm^{-1}$)[e] | 2700 | 1800 | 1700 | 2100 | 5300 | 4100 |

[a] The structures of hydroretinals are represented in conformations resembling 9-*cis*- and 11-*cis*-retinals, i.e., conformations which they presumably adopt when binding to opsin. "B" and "NB" stand for bleachable and nonbleachable pigments, respectively, upon exposure to room light. Although the "NB" pigments derived from 11,12-dihydro- and 9,10,11,12-tetrahydroretinals (**8** and **9**) are stable to room light, they undergo photolysis when exposed to UV light. Hence this differentiation of "B" and "NB" is referred to only in the conventional usage of the word. [b] In MeOH. [c] The maxima of the aldehyde-containing chromophores are listed; the polyene maxima are given in accompanying structures **4**–**9** together with those of the enal moiety. [d] In 0.5% digitonin, 67 mM phosphate buffer, pH 7.0. [e] Difference in $\lambda_{max}$ of rhodopsin and protonated Schiff base, in $cm^{-1}$

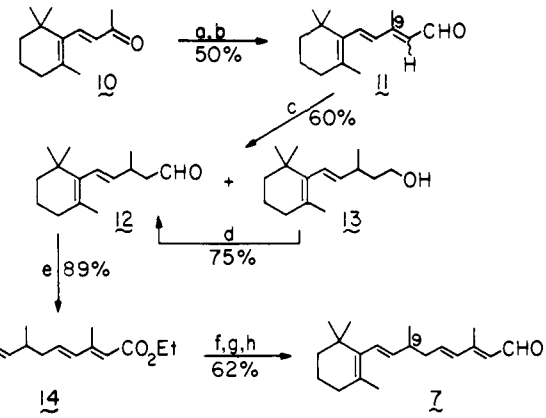
nol.[5] In contrast, the maxima of visual pigments range from 430 to 580 nm,[6] typically 500 nm for cattle rhodopsin, despite the fact that the chromophore is the same. Some pigments, especially those of fresh water animals, contain 3-dehydro-11-*cis*-retinal (see **2**) as well as 11-*cis*-retinal as the chromophore. When these chromophores bind to cone opsin to form pigments responsible for color vision, the maximum is shifted up to 620 nm.[6] The difference between 440 nm of the $SBH^+$ from simple amines and the maxima of natural pigments has been a central theme of interest in vision science. A rationalization of this shift provided by the hydrophobic lipoprotein environment would undoubtedly contribute to a better understanding of one of the most basic problems in this field.

In this and two following communications,[7] we present data on a series of hydrorhodopsins designed to explain the shift, an external point-charge model based on the hydrorhodopsin data, and a simple chemical model which corroborates the theoretical model.

The dihydro- and 9,10,11,12-tetrahydro chromophores **4**–**9** (Table I) described in this communication were chosen for several reasons: (a) the single bonds may bestow sufficient flexibility on the molecule so that they would fit into the binding site and form pigments; (b) the single bonds, except for those located terminally, separate the chromophore into two and thus these hydroretinals and hydrorhodopsins should provide a unique series for theoretical considerations; (c) since the primary event in visual transduction is the double-bond cis–trans isomerization, it may be possible to form a nonbleachable pigment.

Provided that the appropriate double-bond isomer is chosen,[8] we have found that the hydroretinals described in the following do indeed give hydrorhodopsins; furthermore, they bind to the same site as that of natural retinal. The maxima of $SBH^+$ were measured in the leveling solvent methanol,[5] whereas all rhodopsin data, including those of 5,6-dihydro-

**Scheme I.** Synthesis of 9,10-dihydroretinal (7)[a]



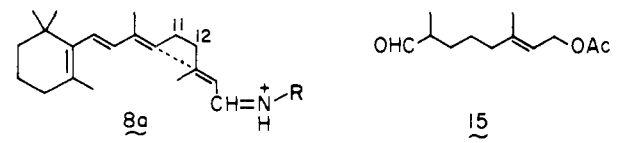

[a] (a) $LiCH_2CH{=}NC_6H_{11}$, −78 °C; (b) $I_2$, THF, −20 °C; (c) $Na_2Fe(CO)_4$/1.5 dioxane, $Fe(CO)_5$, HOAc, THF; (d) $CrO_3 \cdot 2pyr$, $CH_2Cl_2$; (e) $(EtO)_2P(O)CH_2C(CH_3){=}CHCO_2Et$, NaH, THF; (f) Dibal, $Et_2O$, −78 °C; (g) $MnO_2$, 1:1 EtOAc:$Et_2O$, 0 °C; (h) flash chromatography.

9-*cis*-[9] and 11,12-dihydrorhodopsin[10] which had been previously prepared, were all measured in 0.5% digitonin as it was found that the maxima were greatly affected by the detergent.[11] The set of dihydro data thus accumulated (Table I) has led to the external point-charge model.[7]

All dihydroretinals excepting 11,12-dihydroretinal (**8**)[10] were prepared by selective reduction of the $\alpha,\beta$ unsaturation of an appropriate carbonyl precursor followed by further elaboration (Wittig, Emmons, or directed aldol reactions) of the side chain to give the desired compounds. By the use of preparative LC or flash chromatography[12] it was possible to separate gram amounts of certain cis or trans synthetic intermediates; however, the final hydroretinals invariably had to be separated or purified by LC immediately prior to use and only in milligram quantities in the dark, because of the great instability.[13]

The synthesis is exemplified by 9,10-dihydroretinal (**7**) (Scheme I). The $C_{15}$ aldehydes (mixture of 9-cis and trans) **11** were prepared in 50% yield from $\beta$-ionone (**10**) via a directed aldol condensation at −78 °C followed by dehydration.[14] Treatment of **11** with $Na_2Fe(CO)_4$/1.5 dioxane (Collman's reagent)[15,16] gave a 1:1 mixture of the dihydro aldehyde **12** and tetrahydro alcohol **13**, the latter being reoxidized to **12** with $CrO_2$. Emmons reaction of **12** with ethyl phosphonosenecioate gave the ester **14**, which, upon oxidation, reduction, and separation of 13-cis and trans isomers by flash chromatography,[12] afforded the "*all-trans*"-9,10-dihydroretinal (**7**). This was further purified by LC ($\mu$-Porasil, 10% ether in hexane) prior to usage.

The synthesis of 9,10,11,12-tetrahydroretinal (**9**) (Table I) was also required since there was the possibility that the large red shift accompanying pigment formation in the case of 11,12-dihydroretinal (**8**) may be due to a through-space contribution of the type depicted in structure **8a**. The tetrahydroretinal was thus synthesized via condensation of the dihydro




aldehyde **15** derived from geranyl acetate[17] with the triphenylphosphonium salt prepared from $\beta$-cyclocitral. The Schiff bases were prepared by keeping a solution of aldehyde in neat amine over molecular sieves, −20 °C, 12 h, under argon in the dark, evaporating off the excess amine, and dissolving the residue in methanol. Protonation was accomplished by

bubbling dry HCl gas into methanol solution of the Schiff base at −78 °C, under argon in the dark.

Each retinal analogue was incubated at room temperature with bovine opsin at pH 7.0, either in suspension or in 0.5% digitonin[18] for 18–20 h. In the case of suspensions, aliquots were taken at suitable intervals and centrifuged and the pellets were triturated with cold hexane to remove excess chromophore and dissolved in 0.5% digitonin, and the absorption maxima were measured. In the case of reconstitution in 0.5% digitonin, the maxima were simply measured at suitable time intervals. Appearance of a maximum red shifted from that of the corresponding $SBH^+$ was taken as indication of pigment formation. Support for the fact that the retinal analogues bind to the same site as do 11- and 9-*cis*-retinals was secured by the following experiments.

Rhodopsin analogues were formed in suspension, the hexane-washed pellets (see above) were resuspended in pH 7.0 buffer, and the suspension was incubated for 3 h either with 11- or 9-*cis*-retinal. In each case the amount of natural rhodopsin or isorhodopsin formed was only 2–3% as judged from the absorption maxima, i.e., 500 and 480 nm, respectively. Conversely, only a few percent of the rhodopsin analogues were formed upon reincubation of rhodopsin or isorhodopsin with the retinal analogues. In a competitive binding site study, the 7,8-dihydro-9-*cis*-retinal was coincubated with 9-*cis*-retinal in suspension. The hexane-washed pellet was analyzed for bound retinals by the $CH_2Cl_2$-denaturation extraction procedure[19] which showed that the pigment consited of a ~1:3 mixture of 7,8-dihydro-9-*cis*- and isorhodopsins.

The absorption data[20] of the chromophores and pigments thus prepared are summarized in Table I along with the data for 11-*cis*-retinal and bovine rhodopsin. The data in Table I shows several important tendencies: (a) since the $\lambda_{max}$ of the pigment undergoes a progressive shift in going from **4** to **8** (and **9**), clearly it is the enal moiety and not the ene moiety which is responsible for the maxima; (b) since the maximum of the tetrahydroretinal (**9**) derived pigment is similar to that of dihydrorhodopsin, the through-space interaction **8a** can be disregarded; (c) in spite of the shorter chromophore of 11,12-dihydro- (and 9,10,11,12-tetrahydro-) rhodopsin, the difference between the maxima of $SBH^+$ and rhodopsin, in $cm^{-1}$ (see Table I), is more than twice that observed for other cases, including natural rhodopsin.

Analysis of the Table I data has led to the external point-charge model, which in turn is supported by chemical models.[7,21]

**Acknowledgments.** We thank Mr. John D. Carriker for technical assistance.

## References and Notes

(1) A preliminary account of this paper was presented at the 1978 American Society for Photobiology Symposium, Burlington, Vt., June 1978; cf. K. Nakanishi, V. Balogh-Nair, M. A. Gawinowicz, M. Arnaboldi, M. Motto, and B. Honig, *Photochem. Photobiol.*, **29**, 657 (1979).
(2) A. Knowles and H. J. A. Dartnall in "The Eye", Vol. 2B, H, Davson, Ed., Academic Press, 1977, pp 1–689; B. Honig, *Annu. Rev. Phys. Chem.*, **39**, 31 (1978); also a collection of symposium papers,[1] *Photochem. Photobiol.*, **29**, 655–753 (1979).
(3) In view of the recent preparations of various artificial pigments from synthetic model retinals and opsin, we propose the use of the generic name "rhodopsin" prefixed by the name of the specific chromophore, i.e., 5,6-dihydro-9-*cis*-rhodopsin.
(4) The protonated Schiff base ($SBH^+$) structure is the one generally accepted for pigments, especially in view of the resonance laser Raman evidence. For review see R. Callender and B. Honig, *Annu. Rev. Biophys. Bioeng.*, **6**, 33 (1977); G. Eyring and R. Mathies, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 33 (1979). However papers such as the following favor a nonprotonated Schiff base: K. Van der Meer, J. J. C. Mulder, and J. Lugtenburg, *Photochem. Photobiol.*, **24**, 363 (1976); K. Peters, M. L. Applebury, and P. M. Rentzepis, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3119 (1977); J. Shriver, G. Mateescu, R. Fager, D. Torchia, and E. W. Abrahamson, *Nature (London)*, **270**, 273 (1977).
(5) Leveling solvents are defined as those which give the same $\lambda_{max}$ independent of the counteranion: J. O. Erickson and P. E. Blatz, *Vision Res.*, **8**, 1367 (1968).
(6) Review: B. Honig and T. Ebrey, *Annu. Rev. Biophys. Bioeng.*, **3**, 151 (1974).
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(8) In the case of 9,10- and 11,12-dihydroretinals the "all-trans" forms were capable of forming pigments by virtue of the conformational flexibility which enables them to adopt shapes mimicking 9-*cis*- and 11-*cis*-retinals, respectively. In the case of 7,8-dihydroretinal, however, it was necessary to prepare the 9-cis isomer 6. Although DeGrip et al. have shown that 7-*cis*-retinal forms a pigment (W. J. DeGrip, R. S. H. Liu, V. Ramamurthy, and A. Asato, *Nature (London)*, **262**, 416 (1976), the rate of formation was only 1/150 that of rhodopsin derived from 11-*cis*-retinal; thus the "all-trans" form of 7,8-dihydroretinal, which already started to decompose after 3 h under the incubating conditions, was too unstable for the prolonged incubation.
(9) P. E. Blatz, P. B. Dewhurst, B. Balasubramaniyan, P. Balasubramaniyan, and M. Lin, *Photochem. Photobiol.*, **11**, 1 (1970); A. Kropf, P. Whittenberger, S. P. Goff, and A. S. Waggoner, *Exp. Eye Res.*, **17**, 591 (1973).
(10) M. A. Gawinowicz, V. Balogh-Nair, J. S. Sabol, and K. Nakanishi, *J. Am. Chem. Soc.*, **99**, 7720 (1977).
(11) W. H. Waddell, A. P. Yudd, and K. Nakanishi, *J. Am. Chem. Soc.*, **98**, 238 (1976); V. Balogh-Nair and J. Carriker, unpublished work. Although sonicated membranes presumably provide the artificial conditions closest to nature, we have used 0.5% digitonin as detergent owing to unsolved technical difficulties in making sonicated membrane preparations from the various model rhodopsins.
(12) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
(13) The 11,12-dihydroretinal,[10] the least stable of the series, was found to be already contaminated (LC) after a 2-day storage as neat liquid in the dark at −60 °C under argon.
(14) S. P. Tanis, R. H. Brown, and K. Nakanishi, *Tetrahedron Lett.*, 869 (1978).
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(17) J. Meinwald, W. R. Thompson, T. Eisner, and D. F. Owen, *Tetrahedron Lett.*, 3485 (1971).
(18) C. D. B. Bridges, *Vision Res.*, **17**, 301 (1977).
(19) R. Crouch, V. Purvin, K. Nakanishi, and T. Ebrey, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1538 (1975); F. G. Pilkiewicz, M. J. Pettei, A. P. Yudd, and K. Nakanishi, *Exp. Eye Res.*, **24**, 421 (1977).
(20) All pigments showed circular dichroic Cotton effect(s) in the region of the absorption band. These data are being measured at low temperatures and will be reported collectively.
(21) The studies were supported by National Institutes of Health Grant EY 01253.

**Maria Arnaboldi, Michael G. Motto, Kazuo Tsujimoto**
**Valeria Balogh-Nair, Koji Nakanishi***
*Department of Chemistry, Columbia University*
*New York, New York 10027*
*Received May 11, 1979*

## An External Point-Charge Model for Wavelength Regulation in Visual Pigments

*Sir:*

The chromophoric unit of visual pigments is known to consist of 11-*cis*-retinal covalently bound in the form of a protonated Schiff base to the $\epsilon$ amino group of a lysine in the apoprotein opsin.[1] Protonated Schiff bases of retinal absorb at ~440 nm in polar solvents while various salts formed in nonpolar solvents absorb at somewhat longer wavelength (~440–480 nm).[2] The visual pigment bovine rhodopsin has an absorption maximum of ~500 nm while other 11-*cis*-retinal-based visual pigments have maxima as far to the red as 580 nm. The mechanism through which the protein shifts the absorption maximum of the chromophore from its solution value to wavelengths ranging from 440 to 580 nm has been a question of major interest. In this communication we present the first experimentally based model which accounts for the absorption properties of a specific pigment, bovine rhodopsin.

There is considerable support for the suggestion[3] that electrostatic interactions between the chromophore and charged or dipolar groups on the opsin are responsible for wavelength regulation in visual pigments.[2-8] Although models

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