Factors Affecting the Absorption Maxima of Polyene Iminium Systems: A Model Study for Rhodopsin and Bacteriorhodopsin

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Abstract: We had proposed external point-charge models to explain the variance in absorption maxima of cattle rhodopsin and the purple color of bacteriorhodopsin. In the former visual pigment model, a negative counteranion resides ca. 3 Å from the protonated Schiff base (SBH^+) linkage and another point charge is located ca. 3 Å from C-12 and C-14 of the 11-*cis*-retinal chromophore (Figure 1a). In the bacteriorhodopsin model (Figure 1c) a negative counteranion is located ca. 3 Å from SBH^+ and another point charge is present near the ionone ring. A total of 15 nonsteroidal and steroidal di-, tetra-, and hexaene iminium compounds with and without $COOH/COO^-$ groups near the polyene chain have been synthesized in order to structurally mimic the charge distributions within the binding sites of the two pigments. The electronic spectra of these polyene iminium compounds have been measured in different solvents with and without addition of base. The results show that the interplay of the following three factors determines the absorption maxima of these compounds, i.e., the additional negative charge, the distance between the iminium nitrogen and its counteranion, and the solvent polarity. In the case of the natural pigments, the evidence obtained so far shows that the external point charges are important spectroscopic determinants.

Visual pigments are membrane proteins located in special photoreceptor cells, such as the rods and the cones of the retinas of vertebrates. The pigments are embedded in multilayer disklike membrane sheets which are in the cell outer segment and are stacked in a direction perpendicular to the long axis of the cell. The role of visual pigments is to convert light energy into changes of the electrical potential of the cell membrane, which are then transmitted to the brain by the optical nerve through appropriate synaptic processes.

Visual excitation in both vertebrates and invertebrates is initiated via light absorption by the visual pigments consisting of a chromophore covalently bound to an apoprotein, opsin. Biochemical extraction studies have shown that in all pigments the chromophore is 11-*cis*-retinal,[1,2] the parent aldehyde of retinol (vitamin A). The chromophore is bound to the ϵ-amino terminal of a lysine residue of the apoprotein opsin via a protonated Schiff base (SBH^+) linkage.[3,4] In vertebrates, the main chemical action of light is to cleave the chromophore–opsin linkage to yield *all-trans*-retinal and opsin. The process is termed "bleaching" because the pigments absorb in the visible while the retinal chromophore absorbs in the UV. The 11-*cis*-retinal SBH^+ formed from *n*-butylamine has its absorption maximum at 440 nm in methanol[5] whereas the maxima of visual pigments from various sources have maxima as far to the red as 580 nm; the most typical pigment used in vision studies is the bovine rhodopsin which absorbs at 500 nm. We proposed to call these red shifts (in cm^{-1}) from 440 nm, which are due to the effects of the protein environment, the "opsin shift";[6] for example, the opsin shift for cattle opsin would be 22 700 cm^{-1} (440 nm) minus 20 000 cm^{-1} (500 nm) = 2700 cm^{-1}.

Numerous models have been forwarded to account for these opsin shifts, a central problem in vision research.[7] We recently proposed an external point charge model[8] (Figure 1a) for the visual pigment of bovine. This model proposes that in addition to a counteranion near the Schiff base iminium nitrogen, another negative charge is located in the vicinity of C_{11}–C_{12} of the polyene chain. This was based on the experimental maxima of a series of dihydroretinal-derived pigments,[9] theoretical calculations,[8] and synthetic models mimicking the charge distributions in 11,12-dihydrorhodopsin (Figure 1b).[10,11]

The purple membrane is a novel light energy transducing membrane which constitutes part of the plasma of *Halobacterium halobium*.[12,13] It contains a rhodopsin-like protein which uses light energy to translocate protons across the membrane and thereby generates a substantial electrochemical gradient. The cell uses the energy stored in the gradient for ATP synthesis and other vital energy-requiring functions. The color of the purple membrane protein is due to retinal–protein complex of the kind previously described for visual pigments, namely, a protonated Schiff base linkage between a *trans*-retinal and the apoprotein bacterioopsin.[3,4]

We further proposed a model for bacteriorhodopsin (Figure 1c) to account for its maximum at 560 nm or its purple color.[6]

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(1) Wald, G. *Nature (London)* **1968**, *219*, 800. Hubbard, R.; Kropf, A. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 130.

(2) Balogh-Nair, V.; Nakanishi, K. In "Stereochemistry"; Tamm, Ch., Ed.; Elsevier Biomedical Press: Amsterdam, 1982; Chapter 7, pp 283–334.

(3) Callender, R.; Honig, B. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 33. Mathies, R. In "Chemical and Biological Applications of Lasers"; Moore, C. B., Ed.; Academic Press: New York, 1979; Vol. 4, 55–99.

(4) The resonance laser Raman results (ref 3) have been confirmed by Fourier transform IR: Rothschild, K. J.; Marrero, H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4045. Bagley, K.; Dollinger, G.; Einsenstein, L.; Singh, A. K.; Zimanyi, L. *Ibid.*, 4972.

(5) Despite the fact that methanol does not represent the proper environment in the protein binding site, we have taken it as the reference solvent. This is because it has been shown that the maxima of retinal SBH^+ in this solvent is not affected by the counteranion: Blatz, P. D.; Mohler, J. H.; Navangril, H. V. *Biochemistry* **1972**, *11*, 848.

(6) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945.

(7) (a) Kropf, A.; Hubbard, R. *Ann. N.Y. Acad. Sci.* **1958**, *74*, 266. (b) Irving, C. S.; Byers, G. W.; Leermakers, P. A. *Biochemistry* **1970**, *9*, 858. (c) Blatz, P. E.; Mohler, J. H. *Biochemistry* **1972**, *11*, 3240. (d) Ebrey, T. G.; Honig, B. *Q. Rev. Biophys.* **1975**, *8*, 129, and references cited therein. (e) Waddel, W. H.; Schaffer, A. M.; Becker, R. S. *J. Am. Chem. Soc.* **1977**, *99*, 8456. (f) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2558. (g) Ishikawa, K.; Yoshihara, T.; Suzuki, H. *J. Phys. Soc. Jpn.* **1980**, *49*, 1505.

(8) Honig, B.; Dinur, U.; Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G. *J. Am. Chem. Soc.* **1979**, *101*, 7084. See also: Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G.; Honig, B. *Photochem. Photobiol.* **1979**, *29*, 657.

(9) Arnaboldi, M.; Motto, M. G.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 7082.

(10) Results obtained with compounds **1**, **2**, and **4** (Chart I) have been reported earlier as a short communication: Sheves, M.; Nakanishi, K.; Honig, B. *J. Am. Chem. Soc.* **1979**, *101*, 7086.

(11) In view of the numerous rhodopsins and bacteriorhodopsins being prepared from modified retinals, we propose the use of the generic name rhodopsin and bacteriorhodopsin prefixed by the specific modification carried out on the retinal, e.g., 9,10-dihydrobacteriorhodopsin.

(12) Oesterhelt, D.; Stoeckinius, W. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2853.

(13) Ottolenghi, M. *Adv. Photochem.* **1980**, *12*, 97–200. Birge, R. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315–354. Stoeckinius, W.; Bogomolni, R. *Annu. Rev. Biochem.* **1982**, *52*, 587–616.

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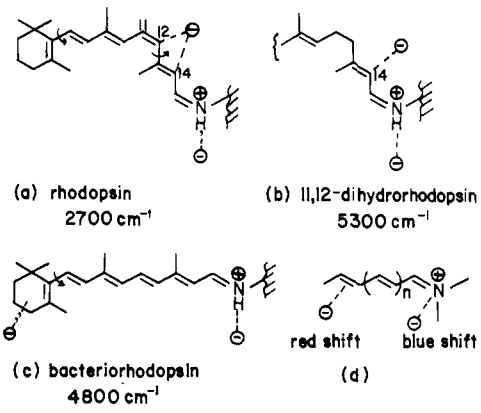
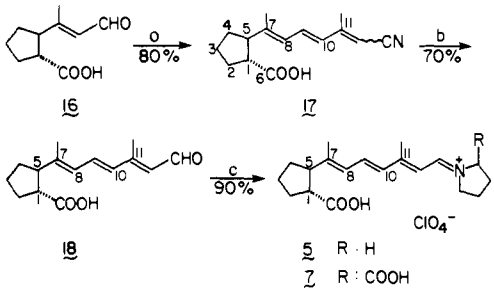


Figure 1. External point-charge models for bovine rhodopsin (a), bovine 11,12-dihydrorhodopsin (b), and bacteriorhodopsin (c). Dashed lines indicate distances of 3–3.5 Å, curved arrows represent nonplanarity, and numerals denote the opsin shifts. Structure d represents schematically the effect of negative charges on the absorption maxima of polyene iminium systems.

Scheme I[a]



[a] (a) $(EtO)_2PO-CH_2-C(Me)=CH-CN$, NaH in THF. (b) Dibal, hexane, −78 °C; then isomer separation. (c) Pyrrolidine perchlorate or proline perchlorate, in EtOH, 0 °C.

This model, which is also based on λ_{max} comparisons of a series of dihydrobacteriorhodopsins,[11] proposes that in addition to a counteranion near the Schiff base iminium nitrogen, another negative charge is located in the vicinity of the ionone moiety.[6] Furthermore, the absorption maxima of bovine rhodopsins and bacteriorhodopsins derived from appropriately designed synthetic retinal analogues were in accordance with the two point-charge models.[14]

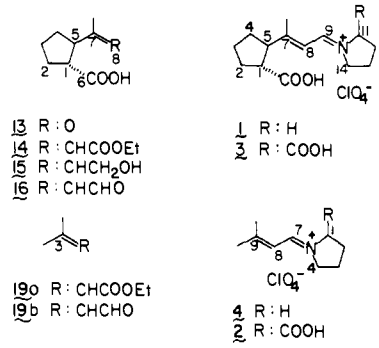
In the present work we have tried to gain further insight into the electrostatic interaction between nonconjugated negative charges (carboxylate groups) and the iminium moiety. We have thus prepared a number of polyene iminium compounds structurally related to the chromophores of visual pigments and bacteriorhodopsin and have checked the influence of negative charges and other factors on their absorption maxima.

Results

The models we have chosen to synthesize were transoid "dienes", "tetraenes", and "hexaenes" containing a quaternary nitrogen at one terminal of the molecule. The influence of a second negative charge was examined by introducing a carboxylate group at a distance of ca. 3 Å from a terminal C=C bond. This distance is close to the distances of the extra point charges proposed in the models for 11,12-dihydrorhodopsin[8] and bacteriorhodopsin.[6] We have also checked the effect of introducing a nonconjugated negative charge located near the $C=N^+$ terminus of the polyene. Thus, by models **1/5/9** (Chart I) we examined the effect of a nonconjugated negative charge located at the C=C terminus of the polyene. Models **2/6/10** gave information about the effect of a nonconjugated negative charge located near the $C=N^+$ terminus of the polyene. Models **3/7/12** gave the combined effects of negative charges located at both termini of the polyene, while **4/8/11** served as reference compounds.

We have also synthesized models **30/34/35** in order to allow more flexibility to the negative charge and thus attain better orbital overlap between the carboxylate anion and the positively charged polyene iminium cation.

I. Preparation of Compounds. A. Cyclopentane Iminium Mono- and Diacid 1 and 3. Reaction of diethyl phosphonoacetate with

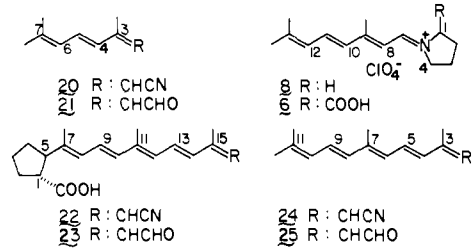


trans-5-acetyl-1-carboxycyclopentane **13**[15,16] gave a mixture of *Z* and *E* esters **14** in a ratio of ~7/3 (by ^1H NMR). Reaction of **14** with Dibal gave *Z* and *E* alcohols **15** which were separated by flash chromatography. Manganese dioxide oxidation of the *E* alcohols yielded the corresponding *E* aldehyde **16**. This aldehyde was condensed with pyrrolidine perchlorate in ethanol[17] to give, after solvent evaporation and trituration with ether and hexane, the carboxyl Schiff base **1**[10] as a colorless oil. Condensation of aldehyde **16** with L(−)-proline perchlorate in ethanol yielded the diacid **3**.[10]

B. *gem*-Dimethyl Enol Schiff Base 2[10] and 4.[10] Compounds **2** and **4** were prepared from acetone via acrylate **19a** and acrolein **19b** by a route similar to that for compounds **1** and **3**.

C. Cyclopentane Tetraene Iminium Mono- and Diacid 5 and 7 (Scheme I). Reaction of *E* enal acid **16** with the C_5 nitrile phosphonate gave a mixture of *E* and *Z* nitriles **17** which were reduced with Dibal. The *E* aldehyde **18** was separated from the *Z* isomer by flash chromatography and condensed with pyrrolidinium perchlorate in ethanol to give the tetraene carboxyl Schiff base **5**. Similar condensation with L(−)-proline perchlorate yielded the diacid Schiff base **7**.

D. Tetraene Iminium Salt with Terminal *gem*-Dimethyl Groups 6 and 8. 3,3-Dimethylacrolein **19b** was condensed with the C_5



nitrile phosphonate to give a mixture of *E* and *Z* nitriles **20** which were reduced with Dibal. The *E* aldehyde **21** was separated and condensed with pyrrolidine perchlorate in ethanol to give the tetraene iminium salt **8**. Similar condensation of aldehyde **21** with L(−)-proline perchlorate yielded iminium salt **6**.

E. Cyclopentane Tetraene Perchlorate Iminium Salt Mono- and Diacid 9 and 11. The *E* isomer of trienal **18** (Scheme I) was condensed with C_5 nitrile phosphonate, and the mixture of *E*/*Z*

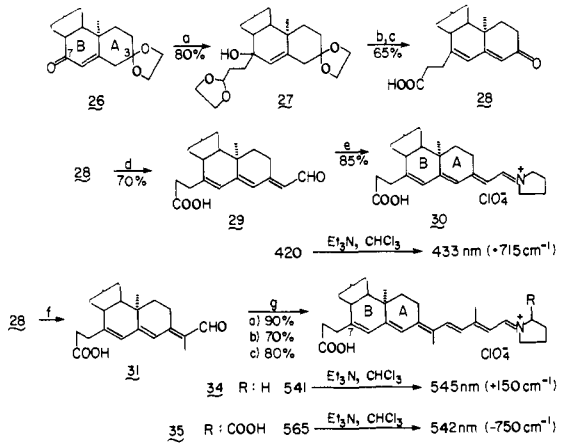
(14) Motto, M. G.; Sheves, M.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 7947.

(15) The numbering systems of compounds in this paper do not follow the IUPAC scheme; they are merely numbered for the sake of convenience in comparing with retinal and assigning NMR peaks.

(16) Mangoni, L.; Belardini, M. *Ann. Chim. (Rome)* **1960**, *50*, 309; *Chem. Abstr.* **1962**, *56*, 9996.

(17) Leonard, N. J.; Ranksteilis, J. V. *J. Org. Chem.* **1963**, *28*, 3021. Irving, C. S.; Beyers, G. W.; Leermakers, P. A. *J. Am. Chem. Soc.* **1969**, *91*, 2141.

Scheme II[a]

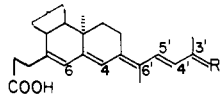


[a] (a) 2-(1-bromoethyl)-1,3-dioxolane, room temperature, THF. (b) TSOH, room temperature, Me_2CO, 2 days. (c) Jones oxidation. (d) $Me_3SiCH_2-CH=N-CMe_3$, LDA/THF, −78 °C; then $(COOH)_2$, 0 °C. Isomer separation. (e) Pyrrolidine perchlorate, EtOH, 0 °C. (f) $Me_3SiCH(Me)-CH=N-CMe_3$, LDA/THF, −78 °C; then $(COOH)_2$, 0 °C. Isomer separation. (g) Steps as in Scheme I; yields comparable for R = H and COOH.

nitrile isomers **22** was reduced with Dibal to the corresponding aldehydes **23**. Condensation of the all-trans aldehyde **23** with pyrrolidine perchlorate in ethanol or with L(−)-proline perchlorate gave compounds **9** and **11** (see Chart I for structures).

F. Hexaene Perchlorate Iminium Salt with Terminal *gem*-Dimethyl Groups 10 and 12. *E* aldehyde **21** was condensed with C_5 nitrile phosphonate to afford the cis and trans nitriles **24**. Reduction of the trans nitrile with Dibal to trans aldehyde **25** and condensation with pyrrolidine perchlorate or L(−)-proline perchlorate gave compounds **10** and **12** (see Chart I for structures).

G. Steroidal Models (with Cholesterol Side Chain) 30, 34, and 35 (Scheme II). Reaction of cholest-5-ene-3,7-dione 3-ethylene ketal (**26**) at room temperature with the Grignard reagent derived from 2-(1-bromoethyl)-1,3-dioxolane yielded bisethylene ketal **27**, which upon treatment with 1% *p*-toluenesulfonic acid in acetone for 2 days and subsequent Jones oxidation gave dienone acid **28**. Condensation with silylated Schiff base[18] gave a mixture of two aldehyde isomers. The *E* isomer **29** was separated by flash chromatography and condensed with pyrrolidine perchlorate to give tetraene acid **30**. The hexaenes **34** and **35** were prepared similarly by condensation of dienone acid **28** with the silylated Schiff base derived from propionaldehyde to give aldehyde **31**. Condensation of aldehyde **31** with the C_5 nitrile phosphonate gave nitrile **32** which was reduced with Dibal to pentenal **33**; con-



densation with pyrrolidine perchlorate and L(−)-proline perchlorate yielded Schiff bases **34** and **35**, respectively.

II. Absorption Spectra (Chart I). We have investigated the influence of carboxylate anion on the absorption spectra of the various chromophores described above. The absorption spectra were measured before and after deprotonation of the carboxylic group. The base had to be carefully selected so that deprotonation would cause neither hydrolysis of the Schiff base nor rearrangement of the iminium compounds to enamines.

The base that was used for compounds **1–4** was NaH in dry CH_3CN. Compound **1** showed a red shift from 276 to 297 nm upon deprotonation of the nonconjugated carboxyl group; this shift could be reversed by addition of acetic acid.

To ascertain that we were dealing with the correct species, we converted aldehyde **16** first to its sodium salt by treating it with NaH in CH_3CN. The resulting salt was then condensed with pyrrolidine perchlorate in ethanol to yield the carboxylate Schiff base identical with **1** prepared previously. Although the salts were unstable the structure was confirmed by ^1H NMR (CD_3CN) and IR (neat) measurements.

The location of the external negative charge is crucial for determining the absorption maxima in visual pigments, bacteriorhodopsin, as well as in the model pigments. Therefore, we checked the effect of deprotonation in compounds **1–3**. The deprotonation was achieved by NaH in CH_3CN. Compound **1** gave a red shift from 276 to 297 nm, compound **2** gave a blue shift from 275 to 268 nm, and **3** showed a red shift from 282 to 293 nm. All shifts were reversible, and by addition of acetic acid, the maxima shifted back to the original wavelength.

The carboxyl groups of compounds with longer polyene chains, namely, **5**, **6**, **7**, **9**, **10**, and **11**, were deprotonated with triethylamine. The system NaH/CH_3CN or $Et_3N/CHCl_3$ was employed because they do not lead to rearrangements in the iminium compounds handled here;[19,20] thus, in the reference compounds **4**, **8**, and **12**, the absorption maxima were neither shifted upon basification with $Et_3N/CHCl_3$ or NaH/CH_3CN systems nor shifted upon acidification with acetic acid. The results are summarized in Chart I.

Because of the rigidity of the trans-1,2-disubstituted cyclopentane system, it is possible that the measured absorption maxima do not represent satisfactory orbital overlap between the carboxylate anion and the positively charged polyene iminium cation. Steroidal compounds **30**, **34**, and **35** were therefore synthesized. Since they contain a flexible 7-CH_2CH_2COOH side chain, the charges could possibly achieve better overlap. The deprotonation was carried out by adding dry triethylamine to a chloroform solution. Here again the shifts were reversible, and by addition of acetic acid to the basic solution the spectra reverted to the original.

The absorption maxima of **30** shifted from 420 nm ($CHCl_3$) to 433 nm (Scheme II, a shift of +715 cm^{-1}). Compound **34** showed a red shift of +150 cm^{-1} (541 → 545 nm) and **35** a blue shift of −750 cm^{-1} (565 → 542 nm).

In comparing the steroidal series with the corresponding nonsteroidal series it is noted that the *shifts upon deprotonation are of comparable magnitude*: i.e., **30** (+715 cm^{-1}) and **5** (Chart I, +600 cm^{-1}), **34** (+150 cm^{-1}) and **9** (+300 cm^{-1}), and **35** (−750 cm^{-1}) and **11** (−400 cm^{-1}).

It is known that the absorption maxima of retynilidene iminium salts are influenced by the polarity of the medium.[21] Thus, in order to check the effect of medium polarity on absorption maxima of iminium salts with different chain lengths, the maxima were measured in H_2O (dielectric constant 78.5) and $CHCl_3$ (dielectric constant 4.7). The data are collected in Table I.

Discussion

From the data of Chart I the following conclusions can be made: (1) Theory predicts that in a conjugated iminium system, a negative charge close to the terminal C=C bond would lead to a red shift due to stabilization of the excited state; in contrast, a negative charge close to the $C=N^+$ bond would lead to a blue shift due to stabilization of the ground state (Figure 1d).[10] This prediction is corroborated by shifts in absorption maxima of compounds **1**, **5**, and **9** accompanying deprotonation of the carboxyl group. This shows that *a nonconjugated terminal negative charge shifts the maxima of iminium salts to the red.* This is a *through-space charge effect.*[10,22] It is also seen that the shorter

(18) Corey, E. J.; Enders, D.; Bock, M. G. *Tetrahedron Lett.* **1976**, 7.

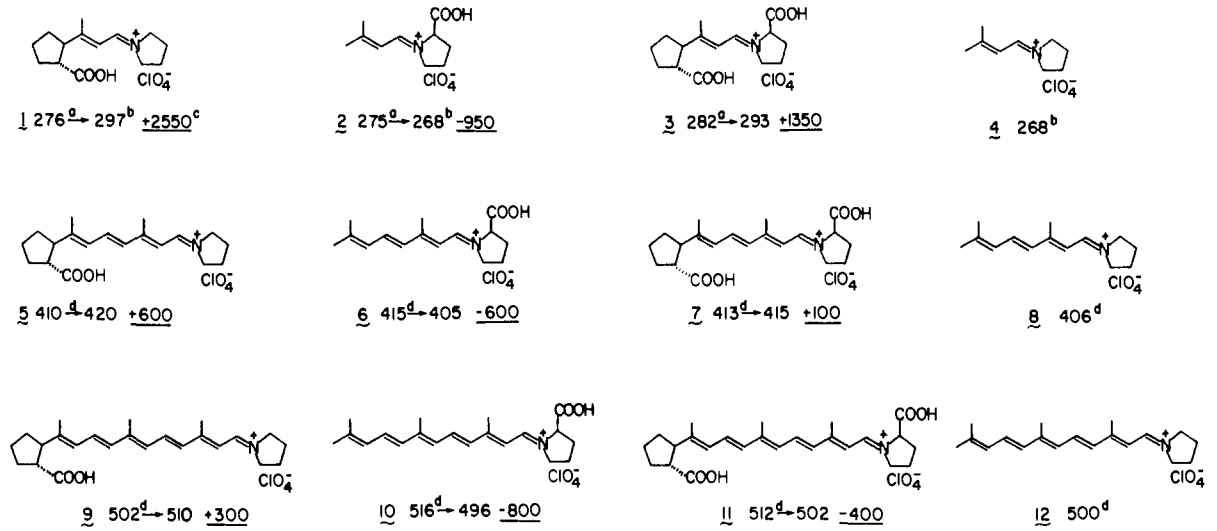
(19) Johnson, J. L.; Herr, M. E.; Babcock, J. C.; Fonken, A. E.; Stafford, J. E.; Heyl, F. W. *J. Am. Chem. Soc.* **1956**, *78*, 430.

(20) Alais, L.; Michelot, M. R.; Tchoubar, B. *C. R. Acad. Sci., Ser. C* **1971**, *273*, 261.

(21) The following deal with solvent effects on maxima of retynilidene iminium salts: (a) Irving, C. S.; Byers, G. W.; Leermakers, P. A. *J. Am. Chem. Soc.* **1969**, *91*, 2141; *Biochemistry* **1970**, *9*, 858. (b) Kliger, D. S.; Miller, S. J.; Dratz, E. A. *Photochem. Photobiol.* **1977**, *25*, 277. (c) Milder, S. J.; Kliger, D. S. *Ibid.*, 287. (d) Das, P. K.; Becker, R. S. *J. Phys. Chem.* **1978**, *82*, 2093. (e) Ottolenghi, M. *Adv. Photochem.* **1980**, *12*, 97–200.

(22) It has been reported that a proximal ammonium ion induces a red shift of the SBH^+ maximum: Tabushi, I.; Shimokawa, K. *J. Am. Chem. Soc.* **1979**, *102*, 5400.

Chart I



[a] In CH_3CN, basified with NaH. [b] Data from ref 10. [c] Shift in cm^{-1} upon basification; positive and negative signs denote respectively red and blue shifts. The shifts are rounded up to the fifties and hundreds. [d] In $CHCl_3$, basified with Et_3N.

Table I. Absorption Maxima of Iminium Salts in Different Solvents

compound	H_2O	$CHCl_3$	Δcm^{-1}
1	276	279	260
5	393	413	1050
9	460	501	1800

the conjugation, the larger the red shift.

(2) Acidic forms of **2**, **6**, and **10** are red-shifted from reference compounds **4**, **8**, and **12** which lack the carboxyl group near the $C{=}N^+$ terminus. This trend can be explained by the fact that in the proline series, the perchlorate anion is more remote from N^+ because of the steric bulk of the COOH group. From these results, we can conclude that *the maxima are influenced by the $C{=}N^+$/counteranion distance and that the more distant the counteranion the larger the red shift* ("*counteranion effect*").[23] It is also seen that deprotonation of compounds **2**/**6**/**10**, i.e., placement of a negative charge close to the $C{=}N^+$ bond, induces blue shifts, which furthermore are not affected by differences in chain lengths.

(3) From deprotonation shifts of **3**, **7**, and **11** we note that the influences italicized in the trends 1 and 2 are approximately additive. Namely, because of the decreasing bathochromic influence of the terminal negative charge with polyene chain length, deprotonation of diene **3** leads to a red shift whereas that of hexaene **11** leads to a blue shift.

(4) In addition to the "through-space charge effect" and the "counteranion effect" there also exists the well documented medium polarity effect. Thus, the data in Table I show that *the less polar the medium the more red-shifted the maxima* and *the shorter the polyene the less pronounced the medium effect*.[21d]

It is not feasible to mimic the binding site environment of visual pigments and bacteriorhodopsin with these simple synthetic models, but the data presented clearly show that at least three effects are operative in shifting the maxima of polyene iminium chromophore, namely, the through-space charge effect, the counteranion effect, and the medium polarity effect.

In binding studies with cattle opsin, the pigment with the shortest chromophore 11,12-dihydrorhodopsin[24] (Figure 1b) exhibited the largest opsin shift of 5300 cm^{-1}, while the rest of the dihydro series all gave rise to opsin shifts of similar amplitudes:[9] 9,10-dihydro (2100 cm^{-1}), 7.8-dihydro (1700 cm^{-1}),[25] and 5,6-dihydro (1800 cm^{-1}).[25] The 5300-cm^{-1} opsin shift of the 11,12-dihydro analogue provided a crucial datum which led to the point-charge model; in this dihydro pigment, contribution of the opsin medium effect is presumably minor because of the short chromophore length (see Table I). The dielectric constant within the binding site is unmeasurable, but the fact that the environment polarity plays only a minor role in a short diene is borne out by the small red shift of only 260 cm^{-1} for compound **1** in two solvents with a dielectric constant difference of 73.8 (H_2O and $CHCl_3$) as was shown above.

We noted earlier (trend 2) that the difference in maxima between unprotonated **2**/**6**/**10** and **4**/**8**/**12** reflects differences in the counteranion distances. This distance effect appears to be independent of chain length as seen in comparisons between **2** and **4** (275 and 268 nm, or 950 cm^{-1}), **6** and **8** (530 cm^{-1}), and **10** and **12** (620 cm^{-1}). In the case of rhodopsins, although the opsin shifts encountered in dihydrorhodopsins are all ca. 2000 cm^{-1}, this is not so for 11,12-dihydrorhodopsin (5300 cm^{-1}). It follows that the exceptionally large opsin shift of the 11,12-dihydro pigment cannot be explained by the distance effect between the $C{=}N^+$ bond and counteranion. In bovine rhodopsin, the medium polarity effect is probably contributing in addition to the external charge and counteranion, because of the longer chromophore length.

In contrast to the cattle rhodopsin, the opsin shifts (in cm^{-1}) of bacteriorhodopsin and its dihydro analogues decrease in an orderly manner, i.e., natural (4870), 5,6-dihydro (2500), 7,8-dihydro (1000), 9,10-dihydro (300), and 11,12-dihydro (nil);[6] this trend, which suggested the presence of a negative charge near the ionone ring besides the counteranion, led to the point-charge model depicted in Figure 1c.[6,26] The medium effect is also probably involved because of the long chromophoric length. As the chromophoric polyene chain in bacteriorhodopsin becomes shorter, both the external negative charge and the medium polarity play a smaller role in shifting the absorption maxima, the former because of its increasing distance from the chromophore and the latter because of its minor influence on short chromophores as was shown in Table I.

Studies with synthetic models have clearly shown that nonconjugated negative charges can indeed cause shifts in absorption maxima of polyene iminiums. However, the shifts encountered in longer polyenes are small relative to natural systems. In syn-

(23) (a) Blatz, P. E.; Mohler, J. H. *Biochemistry* **1972**, *11*, 3240. (b) Ebrey, T. G.; Honig, B. *Q. Rev. Biophys.* **1975**, *8*, 129, and references cited therein. (c) Ishikawae, K.; Yoshihara, T.; Suzuki, H. *J. Phys. Soc. Jpn.* **1980**, *49*, 1505.

(24) Gawinowicz, M. A.; Balogh-Nair, V.; Sabol, I. S.; Nakanishi, K. *J. Am. Chem. Soc.* **1977**, *99*, 7720.

(25) Data for the 9-cis isomer.

(26) Bacteriorhodopsins containing cyanine dyes as chromophores have provided strong independent support for the model shown in Figure 1c: Derguini, F.; Caldwell, C.; Motto, M. G.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 646–648.

thetic models every negative charge is accompanied by a positive countercation, the location of which we cannot control, and this positive charge can weaken the influence of this distal carboxylate group. In the protein, this positive countercation is probably located in a fixed position and the interaction between the negative charge and polyene iminium could be much stronger.

This situation can cause a big difference between synthetic models and the natural system. The counteranion and point charges, besides influencing the absorption maxima of visual pigments and bacteriorhodopsin, probably play a major role in determining the absorption maxima of primary photoproducts bathorhodopsin/hypsorhodopsin/K intermediate and other non-photochemical intermediates. Here the distances between the polyene chain, the counteranion, and the point charges can be different from one intermediate to another and will lead to various absorption maxima.

Experiments carried out with the present series of polyene iminium compounds have shown that their absorption maxima are dependent on additional through-space charge effects, the iminium nitrogen counteranion distance, and solvent polarity. In the case of bovine visual pigment and bacteriorhodopsin, the evidence obtained so far indicates that the external point charges are important spectroscopic determinants.[6,8]

Experimental Section

The spectroscopic measurements were carried out with the following instruments: MS (CI), Finnigan 3300, methane as the carrier gas; UV, JaSCO UVIDEC 505; NMR, Bruker WP-80 or WM-250, chemical shifts were reported in ppm on the δ scale relative to an Me_4Si internal standard. Chromatography was performed by using the flash column technique with Merck silica gel 60 (23–440 mesh) eluted with solvents mentioned.

Carboxycyclopentane Ester (14). Diethyl phosphonoacetate (1.34 g) was added dropwise at room temperature to a slurry of 416 mg of 50% NaH in 50 mL of dry THF. The mixture was stirred under argon for 1 h and then 936 mg of *trans*-5-acetyl-1-carboxycyclopentane **13**[15,16] in 5 mL of dry THF was added. The mixture was stirred for 12 h at room temperature under argon atmosphere. The product was extracted with 200 mL of ether and washed twice with 100 mL of water and then with brine. The ether layer was dried and evaporated to dryness. The product was chromatographed and eluted with a 1:1 mixture of ether and hexane to give 880 mg of **14**, 65% yield. The product appeared to be a 7/3 mixture of *Z* and *E* esters by ^1H NMR. NMR ($CDCl_3$) δ 1.2 (t, 2, J = 7 Hz, Me of ester group), 1.8 (s, 3, 7-Me of *Z* isomer), 2.2 (s, 3, 7-Me of *E* isomer), 4.05 (q, 3, CH_2 of ester group), 5.6 (s, 1, 8-H); IR ($CHCl_3$) 2850, 2800, 1710 cm^{-1}; CI-MS 227 $(M + 1)^+$.

Carboxycyclopentane Alcohol (15). Ester **14** (600 mg) was dissolved in 25 mL of dry ether and cooled to −78 °C under argon. The solution was treated dropwise with 8 mL of 20% diisobutylaluminum hydride, and the mixture was stirred for 30 min at −78 °C and then quenched with ethyl acetate. The solution was warmed to room temperature and 200 mL of ether was added. The solution was washed with water, filtered through Celite, washed with brine, dried, and evaporated to dryness. The crude oil was chromatographed and eluted with a 8/1.9/0.1 mixture of hexane/ether/acetic acid to give 315 mg of the *Z* isomer and 130 mg of the *E* isomer. ***Z* isomer**: NMR ($CDCl_3$) δ 1.75 (m, 9, 2-H, 3-H, 4-H, 7-Me), 2.65 (m, 1, 1-H), 3.25 (q, J = 9 Hz, 5-H), 4.15 (m, 2, CH_2 of alcohol), 5.5 (t, J = 7 Hz, 8-H); IR ($CHCl_3$) 3300, 2850, 2800, 1715 cm^{-1}; CI-MS 185 $(M + 1)^+$. ***E* isomer**: NMR ($CDCl_3$) δ 1.75 (m, 9, 2-H, 3-H, 4-H, 7-Me), 2.65 (m, 2, 1-H, 5-H), 4.15 (d, J = 7 Hz, CH_2 of alcohol), 5.45 (t, J = 7 Hz, 8-H); IR ($CHCl_3$) 3300, 2850, 2800, 1715 cm^{-1}; CI-MS 185 $(M + 1)^+$.

Carboxycyclopentane Aldehyde (16). Each isomer of **15** (100 mg) was dissolved in 5 mL of CH_2Cl_2 and was treated at room temperature for 6 h with 300 mg of active MnO_2. The solution was filtered and evaporated to dryness. The crude oil was chromatographed and eluted with a mixture of hexane/ether, 1/1, to give 80 mg each of (*Z*)- and (*E*)-**16**. ***Z* isomer**: NMR ($CDCl_3$) δ 2.00 (m, 9, 2-H, 3-H, 4-H, 7-Me), 2.90 (m, 1, 1-H), 4.05 (q, J = 9 Hz, 5-H), 6.00 (d, J = 7 Hz, 8-H), 10.20 (d, J = 7 Hz, CHO); IR ($CHCl_3$) 2860, 2810, 1720, 1660 cm^{-1}; CI-MS 199 $(M + 1)^+$. ***E* isomer**: NMR ($CDCl_3$) δ 2.00 (m, 6, 2-H, 3-H, 4-H), 2.20 (s, 3, 7-Me), 6 (d, J = 7 Hz, 8-H), 10.2 (d, J = 7 Hz, CHO); IR ($CDCl_3$) 2860, 2810, 1720, 1660 cm^{-1}; CI-MS 199 $(M + 1)^+$.

Cyclopentane Iminium Perchlorate Acid 1.[10] Each isomer of **16** (20 mg) was dissolved in 3 mL of absolute ethanol and cooled to 0 °C, 15 mg of pyrrolidine perchlorate was added, and the mixture was stirred for 10 h at 0 °C. The solvent was evaporated to dryness and the crude oil was triturated with hexane and ether. ***Z* isomer**: NMR ($CDCl_3$) δ 1.95 (m, 9, 2-H, 3-H, 4-H, 7-Me), 2.15 (m, 4, 11∼14-H), 6.35 (d, J = 1.15 Hz, 8-H), 8.65 (d, J = 11.5 Hz, 9-H); IR (neat) 3160, 2840, 1710, 1620 cm^{-1}; λ_{max} (CH_3CN) 276 nm (ϵ 12000). ***E* isomer**: NMR ($CDCl_3$) δ 1.95 (m, 9, 2-H, 3-H, 4-H, 7-Me), 2.2 (m, 11∼14-H), 6.35 (d, J = 11.5 Hz, 8-H), 8.65 (d, J = 11.5 Hz, 9-H).

Basification of 1 in CH_3CN and UV Measurements.[10] Each isomer of **1** (*Z* and *E*) was dissolved in dry CH_3CN in concentrations of ca. 1.5 OD. An excess of NaH was added and the solution was mixed for 2 min. The UV of both isomers shifted from 276 to 297 nm (Chart I); acidification with acetic acid changed the maxima to 276 nm.

Sodium Salt of 1. *Z* aldehyde **16** (10 mg) was treated in acetonitrile with excess NaH for 20 min. The solution was filtered and evaporated to dryness and the crude oil was dissolved in ethanol, cooled to 0 °C, treated with 5 mg of pyrrolidine perchlorate, and stirred for 1 h. The solvent was evaporated and the crude oil was triturated with hexane: λ_{max} (CH_3CN) 297 nm (Δ 11000); NMR (CD_3CN) δ 2.0 (m, 9, 2-H, 3-H, 4-H, 7-Me), 6.35 (d, J = 11.5 Hz, 8-H), 8.65 (d, J = 11.5 Hz, 9-H); IR (neat) 3200, 2890, 1640, 1580 cm^{-1}.

Schiff Base 3. *E* aldehyde **16** was condensed with L(−)-proline perchlorate similarly to the condensation with pyrrolidine perchlorate to give (*E*)- and (*Z*)-**3** as a mixture: λ_{max} ($CHCl_3$) 282 nm (ϵ 10000); NMR ($CDCl_3$) δ 1.95 (m, 9, 2-H, 3-H, 4-H, 7-Me), 6.35 (d, J = 11.5 Hz, 8-H of *E*), 6.55 (d, J = 11.5 Hz, 8-H of *Z*), 8.50 (d, J = 11.5 Hz, 9-H of *Z*), 8.65 (d, J = 11.5 Hz, 9-H of *E*).

Ethyl 3,3-Dimethylacrylate (19a). This was prepared in a manner similar to that of **14**. The compound was eluted with ether/hexane 5/95: NMR ($CDCl_3$) δ 1.2 (t, 3, J = 7 Hz, Me of ethyl), 1.8 (s, 3, Me), 2.1 (s, 3, Me), 4.05 (q, J = 7 Hz, CH_2 of ethyl), 5.45 (s, 1, 2-H); IR ($CHCl_3$) 3000, 1710 cm^{-1}.

3,3-Dimethylacrolein (19b). Acrylate **19a** (100 mg) was dissolved in 10 mL of dry ether, the solution was cooled under argon to −78 °C, and 3 mL of diisobutylaluminum hydride (20%) was added dropwise. The solution was stirred for 30 min at −78 °C and then 2 mL of ethylacetate was added. The solution was brought to room temperature, 300 mg of active MnO_2 was added, and the mixture was stirred for 6 h and then filtered through Celite. Ether (100 mL) was added and the solution was washed with 100 mL of water and brine. The organic layer was dried and solvent was evaporated. The crude oil was chromatographed and eluted with a mixture of hexane/ether, 9/1, to give 55 mg of **19b**: NMR ($CDCl_3$) δ 1.9 (s, 3, Me), 2.1 (s, 3, Me), 5.8 (d, 1, J = 8 Hz, 2-H), 9.85 (d, 1, J = 8 Hz, 1-H); IR (neat) 2890, 1710 cm^{-1}; CI-MS 83 $(M + 1)^+$.

Condensation of 19b with Pyrrolidine Perchlorate and L(−)-Proline Perchlorate to 4 and 2, Respectively. The acrolein **19b** was condensed with pyrrolidine perchlorate and L(−)-proline perchlorate in a manner similar to that for **16**, to give Schiff bases **4** and **2**, the latter as a mixture of *Z* and *E* isomers. Schiff base **4**: λ_{max} (CH_3CN) 268 nm (ϵ 12000); NMR ($CDCl_3$) δ 2.1 (m, 10, 4-H, 1-H, Me's), 6.35 (d, J = 11.5 Hz, 8-H), 8.75 (d, J = 11.5 Hz, 7-H); IR (neat) 1630 cm^{-1}. Schiff base **2**: λ_{max} (CD_3CN) 275 nm (ϵ 13000); NMR (CD_3CN) δ 2.0 (m, 10, 2-H, 3-H, Me's), 5.00 (t, 1, J = 10 Hz, 1-H), 6.25, 6.55 (d, 1, J = 11.5 Hz, 8-H, mixture of *Z* and *E* isomers), 8.8 (d, 1, J = 11.5 Hz, 7-H); IR (neat) 3400, 1720, 1625 cm^{-1}. Deprotonation of **2** was performed by dissolving the compound in dry CH_3CN and adding excess of NaH.

Carboxycyclopentene Triene Nitrile 17 (Scheme I). The five-carbon phosphonitrile (54 mg) derived from 3,3-dimethacrylonitrile was added dropwise at room temperature to a slurry of 12 mg of 50% NaH in 5 mL of dry THF under argon, the solution was stirred for 30 min, and 45 mg of *E* isomer of carboxycyclopentane enal (**16**) was added. The mixture was stirred for 1 h, 100 mL of ether was added, and the solution was washed twice with 50 mL of water and brine. The organic layer was dried and the solvent was evaporated to dryness. The crude oil was chromatographed and eluted with a mixture of hexane/ether, 1/1, to give 40 mg of a mixture of cis and trans nitriles. UV (EtOH) λ_{max} 315 nm (ϵ 24000); NMR ($CDCl_3$) 1.7 (m, 6, 2-H, 3-H, 4-H), 1.85 (s, 3, 7-Me), 2.05 and 2.20 (s, 3, 11-Me, mixture of cis and trans), 2.7 (m, 2, 1-H, 5-H), 6.1 (m, 3, 8-H, 10-H, 12-H), 6.9 (m, 1, 9-H); IR ($CHCl_3$) 2840, 2200, 1710, 1590 cm^{-1}; CI-MS 246 $(M + 1)^+$.

Carboxycyclopentane Triene Aldehyde 18. Nitrile **17** (35 mg) was dissolved in 6 mL of dry hexane, cooled to −78 °C under argon, and treated with 1 mL of 20% diisobutylaluminum hydride. The mixture was stirred for 30 min and warmed to room temperature, and to this was added 100 mL of ether. The solution was acidified with oxalic acid and then washed twice with 50 mL of water. The organic layer was filtered through Celite, washed with brine, and dried. After evaporation to dryness the crude oil was chromatographed and eluted with a mixture of hexane/ether, 1/1. The two isomers (*Z* and *E*) were separated to give 10 mg of the *E* isomer of **18**: λ_{max} (EtOH) 335 nm (ϵ 24000); NMR ($CDCl_3$) δ 1.7 (m, 6, 2-H, 3-H, 4-H), 1.8 (s, 3, 7-Me), 2.25 (s, 3, 11-Me), 2.7 (m, 2, 1-H, 5-H), 5.9 (d, J = 9 Hz, 12-H), 6.1 (d, J = 12 Hz, 8-H), 6.25 (d, 1, J = 15 Hz, 10-H), 7 (dd, 1, J = 12 and 9 Hz, 9-H), 10.1 (d,

J = 9 Hz, 13-H); IR ($CHCl_3$) 2880, 1710, 1660, 1580 cm^{-1}; CI-MS 249 $(M + 1)^+$.

Condensation of 18 with Pyrrolidine Perchlorate and L(−)-Proline Perchlorate To Give 5 and 7, Respectively (Scheme I). Aldehyde **18** (5 mg) was dissolved in absolute ethanol and 2 mg of pyrrolidine perchlorate was added. The mixture was stirred at 0 °C for 7 h, the solvent was evaporated, and the remaining oil was triturated with hexane. The L(−)-proline derivative was prepared similarly, to give a mixture of Z and E isomers. The UV spectra were measured by dissolving the compound in dry $CHCl_3$, taking the spectra, and then adding dropwise a solution of triethylamine in chloroform until a change in the absorption maxima was observed. The solutions were then acidified with acetic acid and the absorption spectra were taken again. Schiff base **5**: λ_{max} ($CHCl_3$) 410 nm (ϵ 33 000), after addition of triethylamine λ_{max} ($CHCl_3$) 420 nm; NMR ($CHCl_3$) δ 1.8 (s, 3, 7-Me), 2.1 (s, 3, 11-Me), 6 (d, 1, J = 11.5 Hz, 12-H), 6.2 (d, 1, J = 12 Hz, 8-H), 6.25 (d, 1, J = 15 Hz, 10-H), 6.9 (dd, 1, J = 12 and 9 Hz, 9-H), 8.8 (d, 1, J = 11.5 Hz, 13-H); IR ($CHCl_3$) 3400, 3000, 1720, 1615, 1570 cm^{-1}. Schiff base **7** (mixture of Z and E): λ_{max} ($CHCl_3$) 413 nm (ϵ 32 000), after addition of triethylamine λ_{max} ($CHCl_3$) 415 nm (ϵ 22 000); NMR ($CDCl_3$) δ 1.80 (s, 3, 7-Me), 2.1 (s, 3, 11-Me), 5.9 (d, J = 11.5 Hz, 12-H E isomer), 6.1 (d, J = 11.5 Hz, 12-H Z isomer), 6.2 (d, 1, J = 12 Hz, 8-H), 6.3 (d, 1, J = 15 Hz, 10-H), 6.9 (dd, J = 12 and 9 Hz, 9-H), 8.8 (d, 1, J = 11.5 Hz, 13-H Z isomer), 8.95 (d, J = 11.5 Hz, 13-H E isomer); IR ($CHCl_3$) 3400, 3000, 1725, 1615, 1570 cm^{-1}.

3,7,7-Trimethyl-2,4,6-heptatriene-1-nitrile (20). The compound was prepared similarly to compound **17** with 3,3-dimethylacrolein as the aldehyde source: λ_{max} (EtOH) 315 nm (ϵ 26 000); NMR ($CHCl_3$) δ 1.2 (s, 6, 7-Me), 2.1 (s, 3, 3-Me of cis isomer), 2.25 (s, 3, 3-Me of trans isomer), 5.8 (1, s, 2-H), 6.2 (m, 2, 4-H, 6-H), 7 (m, 1, 5-H); IR ($CHCl_3$) 2200, 1590 cm^{-1}; CI-MS 158 $(M + 1)^+$.

3,7,7-Trimethyl-2,4,6-heptatrien-1-al (21). This was prepared similarly to **18** by reducing **20** with diisobutylaluminum hydride. The two isomers were separated by chromatography using a 9.5/0.5 mixture of hexane/ether as the solvent. ***E* isomer**: λ_{max} (EtOH) 335 nm (ϵ 28 000); NMR ($CHCl_3$) δ 1.2 (s, 6, 7-Me), 2.3 (s, 3, 3-Me), 5.9 (d, J = 9 Hz, 2-H), 6.1 (d, 1, J = 12 Hz, 6-H), 6.25 (d, 1, J = 15 Hz, 4-H), 7 (dd, J = 12 and 9 Hz, 5-H), 10.11 (d, J = 9 Hz, 1-H); IR ($CHCl_3$) 1660, 1580 cm^{-1}; CI-MS 151 $(M + 1)^+$.

Condensation of 21 with Pyrrolidine Perchlorate and L(−)-Proline Perchlorate To Give Schiff Bases 8 and 6, Respectively. Schiff base **8**: λ_{max} ($CHCl_3$) 406 nm (ϵ 32 000); NMR ($CHCl_3$) δ 1.2 (s, 6, 13-Me), 5.95 (d, 1, J = 9 Hz, 8-H), 6.1 (d, 1, J = 12 Hz, 12-H), 6.25 (d, 1, J = 15 Hz, 10-H), 7 (dd, 1, J = 12 and 9 Hz, 11-H), 8.9 (d, J = 9 Hz, 7-H); IR ($CHCl_3$) 3400, 3160, 2840, 1615, 1520 cm^{-1}. Schiff base **6**: λ_{max} ($CHCl_3$) 415 nm (ϵ 30 000), dropwise addition of a solution of triethylamine in chloroform to the chloroform solution of **6** shifted the λ_{max} to 405 nm; IV ($CHCl_3$) 3400, 3160, 2850, 1710, 1610, 1520 cm^{-1}.

Carboxycyclopentane Pentaene Nitrile 22. The five-carbon phosphonitrile (27 mg) was added dropwise at room temperature to a slurry of 6 mg of 50% NaH in 5 mL of dry THF under argon. The solution was stirred for 30 min and 20 mg of trans isomer of carboxy triene aldehyde **18** (Scheme I) was added. The mixture was stirred for 1 h and treated with 100 mL of ether, and the solution was washed twice with 50 mL of water and brine. The organic layer was chromatographed and eluted with a mixture of hexane/ether, 1/1, to give 16 mg of a mixture of two nitriles trans and 15-cis: λ_{max} (EtOH) 372 nm (ϵ 40 000); NMR ($CHCl_3$) δ 1.25 (m, 2, 3-H), 1.7 (m, 4, 2-H, 4-H), 1.8 (s, 3, 7-Me), 2.0 (s, 3, 11-Me), 2.2 (s, 3, 15-H), 3.5 (m, 2, 1-H, 5-H), 5.82 (s, 1, 16-H cis isomer), 5.88 (s, 1, 16-H trans isomer), 6.15, 6.6, 6.9 (m, 6, olefinic protons); IR ($CHCl_3$) 2850, 2200, 1720, 1580 cm^{-1}; CI-MS 312 $(M + 1)^+$.

Carboxycyclopentane Pentaene Aldehyde (23). The nitrile **22** (10 mg) was dissolved in dry hexane and cooled to −78 °C under argon. Diisobutylaluminum hydride (0.1 mL) was added dropwise and the mixture was stirred for another 30 min and brought to room temperature. The solution was acidified with oxalic acid, 100 mL of ether was added, and the solution was washed twice with water. The organic layer was filtered through Celite, washed with brine, dried, and evaporated to dryness. The crude oil was chromatographed and eluted with ether/hexane, 1/1. Cis and trans isomers were separated to give 3 mg of trans isomer: λ_{max} (EtOH) 378 nm (ϵ 40 000); NMR δ ($CHCl_3$) 1.25 (m, 2, 3-H), 1.7 (m, 4, 2-H, 4-H), 2.00 (s, 3, 7-Me), 2.15 (s, 3, 11-Me), 2.35 (s, 3, 15-Me), 3.5 (m, 2, 1-H, 5-H), 5.95 (d, J = 9 Hz, 16-H), 6.35 (d, J = 16 Hz, 14-H), 6.65 (dd, 1, J = 11.5 and 16 Hz, 9-H), 7.1 (dd, J = 11.5 and 16 Hz, 13-H), 10.1 (d, 1, J = 9 Hz, CHO); IR (neat) 2890, 1720, 1660, 1580 cm^{-1}; CI-MS 315 $(M + 1)^+$.

Hexaene Schiff Bases 9 and 11. Schiff bases **9** and **11** were prepared by condensation of **23** with pyrrolidine perchlorate and L(−)-proline perchlorate, respectively, as described above for **5** and **7**. Schiff base **9**: λ_{max} ($CHCl_3$) 502 nm (ϵ 42 000), dropwise addition of triethylamine in chloroform shifted the absorption maximum to 510 nm, acidification with acetic acid shifted the band to 504 nm; IR ($CHCl_3$) 3400, 3180, 2860, 1710, 1600, 1580 cm^{-1}. Schiff base **11**: λ_{max} ($CHCl_3$) 512 nm (ϵ 41 000), Et_3N shifted the absorption maximum to 502 nm; IR ($CHCl_3$) 3400, 3180, 2860, 1720, 1600, 1580 cm^{-1}.

3,7,11,11-Tetramethyl 2,4,6,8,10-Pentaene 1-Nitrile (24). The compound was prepared similarly to **22**. The aldehyde used for the condensation was the trans isomer of **21**. The two isomeric cis and trans products were separated by elution with ether/hexane, 9/1. The data for trans isomer are as follows: λ_{max} (EtOH) 372 nm (ϵ 39 000); NMR ($CDCl_3$) δ 1.85 (s, 6, 11-Me), 2.03 (s, 3, 7-Me), 2.25 (s, 3, 3-Me), 6.05 (d, 1, J = 8 Hz, 2-H), 6.5 (d, 1, J = 11.5 Hz, 10-H), 6.2 (d, 1, J = 11.5 Hz, 6-H), 6.3 (d, J = 15 Hz, 8-H), 6.55 (dd, 1, J = 15 and 11.5 Hz, 5-H); IR (neat) 2850, 2200, 1570 cm^{-1}; CI-MS 214 $(M + 1)^+$.

3,7,11,11-Tetramethyl 2,4,6,8,10-Pentaene Aldehyde (25). Nitrile **24** was reduced at −78 °C with diisobutylaluminum hydride as described for **23**. The chromatography was done with a mixture of ether/hexane, 1/9: λ_{max} (EtOH) 378 nm (ϵ 41 000); NMR ($CDCl_3$) δ 1.85 (s, 6, 11-Me), 2.05 (s, 3, 7-Me), 2.35 (s, 3, 3-Me), 5.9 (d, 1, J = 9 Hz, 2-H), 6 (d, J = 11.5 Hz, 10-H), 6.2 (d, J = 11.5 Hz, 6-H), 6.3 (d, 1, J = 15 Hz, 8-H), 6.55 (d, 1, J = 15 Hz, 4-H), 6.65 (dd, J = 15 and 11.5 Hz, 9-H), 7.1 (d, 1, J = 15 Hz, 8-H), 10.1 (d, J = 9 Hz, 1-H); IR (neat) 2840, 1640, 1570 cm^{-1}; CI-MS 205 $(M + 1)^+$.

Schiff Bases 12 and 10 by Condensation of 25 with Pyrrolidine Perchlorate and L(−)-Proline Perchlorate, Respectively. Aldehyde **25** was condensed with pyrrolidine perchlorate and L(−)-proline perchlorate as described for compounds **9** and **11**. Schiff base **12**: λ_{max} ($CHCl_3$) 500 nm (ϵ 42 000); IR (neat) 3420, 3180, 2850, 1600, 1520 cm^{-1}. Schiff base **10**: λ_{max} ($CHCl_3$) 516 nm (ϵ 40 000), the absorption maximum shifted to 496 nm when a chloroform solution of Et_3N was added; IR (neat) 3420, 3180, 2860, 1715, 1600, 1520 cm^{-1}.

3,3-(Ethylenedioxy)-7-[3-(ethylenedioxy)propyl]cholest-5-en-7-ol (27). 2-(1-Bromoethyl)-1,3-dioxolane (150 mg) was added at room temperature to a slurry of 10 mg of magnesium in 5 mL of THF. The solution was stirred for 1 h (the magnesium was consumed) and then 150 mg of cholest-5-ene-3,7-dione 3-ethylene ketal (**26**) was added. The mixture was stirred at room temperature for 2 h, the solution was cooled to 0 °C, treated dropwise with a solution of NH_4Cl in water, and treated with 100 mL of ether, and the organic layer was washed twice with 100 mL of water and brine. After the ether layer was dried and the mixture evaporated to dryness, the crude oil was chromatographed and eluted with a mixture of ether/hexane, 8/2, to give 140 mg of **27** as a mixture of two isomers, 7α and 7β. NMR ($CDCl_3$) δ 0.8 (s, 3, 13-Me), 4.0 (m, 8, ketalic H), 4.95 and 5.20 (s, 1, 6-H of 7α and 7β isomers); CI-MS 545 $(M + 1)^+$.

Dienone Acid 28. Bisethylene ketal **26** (100 mg) was dissolved in 100 mL of acetone and 5 mg of p-toluenesulfonic acid was added. The mixture was stirred at room temperature for 2 days, and then without isolation the mixture was cooled to 0 °C and treated dropwise with 2 mL of Jones reagent. The mixture was stirred for 15 min and treated with 150 mL of ether and then with 100 mL of water. The organic layer was washed with water and brine and dried, and the solvent was evaporated. The crude oil was chromatographed and eluted with ether to give 55 mg of dienone acid **28**: λ_{max} (EtOH) 303 nm (ϵ 24 000); NMR ($CDCl_3$) δ 0.8 (s, 3, 13-Me), 5.6 (s, 1, 4-H), 5.95 (br s, 1, 6-H); IR ($CHCl_3$) 2860, 1710, 1640, 1600 cm^{-1}.

Trienal Acid 29. The silylated Schiff base (0.2 mmol) derived from acetaldehyde[18] was added dropwise to a solution of 0.2 mmol of LDA in 10 mL of dry THF under argon at 0 °C. The solution was stirred for 30 min at 0 °C and then cooled to −78 °C; this was treated with 40 mg of acid dienone **28** and the mixture was warmed to room temperature and quenched with 0.2 mL of water. The pH was adjusted to 4 with oxalic acid and the mixture was stirred for 1 h; 100 mL of ether and 100 mL of water were added and the organic layer was washed twice with water and brine. The ether layer was dried and the solvent was evaporated. The crude oil was chromatographed and eluted with ether. The cis and trans isomers were separated to give 15 mg of trans isomer **29**: λ_{max} (EtOH) 355 nm (ϵ 35 000); NMR ($CDCl_3$) δ 0.8 (s, 3, 13-Me), 0.9 (s, 3, 10-Me), 5.8 (d, 1, J = 9 Hz, 2′-H), 5.9 (s, 1, 6-H), 6.0 (s, 1, 4-H), 10.1 (d, 1, J = 9 Hz, CHO); IR ($CHCl_3$) 2860, 1710, 1640, 1580 cm^{-1}.

Condensation of 29 with Pyrrolidine Perchlorate To Give 30. Aldehyde **29** (5 mg) was dissolved in dry EtOH, cooled to 0 °C, and the solution was treated with 1.5 mg of pyrrolidine and stirred for 6 h at 0 °C. The solvent was evaporated and the remaining oil was triturated with hexane 3 times. The absorption maximum of the product was taken in $CHCl_3$, and then a solution of triethylamine in chloroform was added until no change in absorption was detected. λ_{max} ($CHCl_3$) 420 nm (ϵ 32 000); NMR ($CHCl_3$) 0.85 (s, 3, 13-Me), 0.9 (s, 3, 10-Me), 5.8 (d, 1, J = 10 Hz, 2′-H), 6.05 (s, 1, 6-H), 6.2 (s, 1, 4-H), 8.9 (d, J = 10 Hz, 1′-H); IR

(neat) 3420, 3180, 2850, 1710, 1610, 1520 cm^{-1}.

Condensation of Dienone Acid 28 with Silylated Schiff Base To Give Aldehyde Derivative 31. Compound **31** was prepared similarly to compound **29**. The silylated Schiff base was prepared from propionaldehyde. The trans isomer was separated from the cis by chromatography. Trans isomer: λ_{max} (EtOH) 360 nm (ϵ 32 000); NMR ($CDCl_3$) δ 0.8 (s, 3, 13-Me), 0.9 (s, 3, 10-Me), 2.1 (s, 3, 2'-Me), 5.9 (s, 1, 6-H), 6.2 (s, 1, 4-H), 10.1 (s, 1, 1'-H); IR ($CHCl_3$) 2860, 1710, 1630, 1580 cm^{-1}.

Condensation of 31 with Five-Carbon Nitrile Phosphonate To Give Nitrile 32. The five-carbon nitrile phosphonate (25 mg) derived from 3,3-dimethylacrylonitrile was added at room temperature to a slurry of 6 mg of 50% NaH in 4 mL of dry THF. The solution was stirred under argon for 30 min and then 40 mg of acid aldehyde **31** was added. The mixture was stirred for 20 min. The solution was acidified with oxalic acid and 50 mL of ether and 50 mL of water were added. The organic layer was washed with water and brine and dried, and the solvent was evaporated. The crude oil was chromatographed and eluted with ether to give 44 mg of a mixture of cis and trans **32**: λ_{max} (EtOH) 392 nm (ϵ 38 000); NMR ($CDCl_3$) δ 0.8 (s, 3, 13-Me), 0.9 (s, 3, 10-Me), 2.1 (s, 3, 6'-Me), 2.2 (s, 3, 3'-Me of cis isomer), 2.25 (s, 3, 3'-Me of trans isomer), 5.20 and 5.25 (two s, 1, 2'-H of cis and trans isomers), 5.9 (s, 1, 6-H), 6.2 (s, 1, 4-H), 6.3, 6.4 (m, 2, 4'-H, 5'-H); IR ($CHCl_3$) 2850, 2200, 1710, 1580 cm^{-1}.

Reduction of Pentaene Nitrile 32 to Aldehyde 33. Nitrile **32** was reduced by diisobutylaluminum hydride, as in the case of nitrile **17**, and the product was chromatographed by using ether: λ_{max} (EtOH) 395 nm (ϵ 38 000); NMR ($CDCl_3$) δ 0.8 (s, 3, 13-Me), 0.9 (s, 3, 10-Me), 2.1 (s, 3, 6'-Me), 2.25 (s, 3, 3'-Me of cis isomer), 2.35 (s, 3, 3'-Me of trans isomer), 6 (m, 3, 2'-H, 6'-H, 6-H), 6.3 (m, 3, 4'-H, 5'-H, 4-H), 10.15 (d, J = 9 Hz, 1'-H); IR ($CHCl_3$) 2850, 1710, 1640, 1580 cm^{-1}.

Condensation of 33 with Pyrrolidine Perchlorate and L(−)-Proline Perchlorate To Give 34 and 35, Respectively. The condensation was carried out in EtOH as described for **30**. Compound **34**: λ_{max} ($CHCl_3$) 541 nm (ϵ 44 000), upon addition of triethylamine in chloroform the absorption maximum shifted to 545 nm, acidification with acetic acid caused the maximum to shift back to 542 nm; IR (neat) 3410, 3150, 2850, 1710, 1600, 1520 cm^{-1}. Compound **35**: λ_{max} ($CHCl_3$) 565 nm (ϵ 41 000), addition of triethylamine shifted the absorption maximum to 542 nm; IR (neat) 3420, 3150, 2840, 1715, 1600, 1520 cm^{-1}.

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Registry No. (*E*)-**1**, 85441-22-1; (*Z*)-**1**, 85441-24-3; (*E*)-**2**, 85405-80-7; (*Z*)-**2**, 85405-82-9; **3**, 85405-84-1; **4**, 72471-87-5; **5**, 85405-86-3; **6**, 85405-88-5; **7**, 85405-90-9; **8**, 85405-92-1; **9**, 85405-94-3; **10**, 85405-96-5; **11**, 85405-98-7; **12**, 85406-00-4; **13**, 85441-25-4; (*E*)-**14**, 85406-01-5; (*Z*)-**14**, 85441-26-5; (*E*)-**15**, 85406-02-6; (*Z*)-**15**, 85441-27-6; (*E*)-**16**, 85406-03-7; (*Z*)-**16**, 85441-28-7; (11*E*)-**17**, 85406-04-8; (11*Z*)-**17**, 85441-29-8; (11*E*)-**18**, 85406-05-9; (11*Z*)-**18**, 85441-30-1; **19a**, 638-10-8; **19b**, 107-86-8; (3*E*)-**20**, 85406-06-0; (3*Z*)-**20**, 85406-07-1; (3*E*)-**21**, 85441-31-2; (3*Z*)-**21**, 49831-80-3; (15*E*)-**22**, 85406-08-2; (15*Z*)-**22**, 85441-32-3; (15*E*)-**23**, 85406-09-3; (15*Z*)-**23**, 85441-33-4; (3*E*)-**24**, 85406-10-6; (3*Z*)-**24**, 85441-34-5; (3*E*)-**25**, 80172-51-6; (3*Z*)-**25**, 85441-35-6; **26**, 85406-11-7; 7α-**27**, 85406-12-8; 7β-**27**, 85406-13-9; **28**, 85406-14-0; (*Z*)-**29**, 85406-15-1; (*E*)-**29**, 85406-16-2; **30**, 85406-18-4; (*E*)-**31**, 85406-19-5; (*Z*)-**31**, 85421-51-8; (3'*E*)-**32**, 85406-20-8; (3'*Z*)-**32**, 85441-36-7; (3'*E*)-**33**, 85406-21-9; (3'*Z*)-**33**, 85441-37-8; **34**, 85406-23-1; **35**, 85406-25-3; $(EtO)_2POC(CH_3)CHCN$, 85406-26-4; $Me_3SiCH_2CH{=}NCMe_3$, 73198-78-4; $Me_3SiCH(CH_3)CH{=}NCMe_3$, 58707-01-1; pyrrolidine perchlorate, 22401-44-1; L-proline perchlorate, 67877-19-4; 2-(1-bromoethyl)-1,3-dioxolane, 5267-73-2.

Formation of α-Disulfoxides, Sulfinic Anhydrides, and Sulfines during the *m*-Chloroperoxybenzoic Acid Oxidation of Symmetrical *S*-Alkyl Alkanethiosulfinates[1,2]

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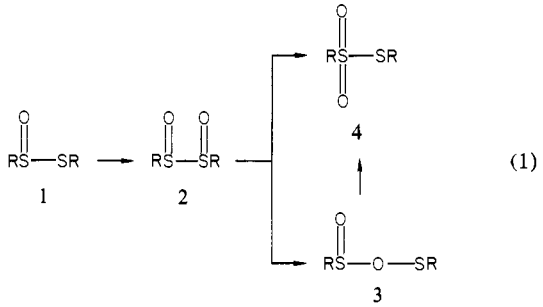
Abstract: The *m*-chloroperoxybenzoic acid (MCPBA) oxidation of *S*-methyl methanethiosulfinate (**33**), *S*-propyl propanethiosulfinate (**34**), *S*-2-propyl 2-propanethiosulfinate (**35**), *S*-butyl butanethiosulfinate (**36**), and *S*-(phenylmethyl) phenylmethanethiosulfinate (**37**) has been studied at low temperatures and compared with the MCPBA oxidation of *S*-(2-methyl-2-propyl) 2-methyl-2-propanethiosulfinate (**26**) and *S*-(2,2-dimethylpropyl) 2,2-dimethylpropanethiosulfinate (**30**). Diastereomeric α-disulfoxides are observed with **33**–**36** at −40 °C, sulfinic anhydrides are observed with **33**, **35**, and **36** at −40 °C, and sulfines are observed on warming the product mixtures from **34**–**37** from −40 °C to −20 °C. The lachrymatory factor ((*Z*)-propanethial *S*-oxide, **47**) of the onion was observed during the oxidation of **35**. The absence of thiosulfonates at −40 °C and their presence at higher temperatures suggest that they are not formed in the initial oxidation process but from subsequent reactions of thiosulfinates and sulfinic acids. Various mechanisms for the formation of intermediates and products are discussed.

Peroxy acids oxidize thiosulfinates (**1**) to thiosulfonates (**4**).[3-14]



(1)

Although α-disulfoxides (**2**) and sulfenyl sulfinates (**3**) have been postulated as transient intermediates, it appears that the mechanism of oxidation varies with the structure of the thiosulfinate

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