If one accepts Benner's evolutionary optimization theory,[13] the present result seems to provide a possibility that the evolution is not a consequence of naturally occurring random phenomena but rather that it lies in the line of scientific inevitability.

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An Artificial Visual Pigment with Restricted C_9-C_{11} Motion Forms Normal Photolysis Intermediates

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The primary event in the photolysis of visual pigments leads to the formation of a new species, bathorhodopsin (batho), characterized by a spectrum which is red-shifted relative to the parent pigment.[1] Batho is stable at temperatures below -140 °C[2] but at higher temperatures is thermally converted in the dark to a series of other intermediates. The sequence of thermal reactions of these intermediates results in an activated form of the pigment which directly initiates visual transduction.[3] There is much evidence that the formation of batho involves a cis-trans isomerization of the 11-*cis*-retinylidene chromophore of the pigment.[4] It has been suggested, however, that such an isomerization would involve a large chromophore geometry change which would be difficult to rationalize with a restricted chromophore pocket of the protein. Thus, models have been proposed in which a number of bonds undergo concerted motions. Warshel has proposed a "bicycle pedal" motion involving several bond rotations which result in isomerization with limited overall shape change of the chromophore.[5] More recently, Liu proposed a model for the isomerization process[6] based on a concerted rotation of the C_{10}—C_{11} single bond and isomerization of the adjacent C_{11}=C_{12} double bond. An analogous approach has been applied to the photocycle of bacteriorhodopsin, suggesting a concerted isomerization (trans → cis) about C_{13}=C_{14} and rotation around C_{14}—C_{15} of its *all-trans*-retinylidene chromophore.[7]

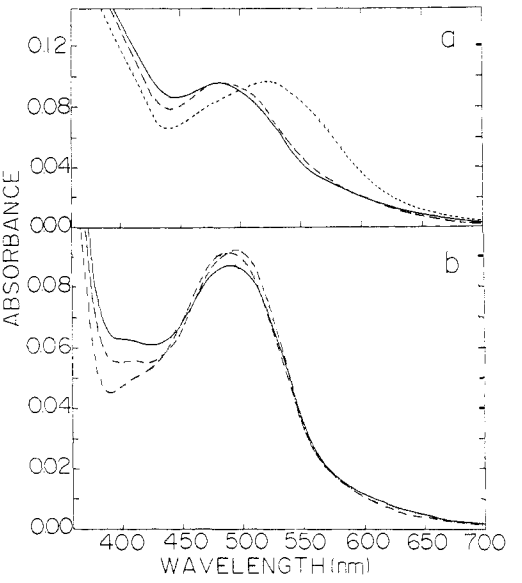
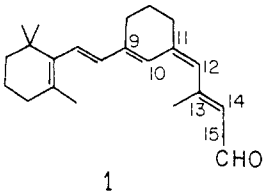


Figure 1. (a) 436-nm illumination of pigment I (2% ammonyx/66% glycerol mixture; pH 6.5) at -130 °C. (—) Spectrum of pigment at -130 °C; (---) illumination for 30 s; (– – –) warming to -85 °C and recooling to -130 °C to monitor the spectrum. (b) 436-nm illumination of pigment I at -85 °C. (—) Absorption of pigment at -85 °C; (---) illumination for 5 min at -85 °C; (–·–) warming to -75 °C and recooling to -85 °C, (•••) warming to -55 °C and recooling to -85 °C.

To test the need for adjacent bond rotation in the photolysis of bovine rhodopsin, we have prepared an artificial visual pigment (I) derived from a chromophore (**1**) whose C_{10}—C_{11} rotation is



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severely hindered by a six-membered ring linking C_9 to C_{11}. A similar approach was first applied by Akita et al.[8] for testing the C_{11}=C_{12} isomerization model. The synthesis of modified retinal **1** has been previously described.[9] Its absorption spectrum in hexane has a λ_{max} of 355 nm and its protonated Schiff base in methanol has a λ_{max} at 445 nm. The extinction coefficients of these transitions are small, about 9500 M^{-1} cm^{-1}. This 11-*cis*-retinal readily forms a pigment when incubated with bovine opsin by standard procedures.[10] No excess of the retinal is needed to form the pigment. The artificial pigment, which absorbs maximally at 485 nm, is stable in 0.1 M hydroxylamine at 20 °C (pH 6.5) and bleaches on exposure to light. The extinction coefficient at 485 nm is about 13000 M^{-1} cm^{-1}, one-third that of native bovine rhodopsin. This extinction coefficient is estimated from the ratio of the 280- and 485-nm absorbances after opsin has been precipitated from an octyl glucoside suspension. This ratio is 3 times as great for the artificial pigment as the equivalent ratio for purified bovine rhodopsin. The "opsin shift" of the artificial pigment, defined as the energy difference between the absorption maximum of a protonated Schiff base in methanol and that of the corresponding pigment,[11] is 1850 cm^{-1}, which approaches that of the native pigment (2650 cm^{-1}) and that of the 11-cis-locked pigment (1950 cm^{-1}).[8] A competition study showed that once a

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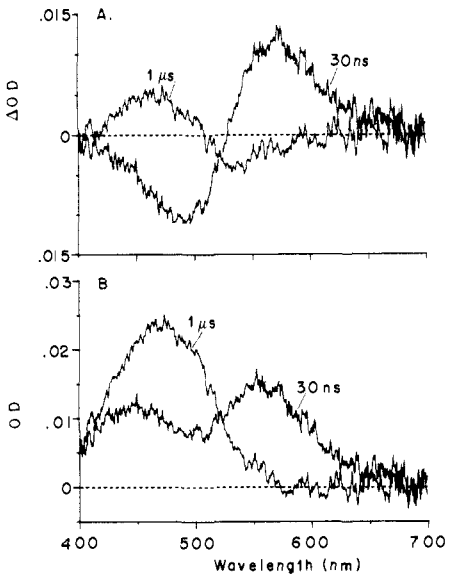

Figure 2. (a) Transient difference spectra observed 30 ns and 1 μs following photolysis of I. (b) Spectra of intermediates present 30 ns and 1 μs after photolysis determined by adding the original pigment spectrum to the difference spectra above. The sample, regenerated ROS solubilized in 2% octyl β-D-glucopyranoside (octyl glucoside), was photolyzed with a 532-nm, 7-ns pulse from a frequency doubled Nd:YAG laser.

pigment is formed, the synthetic chromophore is not displaced from its binding site by 11-cis-retinal. Further, the hypothesis that the synthetic chromophore occupies the true pocket is supported by the fact that the pigment is stable in 1% ammonyx detergent which destroys metarhodopsin III formed from the native pigment.

To test the photochemical behavior of pigment I, we looked for photolysis intermediates using both low-temperature spectra and nanosecond laser photolysis. Low-temperature trapping studies were performed by standard procedures.[12] As shown in Figure 1, 436-nm illumination at −130 °C yields a red-shifted absorption (λ_{max} = 530 nm), which is analogous to that due to the formation of batho in the case of native bovine rhodopsin.[13] Illumination at −85 °C and subsequent warming to −55 °C yield spectra with λ_{max} = 495 and 480 nm, respectively, and with slightly increased extinction. These spectral maxima are very close to those of the lumirhodopsin (lumi) and the metarhodopsin I stages of cattle rhodopsin.[13]

Laser photolysis studies were carried out as described previously,[10] except that 90° excitation was employed with path lengths through the sample of 2 mm for actinic light and 1 cm for the probe beam. Also, to maximize information obtained in the experiment with a minimum amount of material, spectra were obtained using multichannel detection. Figure 2 shows the transient difference spectra and the corrected spectra of the intermediates formed 30 ns and 1 μs after photolysis, which for bovine rhodopsin represent the time scales of batho and lumi.[10,14,15] It is evident that the spectral changes observed in the case of I (with room temperature spectral maxima of batho and lumi intermediates at 560 and 470 nm) are very similar to those of native rhodopsin (560 and 475 nm, respectively[10]). Moreover, as shown in Figure 3, the kinetics of the decay of the 575-nm absorption are very similar to those seen in native rhodopsin.

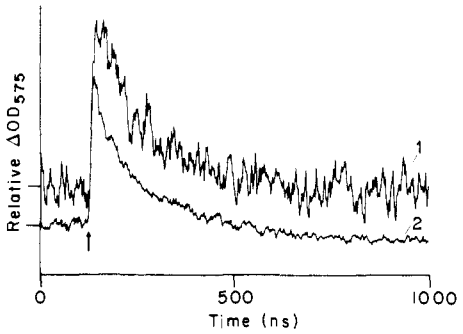

Figure 3. Decay kinetics following photolysis at room temperature of the 575-nm absorption in I (curve 1) compared with that in native rhodopsin (curve 2). Photolysis conditions were the same as those in Figure 2 with the photolysis pulse occurring at the time indicated by the arrow. The signal from I is noisier since it is small due to the low extinction of this pigment. The signal from native rhodopsin is scaled and offset for ease of comparison. The kinetics can be fitted by a single exponential decay of 135 ns.

The major conclusion of this work is that a visual pigment which has C_9=C_{10} and C_{10}—C_{11} bonds locked in their trans configurations produces photolysis intermediates which are very similar to those produced in native rhodopsin. This indicates that a significant rotation of the C_{10}—C_{11} bond is not an essential requirement for the bleaching sequence of rhodopsin to occur. The possibility that other bond rotations accompany C_{11}=C_{12} isomerization will be investigated in further studies.

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Reorientation of the CH_3 Groups and Its High Activation Energy in σ Radical Cations of Alkanes: ESR Observation for *n*-Butane$^+$

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In our first ESR observation of $C_2H_6^+$ and other prototype alkane radical cations,[1,2] we have shown that the methyl group is rather rigid in the σ radical cations such as propane,$^+$ *n*-butane,$^+$ isobutane,$^+$ neopentane,$^+$ etc. This was somewhat surprising because of the deficiency of the C–C σ-bonding electrons, which might make the molecular framework somewhat more flexible. Information on the internal rotation of such one electron-loss species from the σ-bonding orbital must be of fundamental significance in chemistry. In the present work, we wish to report the hindering potential barrier for the CH_3 reorientation in *n*-butane radical cations as the first example for such σ radical cations.

As is presented in our previous paper,[2,3] *n*-$C_4H_{10}^+$ in $CFCl_3$ or $CFCl_2CF_2Cl$ exhibits the three-line spectrum with a coupling constant of 60.0 and 61.3 G, respectively, arising from the two in-plane end protons in the planar extended structure. Shortly later, Wang and Williams have shown that the spectrum of *n*-$C_4H_{10}^+$ in $CFCl_3$ gives resolvable substructure at 150 K and have obtained the coupling constant of 75.6 G,[4] which is considerably

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