# Models for Bacteriorhodopsin-Catalyzed Dark Cis-Trans Isomerization of Bound Retinal

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**Abstract:** The syntheses of *syn*- and *anti*-2-aza-6-carboxymethylbicyclo[2.2.2]octane and *syn* and *anti*-2-aza-6-carboxypropyl[2.2.2]octane zwitterions are described. These compounds, which have secondary amines to form positively charged Schiff bases with retinal and a pendant carboxylate anion to interact with the polyeniminium system, mimic the presence of aspartate-212 and lysine-216 at the retinal binding/cis-trans isomerization site of bacteriorhodopsin. Kinetics of retinal cis-trans isomerization of these model compounds are reported and are discussed in light of previous MNDO calculations (*J. Am. Chem. Soc.* **1987**, *109*, 1627-1631) of isomerization barrier heights affected by the movement of an anion to different positions with respect to the polyeniminium system.

The purple membrane of *Halobacterium holobium* is an energy transducer, converting solar energy to chemical energy through its light-driven proton pump.[1] The membrane contains a protein, bacteriorhodopsin, bR, a single polypeptide chain of 248 amino acids, woven into the lipid bilayer as seven, approximately parallel, rods, each with a high degree of $\alpha$-helical character. The purple color derives from the binding of 1 equiv of *all-trans*-retinal, through a protonated Schiff base to lysine-216 of the protein. The color has been the subject of intense investigation since *all-trans*-retinal itself has an absorption maximum of 368 nm in hexane, while the light-adapted form of bR ($bR^{LA}$) absorbs at 568 nm.[2] Recent experiments suggest that the protonated retinal Schiff base, in a protein pocket within the membrane, interacts with negatively charged groups of the protein to produce the observed bathochromic shift. Negative charges in close proximity to the Schiff base's positively charged nitrogen and the C5 and C7 of the retinal chain appear to account for the wavelength shift.[3]

A photocycle is initiated by the membrane's absorption of a photon the first step of which is a rapid cis-trans photoisomerization of the *all-trans*-retinal to its bound 13-cis isomer.[4] Subsequent steps in the cycle, leading to spectroscopically distinct intermediates, occur thermally and do not require light. During this process protons are ejected from the extracellular side of the membrane followed by the uptake of protons on the opposite side. It has been demonstrted that photoisomerization of the bound *all-trans*-retinal is obligatory for proton pumping.[5] It is obvious therefore that continuous proton pumping depends on the ability of the membrane's bound 13-*cis*-retinal to reisomerize to its all-trans isomer in a nonphotolytic process.

Another dark *cis-trans*-retinal isomerization occurs when light is removed from $bR^{LA}$. Resonance Raman and NMR studies indicate that the configuration about the C15-$NH^+$ double bond in $bR^{LA}$ is anti.[6] In the absence of light, $bR^{LA}$ slowly isomerizes to a dark-adapted form ($bR^{DA}$) in which all-trans and 13-cis are both present. The same studies have identified the configuration about the C15-$NH^+$ for the latter isomer as syn. That neither the all-trans-15-syn nor the 13-cis-15-anti have been reported suggests that a concerted double isomerization occurs presumably by a bicycle-pedal isomerization mechanism. Double isomerization by such a mechanism requires less reorganization of the surrounding protein than would otherwise be necessary were the two double bonds to isomerize in stepwise fashion.[7] Other examples of double isomerization, apparently by a bicycle-pedal mechanism, have been reported from this laboratory.[8] Previous studies have shown that retinal cis-trans isomerization about the C13-C14 double bond, and presumably in concert with isomerization about the C15-N double bond, is a dynamic process, while bacteriorhodopsin is in the dark-adapted state.[9]

Neutron scattering and other studies suggest that the bound retinal resides well inside the membrane and is presumably shielded from water.[10] There is, however, communication between solvent water and the proton of the protonated Schiff base.[11] Because retinal remains bound to the protein throughout the cycle and because of its location in this suprastructure it is reasonable to suggest that dark cis-trans isomerizations are catalyzed by the host protein. Parallel studies of enzyme-catalyzed cis-trans isomerization have prompted us to suggest that nucleophilically catalyzed isomerization is a likely path.[12]

In previous papers we have suggested that a carboxylate anionic group of an aspartate residue, serving as a counterion to the lysine-protonated nitrogen of the retinal Schiff base, could also act as a catalyst in cis-trans isomerizing the bound retinal.[9,13] Catalysis could be brought about by the movement of the carboxylate group from near the positively charged nitrogen to the C13 atom of retinal. MNDO calculations of barriers for cis-trans

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isomerization about the C13–C14 double bond (ordinary) or concerted isomerization about the C13–C14 and C15–N double bonds (double) are high (38 and 50 kcal/mol, respectively) when an anion (e.g., $Cl^-$ or acetate) is held 2.5 Å above the nitrogen, perpendicular to the polyene plane. These calculations also indicate that if the anion is removed from the positively charged nitrogen and placed an equal distance above retinal's C13 and again perpendicular to the polyene plane the barriers to ordinary and double isomerization fall to 11.6 and 15–16 kcal/mol, respectively. If, however, the anion is removed to infinity, the barriers to isomerization are intermediate between the two. This report deals with model systems which attempt to explore the effects on the rate of cis–trans isomerization brought about by changing the position of negative charge with respect to the position of a positively charged retinal Schiff base.

## Experimental Section

**General Methods.** NMR spectra were taken on an AM-300 Bruker spectrometer except where noted otherwise. An HP 5890A gas chromatograph with a megabore capillary column was used for analysis. The gas chromatograph was fitted with a flame ionization detector.

***N*-Benzyl-2-azabicyclo[2.2.2]octan-6-one (1).** Methyl 3-cyclohexenecarboxylate (Frinton Laboratories), treated with *m*-chloroperoxybenzoic acid, was converted to a mixture of *exo*- and *endo*-3-carbomethoxy-7-oxabicyclo[4.1.0]heptane by the method of Henbest[14] and subsequently converted with benzylamine to 3-aza-*N*-benzyl-5-*endo*-hydroxybicyclo[2.2.2]octan-2-one by the method of Huffman:[15] $^1H$ NMR ($CDCl_3$) δ 1.3–2.12 (br m, H-5, H-7, H-8), 2.56 (br s, H-4), 3.36 (H-1), 3.81 (br s, H-6), 4.52 (s, $CH_2C_6H_5$), 7.28 (s, $C_6H_5$); MS 231 ($M^+$). Reduction of the amide with Red-Al (Aldrich Chemical Co.) and oxidation of the resulting hydroxyamine with benzophenone and potassium *tert*-butoxide in toluene by the method of Borne[16] yielded *N*-benzyl-2-azabicyclo[2.2.2]octan-6-one: bp 122/0.35 Torr, yield 62% from the alcohol; IR 1730 $cm^{-1}$; GC/MS $M^+$ 215.

**Diethyl methyl phosphonoacetate** was prepared by the method of Wolinsky and Erickson[17] from 21.7 g of triethyl phosphite and 20 g of methyl bromoacetate: bp 85 °C/0.6 Torr; $^1H$ NMR ($CDCl_3$, 80MHz) δ 1.35 (t, $J$ = 7.1, $CH_3CH_2$), 2.98 (d, $J$ = 21.6, $PCH_2$), 3.75 (s, $CH_3$), 4.18 (d, q, $J$ = 7.1, 8.0, $OCH_2CH_3$); GC/MS 183 ($M^+$ − 27).[18] Also present in a separate GC peak was mass 197 ($M^+$ − 27) indicating the presence of triethyl phosphonoacetate (5%) as an impurity.

***N*-Benzyl-2-aza-6-(carboxymethylene)bicyclo[2.2.2]octane Methyl Ester (2a).** The assembled apparatus which included a three-necked flask, dropping funnel, gas inlet, magnetic stirrer, and thermometer was flamed in a stream of argon. Sodium hydride (1.76 g, 36.6 mmol, 50% in oil) was added followed by 20 mL of dried ether. Diethyl methyl phosphonoacetate (6.84 g, 32.5 mmol) was allowed to drip in slowly so that the temperature did not rise above 20 °C. After addition was complete, the mixture was allowed to stir for 0.5 h. Then 6.2 g (28.8 mmol) of *N*-benzyl-2-azabicyclo[2.2.2]octan-6-one was added dropwise so that the temperature remained below 20 °C. It was allowed to stir for an additional hour after addition was complete. Water (115 mL) was added, and the mixture was extracted with ether (4 × 75 mL) and dried over sodium sulfate. The residue after filtering and ether evaporation was flash chromatographed ($SiO_2$, 70% hexanes/30% ether). GC/MS detected four compounds; the major two had $m/e$ 271 ($M^+$, methyl ester) and two minor had $m/e$ 285 ($M^+$, ethyl ester). The mixture was transesterified by refluxing its solution in 100% ethanol containing 3.3 mL of concentrated sulfuric acid for 23 h. The bulk of the ethanol was removed under reduced pressure and then treated carefully with anhydrous potassium carbonate until near neutrality and then with 50 mL of water followed by more potassium carbonate until strongly alkaline. The mixture was extracted with ether (5 × 50 mL), and the ether extract was dried over sodium sulfate overnight and filtered, and the ether evaporated leaving 7.1 g of an oil. GC/MS: two major peaks were observed in the ratio (early/late) of 1:3.6 with $m/e$ 285 ($M^+$).

***syn*- and *anti*-2-Aza-6-(carbethoxymethyl)bicyclo[2.2.2]octane (3a).** The mixture of early and late isomers from transesterification (4 g) in 200 mL of glacial acetic acid was shaken in a Paar apparatus under a pressure of 3 atm of hydrogen in the presence of 210 mg of 10% Pd/C for 41 h. After filtration and removal of the solvent at reduced pressure, the residual oil was taken up in 50 mL of ether, and saturated potassium carbonate was added to pH 12. The layers were separated, and the aqueous layer was extracted with ether (4 × 50 mL). After drying and removal of solvent 2.3 g of a light yellow oil remained. GC/MS: two major peaks with $m/e$ 197 ($M^+$) were observed with GC area ratio of 1:1.65. The isomer mixture was twice flash chromatographed on $SiO_2$ with 10% diethylamine/90% chloroform as solvent. GC/MS of the separated isomers indicated that the early and late isomers were 97.4 and 97.8% pure, respectively, each with $m/e$ 197 ($M^+$): $^1H$ NMR ($CDCl_3$, 80 MHz) anti isomer δ 1.25 (distorted t, $J$ = 7.1, 4 H), 1.65 (br s, 5 H), 2.32 (m, 4 H), 2.67 (m, 1 H), 3.00 (br s, 2 H), 3.35 (br s, 1 H), 4.08 (q, $J$ = 7.2, 2 H); syn isomer 1.24 (t, $J$ = 7.1), 1.63–1.71 (br s), 1.82–2.33 (multiplet), 2.45 (br s), 2.52 (br s), 2.67 (br s), 2.96 (br s) 3.45 (br s) 4.08 (q, J 7.2).

**NMR Shift Reagent Study.** *anti*-2-Aza-6-(carbethoxymethyl)bicyclo[2.2.2]octane (7.86 mg) was dissolved in 0.772 mg of $CDCl_3$. To this solution was added $Eu(fod)_3$ so that the total concentration of shift reagent was 0.0, 2.64, 5.12, and 7.07 μequiv. A $^1H$ NMR spectrum was taken after each addition. A similar study was carried out with the syn isomer: 6.47 mg in 0.670 mg of $CDCl_3$ followed by total additions of 0.0, 1.64, 2.77, 6.23 μequiv of $Eu(fod)_3$.

***syn*-2-Aza-6-(carboxymethyl)bicyclo[2.2.2]octane Hydrochloride (*syn*-4a).** A heavy walled glass ampoule to which *syn*-2-aza-6-(carboxymethyl)bicyclo[2.2.2]octane ethyl ester and excess 6 N HCl were added was attached to a vacuum line where the contents were degassed by freeze–pump–thaw cycles before the ampoule was sealed. The contents were heated at 110 °C for 48 h. The HCl was evaporated at reduced pressure. The residue was dissolved in water, and the solvent was evaporated. The addition of water and evaporation was repeated twice. The residual solid was recrystallized from isopropyl alcohol–ether (1:1) and decolorized with carbon: mp 211.3–212.6 °C; $^1H$ NMR ($D_2O$) δ 1.30 (dd, 1 H, anti-H-5), 1.72 (m, 2 H, H-8), 1.82–1.99 (m, 3 H, H-7 and syn-H-5), 2.12 (br t, 1 H, H-4), 2.32–2.43 (m, 1 H, anti-H-6), 2.60 (dd, 2 H, $CH_2CO_2D$), 3.18 (br s, 2 H, H-3), 3.42 (br m, 1 H, H-1). Anal. Calcd for $C_9H_{16}NO_2Cl$: C, 52.56; H, 7.84; N, 6.81; Cl, 17.24. Found: C, 52.47; H, 8.54; N, 6.77; Cl, 16.9.

***anti*-2-Aza-6-(carboxymethyl)bicyclo[2.2.2]octane hydrochloride (*anti*-4a)** was prepared by acid hydrolysis of the corresponding ethyl ester as described above for the syn isomer: mp 203.4–204.8 °C; $^1H$ NMR ($D_2O$) δ 1.31 (br d, 1 H, anti-H-5), 1.68 (br d, 2 H, H-8), 1.91 (br t, 2 H, H-7), 2.02 (br s, 1 H, H-4), 2.08 (br m, 1 H, syn-H-5), 2.52 (br s, 3H, $CH_2CO_2D$ and syn-H-6), 3.23 (br s, 2 H, H-3), 3.41 (br s, 1 H, H-1). Anal. Calcd for $C_9H_{16}NO_2Cl$: C, 52.56; H, 7.84; N, 6.81; Cl, 17.24. Found: C, 52.28; H, 8.37; N, 6.67; Cl, 17.2.

**2-Aza-6-(carboxyalkyl)bicyclo[2.2.2]octane zwitterions (5a)** were prepared by ion exchange on Bio-Rad AG 3 × 4A in its hydroxide form. The amino acid hydrochloride (15 mg) in about 1 mL of water was applied to the column (0.7 × 13 cm), and the zwitterion was eluted with 200 mL of water. The bulk of the water was removed at reduced pressure, and the remaining solution was lyophilized to yield a white solid: $^1H$ NMR ($D_2O$) *syn*-2-aza-6-(carboxymethyl)bicyclo[2.2.2]octane δ 1.32 (br dd, 1 H, H-1), 1.70 (br d, 2 H, exo-H-8 and H-4), 1.8–2.1 (m, 4 H, endo-H-8, H-7, and syn-H-5), 2.18–2.32 (m, 1 H, H-6), 2.43 (d, $J$ = 6.7, 2 H, $CH_2CO_2^-$), 3.15 (br s, 2 H, H-3), 3.33 (br s, 1 H, H-1); *anti*-2-aza-6-(carboxymethyl)bicyclo[2.2.2]octane zwitterion δ 1.25 (dd, 1 H, anti-H-5), 1.67 (m, 2 H, H-8), 1.87 (m, 2 H, H-7), 1.99 (m, 2 H, H-4 and syn-H-5), 2.26 (m, 2 H, $CH_2CO$), 2.42 (m, 1 H, H-6), 3.19 (br s, 2 H, H-3), 3.30 (br s, 1 H, H-1).

**(3-(Ethoxycarbonyl)propyl)triphenylphosphonium Bromide.** To 53.7 g (0.205 mol) of triphenylphosphine (Sigma Chemical) in 200 mL of benzene was added 43 g (0.209 mol) of ethyl 4-bromobutyrate (Aldrich). It was refluxed for 51 h. The solvent was removed at reduced pressure, and the solid was extracted continuously with ether leaving behind 64.7 g of solid. The salt was dissolved in $CH_2Cl_2$ at ambient temperature and then precipitated with the addition of half the volume of ether. The solid was dried overnight under vacuum at 120 °C: mp 171–172 °C, lit.[19] 164 °C; $^1H$ NMR ($CDCl_3$) δ 1.23 (t, 3 H, $CH_3$), 1.93 (m, 2 H, $CH_2CH_2CH_2$), 2.91 (t, 2 H, $CH_2CO$), 4.10 (m, 4 H, $OCH_2$ and $PCH_2$), 7.80 (m, 15 H, aromatic).

***N*-Benzyl-2-aza-6-(3-(ethoxycarbonyl)propylidene)bicyclo[2.2.2]octane (2b).** To sodium hydride (Alfa, 5.2 g of a 50% dispersion in oil, 108.3 mmol) in 10 mL of previously dried DMF at 10 °C under an atmosphere of argon was added dropwise a solution of 39.9 g (87 mmol) of (3-(ethoxycarbonyl)propyl)triphenylphosphonium bromide in 60 mL of dry DMF. The mixture was stirred at 10 °C for another 40 min after addition was complete. Then 10.3 g (47.8 mmol) of *N*-benzyl-2-azabicyclo[2.2.2]octan-6-one in 10 mL of DMF was added dropwise. Stirring was continued for 4 h at 10 °C after which the temperature was allowed to rise to an ambient one, and stirring continued for 14 h. The bulk of the DMF was removed under reduced pressure, and then the mixture was

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triturated with 100 mL of ether. Water (25 mL) was added, the solution was shaken, and the layers were separated. The aqueous layer was extracted with ether (5 × 75 mL), and the ether layers were dried over sodium sulfate. The ether was removed, and the residue was flash chromatographed ($SiO_2$) by using hexane/ether (75/25) as the eluting solvent. The product eluted shortly after the starting material. A second and third chromatographic separation was used to purify the product: yield 5.0 g (96%, capillary GC, 16 mmol); GC/MS *m/e* 313 (5.6, $M^+$), 284 (12, $M^+ - Et$), 268 (3.4, $M^+ - OC_2H_5$), 240 (1.5, $M^+ - CO_2C_2H_5$), 226 (21, $M^+ - CH_2CO_2CH_2H_5$), 212 (7.1, $M^+ - CH_2CH_2CO_2C_2H_5$), 91 (100, $C_7H_7^+$); $^1H$ NMR δ 1.25 (t, 3 H, $CH_3$), 1.4–1.8 (m, 4 H, H-7 and H-8), 2.0–2.3 (m, 8 H, $CH_2CH_2CO$, H-5, and H-3), 3.05 (br d, 1 H, H-4), 3.27 (br s, 1 H, H-1), 3.53 (q, 2 H, $CH_2$phenyl), 4.11 (q, 2 H, $OCH_2$), 5.24 (t, 1 H, =CH), 7.22–7.37 (m, 5 H, aromatic). Anal. Calcd for $C_{20}H_{27}NO_2$: C, 76.64; H, 8.68; N, 4.47. Found: C, 76.57, 76.54; H, 8.89, 8.66; N, 4.37, 4.00.

**2-Aza-6-(3-carbethoxypropyl)bicyclo[2.2.2]octane (3b).** *N*-Benzyl-2-aza-6-(3-(ethoxycarbonyl)propylidene)bicyclo[2.2.2]octane (2.65 g, 8.45 mmol) in 100 mL of glacial acetic with 200 mg of 10% Pd/C was reduced on a Paar shaker under 3 atm of hydrogen at 72 °C for 62 h. The catalyst was filtered off, and the solvent was removed by rotorary evaporation. Capillary GC (HP-1, 30 m × 0.53 mm) indicated the presence of an early (5.9%) and late isomer (89%) with retention times differing by about 0.15 min. The residue was dissolved in 50 mL of absolute ethanol followed by 3 mL of diethylamine. The ethanol and excess diethylamine were evaporated off, and the residue was flash chromatographed on silica gel with 10% diethylamine (previously distilled)/90% chloroform. Fractions were analyzed for desired product both by TLC using an iodide–$PtCl_4$ spray and by GC. Purification and separation of isomers required subsequent flash chromatography. The impure early isomer was chromatographed with 6%, 5%, 4%, and then 2% diethylamine at which time GC analysis indicated it to be 100% pure: GC/MS *m/e* 225 (13, $M^+$), 180 (14, –OEt), 138 (15, $-CH_2CO_2Et$), 124 (100, $-CH_2CH_2CO_2Et$), 110 (15), 96 (30), 83 (39), 82 (88), 68 (43); high resolution mass spectrocopy calcd for $C_{13}H_{23}NO_2$ 225.1729, found 225.1735. The more abundant late isomer, similarly purified by repeated flash chromatography (10% diethylamine/90% chloroform), analyzed for 99% purity: GC/MS 225 (22), 180 (18), 138 (22), 124 (100), 110 (18), 96 (32), 82 (63), 68 (24).

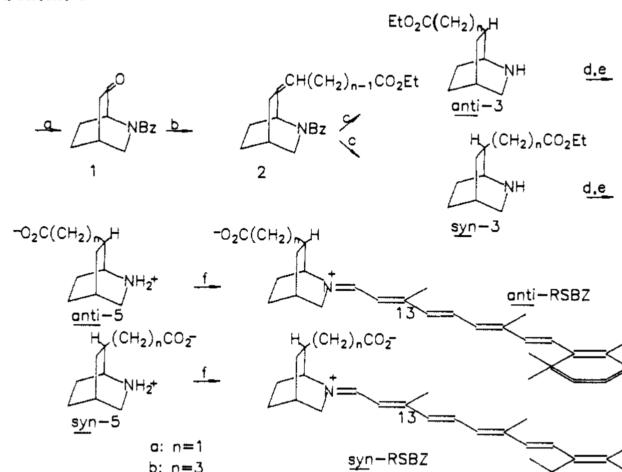
***syn*- and *anti*-2-Aza-6-(3-carboxypropyl)bicyclo[2.2.2]octane hydrochlorides (4b)** were prepared from the corresponding amino acid ethyl esters by hydrolysis in 6 N HCl as described above: anti isomer. Anal. Calcd for $C_{11}H_{20}ClNO_2$: C, 56.53; H, 8.62; N, 5.99. Found: C, 56.03; H, 8.63; N, 5.90. The small quantity of the syn isomer·HCl precluded analysis by combustion, but see high resolution mass spectroscopy analysis above for its precursor.

***syn*- and *anti*-2-Aza-6-(3-carboxypropyl)bicyclo[2.2.2]octane zwitterions (5b)** were prepared by the use of BioRad AG3 × 4A resin as described above.

**Retinal 2-Aza-6-(carboxyalkyl)bicyclo[2.2.2]octane Schiff Bases.** The amino acid zwitterion or its hydrochloride was dissolved in a small amount (250 μL) of ethanol or methanol previously distilled from lithium aluminum hydride. Subsequent operations were performed under dim red light. One-half to one equivalent of *all-trans*-retinal in dry THF was added, and the UV absorption was monitored to ascertain the complete conversion of retinal to its Schiff base as noted by the loss of absorption at 380 nm, the gain in absorption at 450 ± 2 nm, and a minimum peak width at half height at the absorption maximum.

**Kinetics of Retinal Schiff Base Isomerization.** The analysis of isomers was carried out by a method adapted from Groenendijk et al.[20a] and from Lukton and Rando.[20b] The retinal Schiff base zwitterions were formed in situ as described above and diluted with the appropriate volume of solvent to attain the desired concentration. The flask containing the reaction solution was stoppered with a serum cap to avoid solvent evaporation. Aliquot (50–200 μL) were removed periodically with a hypodermic syringe and added directly to 200 μL of a hydroxylamine solution in methanol contained in an Eppindorf microcentrifuge tube. The hydroxylamine solution was prepared by titrating a saturated methanolic hydroxylamine·HCl solution with saturated sodium hydroxide in methanol to near neutrality with use of EM Science colorpHast indicator strips. The solutions were mixed with a Vortex mixer and allowed to stand for 5 min at which time 0.8 mL of heptane was added. The mixture was centrifuged for about 15 s, and the bottom layer was drawn off with a Pasteur pippete. The heptane layer was washed twice, each time with 200 μL of water, and centrifuged. The heptane layers of each aliquot were transferred to vials for automatic injection into the HPLC system. Heptane layers from runs in 1-octanol were washed twice with 200 μL of 90% methanol–10% water to remove the bulk of the octanol and then washed with 200 μL of water before the heptane was injected into the HPLC. Duplicate analyses were carried out for each of 10–14 aliquots taken per kinetic run. A column (0.2 × 25 cm) of 5 μm Nucleosil 50 was used with 90% hexane–10% ether as solvent to separate isomeric retinal oximes. Detection was at 340 nm with an Altex 153 detector. The variation of the percent of the all-trans isomer with time was fitted to a first-order reaction by using the nonlinear least-squares routine of the ASYST software package.

**Scheme I**[a]




[a] Reagents: (a) Four steps from methyl 3-cyclohexenecarboxylate (refs 14–16); (b) for *n* = 1, $NaH/(EtO)_2P(O)CH_2CO_2Et$/ether; for *n* = 3, $NaH/(C_6H_5)_3P(CH_2)_3CO_2Et^+Br^-$/DMF; (c) 10% Pd/C/$H_2$/HOAc//50 °C; (d) 6 N HCl/110 °C; (e) BioRad AG 3 × 4A; (f) *all-trans*-retinal.

**Test for Enamine Formation.** The preparation of the retinal Schiff base zwitterion of anti-**5a** was carried out in 0.5 mL of absolute ethanol as described above. Then 4.5 mL of ethanol-*O-d* (Stohler Chem Co., 99 atom % D, anhydrous), previously distilled from lithium aluminum hydride, was added when the UV spectrum indicated that the Schiff base was fully formed. Reaction was allowed to proceed for 435 min when 2 mL of the reaction mixture was removed and partitioned between 5 mL of $H_2O$ and 4 mL of heptane. The heptane layer was separated and dried over sodium sulfate overnight in the freezer. The heptane solution was applied to the filament of the direct exposure probe of a Finnigan 5100 mass spectrometer and analyzed for masses 284, 285, and 300. A sample of *all-trans*-retinal (Fluka, 95%) was also analyzed in the same way.

In another run the Schiff base was prepared and diluted with anhydrous natural ethanol. It was allowed to proceed for 415 min when 1.75 mL was withdrawn and added to 5 mL of $D_2O$ and allowed to stand for 1 min before 5 mL of heptane was used to extract the retinal. The heptane layer was dried overnight in the freezer over sodium sulfate. *all-trans*- and 13-*cis*-Retinals were separated by HPLC on silica gel 4% ether/hexane solvent. Mass spectrometric analysis was carried out as described above.

## Results

The syntheses of *syn*- and *anti*-2-aza-6-(carboxyalkyl)bicyclo[2.2.2]octanes were carried out according to that shown in Scheme I. *N*-Benzyl-2-aza-6-oxobicyclo[2.2.2]octane (**1**) was prepared according to published procedures. Emmons–Horner reaction was utilized to produce **2a** (*n* = 1) or the Wittig reaction to produce **2b** (*n* = 3). Catalytic hydrogenation in glacial acetic acid with 10% Pd/C at about 70 °C and about 3 atm of hydrogen resulted in reduction and debenzylation to furnish syn and anti isomers of 2-aza-6-(carbethoxyalkyl)bicyclo[2.2.2]octane (**3a**, *n* = 1 or **3b**, *n* = 3). Under these conditions **3a** is produced as a 1:12 syn–anti mixture, whereas for **3b** the ratio is about 1:10, as determined by GC/MS. Isomers were separated and purified by repeated flash chromatography. Subsequent hydrolysis at 110 °C with 6 N HCl gave the amino acid hydrochlorides (**4**). The

(18) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. *Mass Spectrometry of Organic Compounds*; Holden-Day, Inc: San Francisco, CA, 1967; pp 647–650.
(19) Bestmann, H. J.; Koschatzky, K. H.; Schatzke, W.; Susz, J.; Vostrowsky, O. *Liebig Ann. Chem.* **1981**, 1705–1720.
(20) (a) Groenendijk, G. W.; DeGrip, W. J.; Daemen, F. J. M. *Biochem. Biophys. Acta* **1980**, *617*, 430–438. (b) Lukton, D.; Rando, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 4525–4531.

**Table I.** Fragmentation Pattern of *syn*- and *anti*-2-Aza-6-carbethoxymethylbicyclo[2.2.2]octane

| *m/e* | 124 | 152 | 168 | 197 |
|---|---|---|---|---|
| syn | 100 | 33.4 | 9.55 | 32.5 |
| anti | 100 | 4.53 | 9.26 | 24.6 |

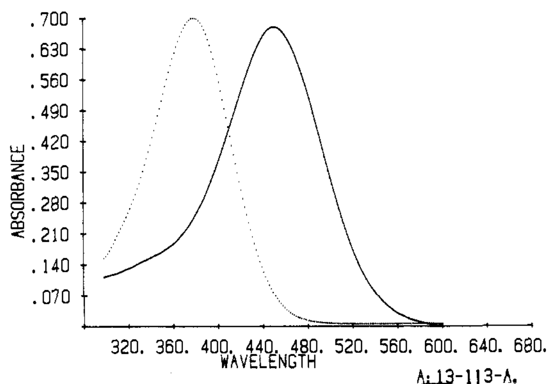



**Figure 1.** Comparison spectra in absolute ethanol of *all-trans*-retinal (dotted line) and *anti*-RSBZ ($n$ = 3, 80% *all-trans*/20% 13-*cis*, solid line) after a 30-min reaction between *anti*-**5b** and *all-trans*-retinal.

zwitterionic bicyclic amino acids (**5**) were obtained through dehydrochlorination on an ion exchange resin. Structural assignments are in accord with elemental analysis and mass and NMR spectral data.

The syn and anti configurations of **3a** were assigned on the basis of their behavior with Eu(fod)$_3$. The rate of change of chemical shift of the ethyl's methylene quartet with increasing concentration of shift reagent was measured. The isomer formed in the smaller amount on reduction of the Emmons–Horner adduct had a slope of 1.09 ppm/mequiv of Eu(fod)$_3$/mequiv of ester (intercept −0.013), while the isomer formed in the larger amount had a slope of 0.336 (intercept −0.0012) measured the same way. The compound exhibiting the larger sensitivity to shift reagent is assigned the syn structure because of the probable chelation effect by nitrogen and oxygen on the same side of the molecule which would increase the association of shift reagent with substrate.

The *syn*- and *anti*-2-aza-6-(carbethoxymethyl)bicyclo[2.2.2]octane have significantly different mass spectral fragmentation patterns (Table I) which appear to be structure-dependent. The parent ion ($M^+$ 197) undergoes loss of ethyl to furnish the 168 peak to about the same abundance for each isomer. The loss of ethoxyl, however, is greater from the less abundant syn isomer, by a factor of about 7.5, than from the anti isomer which suggests an enhanced stabilization of the $M^+$ − OEt ion derived from the syn isomer. These results strengthen the syn/anti assignment since it would appear that the syn isomer $M^+$ − OEt ion could be stabilized by a carbonyl–amine interaction which would be absent in the corresponding ion derived from the anti isomer.

Assignment of stereochemistry for *syn*- and *anti*-**3b** was made on the basis of X-ray crystallographic analysis (results not shown), carried out by Dr. Joseph W. Lauher, SUNY, Stony Brook, on the amino acid hydrochloride derived from the more abundant isomer of **4b**. This was found to have anti stereochemistry, and consequently the less abundant isomer has the syn stereochemistry as in the case of **4a**.

Reaction of each zwitterionic bicyclic amino acid with *all-trans*-retinal in absolute ethanol, distilled from lithium aluminum hydride, leads to the formation of the corresponding zwitterionic Schiff base. Formation of the Schiff base was followed by monitoring the disappearance of the retinal absorption at 380 nm with the corresponding rise in absorption of the Schiff base at about 450 nm as shown in Figure 1.

During Schiff base formation cis–trans isomerization about the C13–C14 double bond also occurs but at a slower rate. Isomerization in these systems proceeds with first- and second-order kinetics (vide infra). By carrying out Schiff base formation at relatively high concentrations of retinal and zwitterion the rate

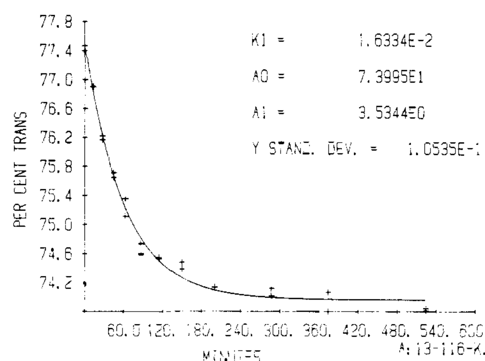



**Figure 2.** Sample kinetics of isomerization of *anti*-RSBZ ($n$ = 3) in octanol/ethanol solvent. Data are fitted to first-order kinetics.

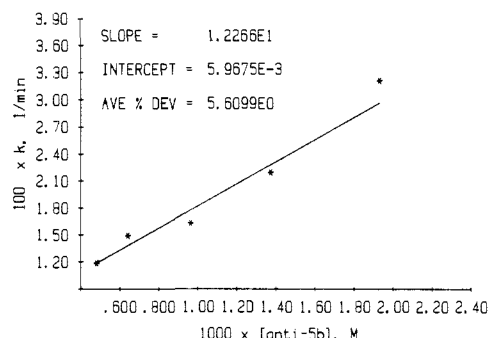



**Figure 3.** Nonlinear least-squares fit of observed pseudo-first-order rate constants vs concentration of *anti*-**5b** in octanol/ethanol solvent.

of formation is enhanced, while the rate of first-order isomerization is unaffected. The rate of second-order isomerization, unfortunately, is also enhanced by the high concentration condition. The latter process is substantially curtailed, however, when after the Schiff base is fully formed, the reacting solution is substantially diluted. First-order isomerization then becomes competitive with the second-order process. Isomerization kinetics were measured on the diluted solutions.

The kinetics of cis–trans isomerization was followed by quenching aliquots of the reaction mixture with hydroxylamine and determining the 13-*cis*/*all-trans*-retinal oxime ratio by HPLC on silica gel upon which syn and anti isomers of each oxime could be separated. It has been shown previously that the integrity of retinal isomer stereochemistry is preserved during and after oxime formation.[20a] Isomer ratios for each aliquot were determined in duplicate. The approach of a cis/trans mixture of Schiff bases to an equilibrium value with time fit pseudo-first-order kinetics. Observed first-order rate constants ($k_{forward} + k_{reverse}$) were obtained by nonlinear least-squares fitting of the data. Sample data for a single run are shown in Figure 2. Observed rate constants are, however, dependent on the initial concentration of bicyclic compound. Typical data for the isomerization of retinal Schiff base zwitterions (RSBZ) are shown in Figure 3, in this case that formed from *anti*-**5b** ($n$ = 3). These data as well as the others show a linear dependence of the observed rate constant on the concentration of bicyclic compound; $k_{obs} = k_1 + k_2$[bicyclic]. The data allow separation of $k_2$ (slope) and $k_1$ (intercept). In addition to kinetic studies in absolute ethanol rates were also measured in 90% 1-octanol/10% ethanol. The data are separated into first- and second-order rate constants, and these are shown in Tables II and III, respectively. Those first-order rate processes which are much slower than their corresponding simulatneous second-order process and which account for a small portion of the total reaction have sizeable errors associated with them.

The possibility that cis–trans isomerization of the retinal Schiff base proceeds through reversible formation of an enamine intermediate was investigated by carrying out the isomerization in ethanol-*O-d*. In ethanol-*O-d*, enamine formation and return to the positively charged Schiff base would be accompanied by retinal–hydrogen exchange. Deuterium incorporation was ex-

**Table II.** First-Order Rate Constants for Cis-Trans Isomerization of Bound Retinal in RSBZ[a]

| | 100% ethanol | | 90% 1-octanol/10% ethanol |
|---|---|---|---|
| | $n = 1$ | $n = 3$ | $n = 3$ |
| syn | $2.9 \pm 1.4 \times 10^{-5}$ (4) | $8 \pm 11 \times 10^{-5}$ (4) | $1.7 \pm 0.03 \times 10^{-3}$ (4) |
| anti | $2.4 \pm 0.3 \times 10^{-3}$ (4) | $2.6 \pm 0.3 \times 10^{-3}$ (6) | $6.0 \pm 1. \times 10^{-3}$ (5) |

[a] Isomerization studies for *all-trans* to an equilibrium mixture of *all-trans*/13-*cis* at 23 °C. Rate constants are for the sum of forward and backward reactions and are in $min^{-1}$. Numbers in parentheses indicate how many kinetic experiments (as in Figure 2) were carried out to disect the $k_{obs}$ vs concentration dependence (as in Figure 3) into first- and second-order isomerization rate constants. Probable errors shown are calculated according to the method given in the following: Worthing, A. G.; Geffner, J. *Treatment of Experimental Data*: J. Wiley & Sons: NY, 1943, pp 249-50.

**Table III.** Second-Order Rate Constants for Cis-Trans Isomerization of Bound Retinal in RSBZ[a]

| | 100% ethanol | | 90% 1-octanol/10% ethanol |
|---|---|---|---|
| | $n = 1$ | $n = 3$ | $n = 3$ |
| syn | $1.7 \pm 0.03$ (4) | $7.0 \pm 0.1$ (4) | $2.7 \pm 0.03$ (4) |
| anti | $5.8 \pm 0.2$ (4) | $5.1 \pm 0.2$ (6) | $12. \pm 0.08$ (5) |

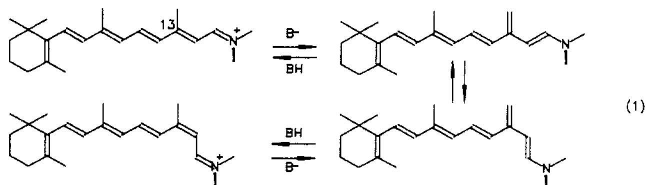
[a] Isomerization for *all-trans* to an equilibrium mixtures of *all-trans*/13-*cis* at 23 °C. Rate constants are for the sum of forward and backward reactions and are in units of $M^{-1}$ $min^{-1}$. Numbers in parentheses indicate how many kinetic experiments (as in Figure 2) were carried out to disect the $k_{obs}$ vs concentration dependence (as in Figure 3) into first- and second-order isomerization rate constants. Probable errors shown are calculated according to the method given in the following: Worthing, A. G.; Geffner, J. *Treatment of Experimental Data*; J. Wiley & Sons: NY, 1943; pp 249-50.

amined by comparing the mass 285/284 intensity ratio of retinal isolated after reaction in the deuterated solvent to that for the untreated retinal. It has been shown, however, that retinoic acid, a contaminant, often found as a result of manipulations during introduction of solid retinal samples into the mass spectrometer, contributes about 30% of its $m/e$ 300 to mass 285,[21] otherwise mainly due to the $^{13}C$ natural abundance of retinal. The 285/284 data were therefore corrected for the presence of retinoic acid. The corrected 285/284 intensity ratio for commercial *all-trans*-retinal (Fluka, 95%) was found to be 0.221.

Isolation of the retinal for these studies requires Schiff base hydrolysis and heptane extraction. Whether deuterium exchange occurs during Schiff base hydrolysis was checked by carrying out that step in $D_2O$. The deuterium incorporation in retinal during isomerization of the retinal Schiff base zwitterion, measured by mass spectrometry, appears to be nil (see Table IV).

## Discussion

In the present study the role of a nearby carboxylate anion in catalyzing cis-trans isomerization of a positively charged retinal Schiff base is investigated. At the outset one mechanism that should be considered is general base catalysis to form an enamine with consequent cis-trans isomerization (eq 1). Such a mech-



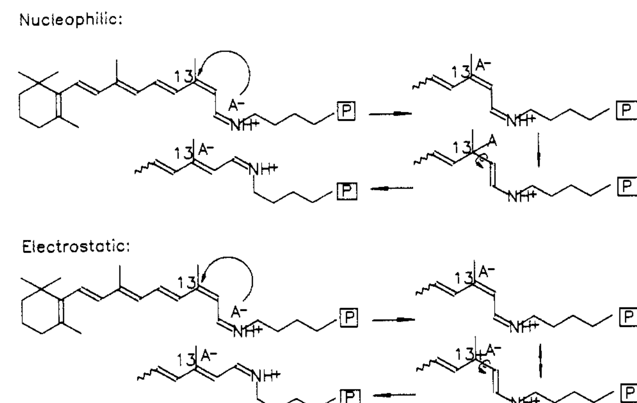

anism appears very unlikely in the native system in view of the finding that after thousands of light-dark adaptations and $10^7$

**Table IV.** Mass Spectrometric Patterns of Retinal Isolated from Reactions in Deuterated Solvent

| solvent | rxtime | workup | $I_{284}$ | $I_{285}$ | $I_{300}$ | corr 285/284 |
|---|---|---|---|---|---|---|
| ethanol-*O-d* | 435[a] | $H_2O$ | 3904 | 912 | 34 | 0.231 |
| ethanol | 415[a] | $D_2O$ | 716 | 151 | 37 | 0.195[b] |
| ethanol | 415[a] | $D_2O$ | 403 | 106 | 56 | 0.221[c] |

[a] Min. [b] *all-trans*-Retinal isolated by HPLC. [c] 13-*cis*-Retinal isolated by HPLC.

**Scheme II**




cycles in light in $D_2O$, no significant deuterium incorporation can be detected in the retinal of bacteriorhodopsin.[22] In model studies of amine-catalyzed retinal cis-trans isomerization the observation of triethylamine catalysis in the isomerization of retinylidene *n*-butylamine suggested an enamine mechanism.[23] Subsequent studies, however, demonstrating a retardation of isomerization of neutral retinal Schiff base by added triethylamine casts strong doubt on enamine formation in that system.[24] In the present study of carboxylate-catalyzed positively charged retinal Schiff base isomerization, the question of an enamine mechanism arises again. An enamine mechanism is ruled out here too. Isomerization in ethanol-*O-d* leads to no deuterium incorporation. Moreover, possible loss of hydrogen label during workup to form retinal oximes for HPLC isomer analysis is discarded since workup in the presence of $D_2O$ indicates no hydrogen exchange in the retinal. The isomerizations observed here appear to proceed through a nucleophilic or an electrostatic mechanism.

In parallel studies of enzyme-catalyzed cis-trans isomerization of maleylacetone, glutathione (GSH), a sulfhydryl coenzyme required for reaction, has been shown to function as a nucleophilic catalyst.[12] The GS group adds to the $\delta$-carbon of an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl system which results in a shift of double bonds to form a dienol intermediate thereby to allow facile rotation about the $\gamma,\delta$ or concerted double rotation about the $\alpha,\beta$- and $\gamma,\delta$-bonds in the intermediate. Loss of the GS group returns the double bonds to their original position but in an isomerized skeleton. For such a mechanism to operate it would seem that nucleophilic attack from above or below the skeletal plane would be preferred to attack from other directions since maximum overlap of the $\pi$-system with the nucleophile can be realized with the perpendicular approach.

A similar mechanism has been proposed for the rapid nonphotolytic 13-cis/all-trans isomerization of retinal.[9,13] As shown in Scheme II, a nucleophile, presumably the carboxylate of aspartate-212 (vide infra), suggested to act as a counterion in stabilizing the positive charge on the Schiff base nitrogen in the low dielectric environment of the membrane, moves from that position to one above or below the C13 atom of retinal. There it can add to C13 to convert double bonds between C13 and the

(21) (a) Lin, R. L.; Waller, G. R.; Mitchell, E. D.; Yang, K. S.; Nelson, E. C. *Anal. Biochem.* **1970**, *35*, 435-441. (b) Seltzer, S.; Lin, M. *Methods Enzymol.* **1982**, *88, Part I*, 543-546.

(22) Lin, M.; Seltzer, S. *FEBS Lett.* **1979**, *106*, 135-138.
(23) Mowery, P. C.; Stoeckenius, W. *J. Am. Chem. Soc.* **1979**, *101*, 414-417.
(24) Lukton, D.; Rando, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 258-259.

nitrogen to single bonds and single bonds to double bonds to permit internal rotation. By reacting with C13 only rotation about the C13–C14 and/or C15–N bonds are facilitated thereby providing a scheme for the strict regiospecificity observed in isomerization in the bacteriorhodopsin system.

Instead of nucleophilic catalysis the carboxylate may function as an electrostatic catalyst in a related mechanism. The carboxylate here is again suggested to move from above the positively charged nitrogen to above retinal's C13. Were this to occur, a large portion of the positive charge, localized on nitrogen in the reactant, would be found on C13 in the intermediate. The negatively charged carboxylate group acts to stabilize charge localization at C13. This in effect makes double bonds between C13 and nitrogen more single bond-like and single bonds more double bond-like with the net effect of a lower barrier to rotation about the original double bonds between these two points. Previous MNDO calculations bear this out.[13] While the nucleophilically and electrostatically catalyzed mechanisms are presented here as two extremes, it is recognized that a mixed mechanism is possible. Because of steric constraints in the protein, the carboxylate of aspartate-212 and retinal's C13 may only partially bond. Charge neutralization in such an intermediate would be incomplete, but additional stabilization would be provided by the electrostatic interaction of the residual charges in the low dielectric environment.

Structural studies suggest a high degree of $\alpha$-helicity for bacteriorhodopsin making it likely that lysine-216 and aspartate-212 lie one above the other.[25] In the low dielectric environment of protein surrounded by lipid the carboxylate of aspartate-212 and the positively charged nitrogen of lysine-216, used to form the retinal Schiff base, would presumably be close enough to form a salt bridge. Movement of the aspartate carboxylate from the nitrogen to retinal's C13 could initiate catalysis as depicted in Scheme II.

In an attempt to test the proposed mechanism and the conclusions drawn from previous MNDO calculations, model compounds with syn geometry were synthesized which incorporate the following features used to mimic the functionality and stereorelationship of aspartate-212 and lysine-216 suggested to be present at the binding/isomerization site of bacteriorhodopsin: (1) a secondary amine to form a positively charged Schiff base with retinal which will maintain its charge independent of solvent basicity, (2) a carboxylate anion group to mimic the presence of aspartate-212, located above the retinal skeletal plane in the Schiff base so that it can interact with the chromophore's $\pi$-system, (3) the carboxylate is bonded to the model's skeleton through a variable length tether to help direct the carboxylate to interact predominantly with specific atoms of the retinal Schiff base (viz. N or C13) in different models. *The syntheses were designed to provide the zwitterions of these compounds so that the only ionic species after reaction with retinal would be the possitively charged Schiff base with its pendant counterion, the carboxylate group.* Exclusion of extraneous ions would eliminate the possibility that any observed catalysis could be caused by agents unrelated to the models. The parallel synthesis of model compounds with amino and carboxylate groups with anti geometry provides a further opportunity to test the effect of an anion held at a more distant site from the Schiff base.

Both *syn*- and *anti*-2-aza-6-(carboxyalkyl)bicyclo[2.2.2]octanes when combined with retinal catalyze cis–trans isomerization about the C13–C14 of the bound chromophore. Isomerization of all the retinal Schiff base zwitterions in the two solvent systems studied, absolute ethanol and 90% 1-octanol/10% absolute ethanol, exhibit kinetics which are dependent on both the first and second power of the zwitterion concentration. The first- and second-order rate constants extracted from these studies are shown in Tables II and III, respectively.

First- and second-order processes are interpreted here to represent the unimolecularly and bimolecularly catalyzed pathways for retinal isomerization. While we have proposed an intramolecular interaction of a nearby carboxylate anion with retinal's C13 atom as a mechanism for catalysis in the protein and in suitable models, it is reasonable to expect that the interaction of a carboxylate ion of one molecule with the polyeniminium group of a second molecule in model compounds would also lead to catalysis. Our primary aim, however, is to test the proposed spatial relationship of aspartate-212 and lysine-216 and its effect on catalysis of isomerization, and therefore the first-order process is the more pertinent of the two to the present discussion.

With a single methylene group in the tether of the *syn*-RSBZ ($n = 1$), simple calculations (i.e., ball and stick-type) with the program MOLPIX using standard bond lengths and angles indicate that on close approach the distance of the carboxylate from C13 is always greater than the corresponding distance of the carboxylate to the positively charged nitrogen.[26] For example, *without regard for attractive or repulsive interactions*, a MOLPIX calculation indicates that on closest approach a carboxylate–oxygen can be 2.7 Å from C13, while at the same time the oxygen would be only 1.7 Å from nitrogen which suggests a stronger interaction of carboxylate with nitrogen than with C13. MNDO calculations predict a very high barrier (38 kcal/mol) for isomerization about the C13–C14 bond when a chloride ion is directly above the nitrogen at a distance of 2.5 Å. (For the most part calculations were carried out with chloride ion instead of acetate as the negative ion to reduce computation time but were found to have almost identical effects on calculated barriers to concerted double isomerization of C13–C14 C15–N double bonds by bicycle pedal rotation.)[13]

In the corresponding *anti*-RSBZ ($n = 1$) the carboxylate is far from the nitrogen and considerably further from retinal's C13. Again using MOLPIX to estimate distances for close approach, the nearest carboxylate oxygen can be 4.0 Å from nitrogen, while at the same time it is 7.0 Å from C13. When the counteranion is absent from the environment of a positively charged Schiff base, the positive charge is delocalized. MNDO calculations predict a lower barrier (15.8 kcal/mol) to isomerization when the anion is absent.[13] We suggest that in the *anti*-RSBZ ($n = 1$) the carboxylate is sufficiently distant so that it is effectively at "infinity". The solvent polarity must also play a role in diminishing the effective interaction between opposite charges distant from one another. The relative magnitudes of the first-order rate constants for *syn*- and *anti*-RSBZ ($n = 1$) mirror the results of the semiempirical calculations.

The tether to the carboxylate was increased to three methylene groups providing *syn*- and *anti*-RSBZ ($n = 3$). The longer arm in the syn isomer could allow closer interaction of the carboxylate with C13 than with nitrogen thereby providing greater catalysis for isomerization than what is observed for *syn*-RSBZ ($n = 1$). MNDO calculations yield a barrier of only 11.8 kcal/mol for isomerization about the C13–C14 bond when chloride ion is held 2.5 Å above C13. The increase in first-order rate constant in ethanol, however, is only modest suggesting that the primary interaction of the carboxylate is still with the positive nitrogen as would be allowed by the flexible arm. A stiffer or longer tether might help direct the carboxylate to approach the C13 more closely than to nitrogen.

The first-order rate constant for isomerization of the *anti*-RSBZ ($n = 3$) under the same conditions is essentially the same as that for the *anti*-RSBZ ($n = 1$) and greater than that for the *syn*-RSBZ ($n = 3$). That the rate constants for *anti*-RSBZ ($n = 1$) and *anti*-RSBZ ($n = 3$) are the same is in accord with the suggestion that the carboxylate ion is already at "infinity" with respect to the polyeniminium system in the *anti*-RSBZ ($n = 1$) arrangement.

(25) (a) Vogel, H.; Gartner, W. *J. Biol. Chem.* **1987**, *262*, 11464–11469. (b) Gibson, N. J.; Cassim, J. Y. *Biochemistry* **1989**, *28*, 2134–2139. (c) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: 1960; p 499. (d) Nemethy, G.; Phillips, D. C.; Leach, S. J.; Sheraga, H. A. *Nature* **1967**, *214*, 363–365.

(26) MOLPIX (Johnson, M. D., Jr. Department of Chemistry, University of South Florida, Tampa, FL 33620) was used to calculate Cartesian coordinates of the atoms for bond lengths and angles entered. Close approach geometries were achieved by allowing stepwise rotations about C–C bonds in the tether.

An increase of the separation distance would have no further effect on increasing the degree of charge delocalization in the conjugated system.

The kinetic studies, described up to this point, of models for cis–trans isomerization of retinal in bacteriorhodopsin were carried out in ethanol ($\epsilon$ = 23.8). Barrier heights to isomerization, however, are calculated for a medium which is a vacuum ($\epsilon$ = 1). Moreover, cis–trans isomerization in bacteriorhodopsin occurs within a membrane where the dielectric is probably lower than it is in ethanol. It is pertinent to ask what the dielectric of the medium at the bacteriorhodopsin site of isomerization might be and what the effect of such a medium will have on the rate of isomerization of the present model compounds. Our proposition that aspartate-212 is involved focusses on the polarity of the medium around retinals's C13 and of the nearby salt bridge of aspartate-212 with the protonated nitrogen of the Schiff base in the reactant. Warshel and co-workers concluded that, in general, the microregion surrounding a salt bridge will be of intermediate polarity.[27] Honig and Hubbel, however, find that a salt bridge will be sufficiently stable in a medium with a dielectric as low as $\epsilon$ = 2–4.[28] It was of interest therefore to study rates of isomerization of the model compounds in a solvent of lower polarity than ethanol.

If catalysis in *syn*-RSBZ ($n$ = 3) is brought about by either a nucleophilic or an electrostatic interaction of an anion with the charged retinal Schiff base, then a decrease in activation energy, consequently an increase in rate, would be predicted if the reaction were measured in a lower dielectric solvent. The reactants, being charged, would become less stable in a lower polarity solvent. In a nucleophilic transition state where reactants are oppositely charged ions, charges are partially destroyed, and thus the transition state with a lower charge density is not destabilized as much as the reactant in going to a lower polarity solvent. The net effect is a reduced activation energy compared to that in a more polar solvent. A similar effect is predicted for an electrostatic mechanism. The transition state is fully charged, but the proximity of positive and negative charge results in an electrostatic stabilization energy which is inversely proportional to the medium dielectric.[29]

First-order rate constants for cis–trans isomerization of *syn*- and *anti*-RSBZ ($n$ = 3) in 90% 1-octanol/10% ethanol are reported in Table II. The rate increase which is anticipated for either mechanism is more than 20-fold for *syn*-RSBZ ($n$ = 3) when the solvent dielectric is halved ($\epsilon$ = 12).[30] The corresponding increase for the *anti*-RSBZ ($n$ = 3) is a little more than two. Note that in this lower polarity solvent the first order rate constant for the syn isomer has increased substantially so that it is within a factor of 3.5 of the rate constant for the anti isomer. Further halving of the medium dielectric could very well make the isomerization of the syn isomer proceed at a faster rate than that exhibited by the anti isomer in that same solvent. That the solvent effect rate increase for syn and anti differ by a factor of ten affirms that there is a difference in isomerization mechanism for the two.

Second-order rate constants (Table III) are suggested to represent bimolecular-catalyzed isomerization paths. These probably involve nucleophilic attack and/or electrostatic stabilization by the carboxylate anion of one molecule interacting with C13 of a positively charged retinal Schiff base of another molecule, or, in the case of *syn*-RSBZ molecules, the carboxylate of one may be paired with the positive charge of a second molecule thereby allowing greater positive charge delocalization in the first and leading to a weaker C13–C14 double bond. Detailed comments concerning individual constants are best withheld at this point since variation of these kinetic constants with structure or solvent polarity is considerably smaller than those encountered in first-order processes.

**Conclusions.** The studies presented here were carried out to test the idea, supported by previous MNDO calculations, that cis–trans isomerization of retinal bound to lysine-216 of bacteriorhodopsin is catalyzed by the carboxylate anion of aspartate-212 moving from a salt bridge with the positively charged nitrogen of the Schiff base where its position is anticatalytic to a position above retinal's C13 where it exerts its catalytic effect. Model systems to represent these two extreme stereochemistries were synthesized. The first-order kinetics of isomerization of these model compounds fit the above picture. The *anti*-RSBZ model compounds represent another possible path that bacteriorhodopsin might use in isomerizing retinal. As predicted by the MNDO calculations, these compounds show that catalyzed isomerization can be achieved when the anion, normally involved in the salt bridge, is moved to a distant position. The effect appears to be due to greater positive charge delocalization in the polyeniminium group.

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