The comparison between polarity influences on $O_2$ and CO binding is quite intriguing. In the bis-pocket porphyrin, where both $P_{1/2}(O_2)$ and $P_{1/2}(CO)$ can be determined in a range of solvents, ln $M$ (which is proportional to the differences in the free energies of the $O_2$ and CO complexes) correlates well with solvent polarity, as shown in Figure 2. Therefore, *this iron complex discriminates against CO binding as binding site polarity increases.* If it is accepted that $O_2$ affinities increase with polarity due to stabilization of charge separation, then it is reasonable to suggest that the dipole moment of the 6-coordinate CO complex must be smaller than the combined moments of free CO and the 5-coordinate complex. This suggests another mechanism by which heme proteins may differentiate between $O_2$ and CO and indicates the importance of the distal binding sites polarity to the ligand affinities of heme proteins.

These observations may also be used to explain in large measure the discrepancies between previous synthetic analogues: those with only moderately polar pockets in nonpolar solvents[3] (e.g., "picket-fence" porphyrins) will have much larger $M$ values than those with open binding sites in polar media[7] (e.g., "chelated protoheme" in aqueous micelles). In addition, our observations are consistent with the change in $M$ reported for the open-faced chelated protoheme in micellar water compared to benzene.[7c,21]

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**Registry No.** FeTPP(Im), 67077-69-4; FeTPP(2-MeIm), 48243-44-3; FeTPP(1,2-$Me_2$Im), 72186-60-8; FeTPP(2-EtIm), 90555-51-4; FeTPP(2-*i*-PrIm), 90583-04-3; FeTPP($Me_4$Im), 90583-05-4; FeTPP, 16591-56-3; FeTTPPP(1,2-$Me_2$Im), 90583-06-5; CO, 630-08-0; $O_2$, 7782-44-7.

# Catalysis of Vitamin A Aldehyde Isomerization by Primary and Secondary Amines

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**Abstract:** Model studies were conducted to quantitatively assess the role of Schiff base formation in catalyzing thermal isomerizations of vitamin A aldehydes (retinaldehyde), processes that are known to be critical for vertebrate vision and proton pumping in certain halophilic bacteria. Schiff base formation by itself, between vitamin A aldehydes and either saturated or aromatic amines, does not strongly enhance the measured thermal rates of isomerization. However, protonation of the Schiff bases strongly enhances their rates of isomerization; at 65 °C the first-order rates of thermal isomerization of 11-*cis*-retinal, the *n*-butylamine Schiff base, and the aniline Schiff base are $2.4 \times 10^{-6}$, $8.0 \times 10^{-6}$, and $2.8 \times 10^{-6}$ $s^{-1}$, respectively. At 25 °C the HCl-catalyzed rates of isomerization of the *n*-butylamine Schiff bases of 11-*cis*-, 13-*cis*-, and 9-*cis*-retinal are $2 \times 10^{-2}$, $3 \times 10^{-2}$, and $9.4 \times 10^{-4}$ $s^{-1}$, respectively. However, the rates of these isomerization reactions appear to be dependent on the strength of the conjugate base because base catalysis is probably required. Trifluoracetic acid proved to be a much weaker catalyst than HCl. Under conditions of approximately equal protonation, the first-order rates of isomerization of the *n*-butylamine and aniline Schiff bases of 11-*cis*-retinal are $2.6 \times 10^{-6}$ and $7.9 \times 10^{-4}$ $s^{-1}$ at 25 °C. This result is most easily understood in terms of the greater nucleophilicity of chloride vs. trifluoroacetate. Adding nucleophilic bases to the protonated primary amine Schiff bases to enhance the rate of isomerization is not possible because the deprotonation of the Schiff base renders base catalysis ineffective. However, Schiff bases formed with secondary amines, such as piperidine, can obviate this problem because their positive charge cannot be neutralized by proton transfer. It is shown here that piperidine also catalyzes the isomerization of vitamin A aldehydes with a pseudo-first-order rate constant of $k = 4.1 \times 10^{-5}$ $s^{-1}$ at 37 °C, but here the rate-limiting step is Schiff base formation itself, rather than the isomerization reactions. The model studies reported here suggest that the physiological mechanism of vitamin A aldehyde isomerization will involve positively charged Schiff base formation followed by nucleophilic attack on the relevant carbon–carbon double bond. The fact that biological molecules, such as reduced flavins and phosphatidylethanolamine (PE), catalyze the isomerizations of the vitamin A aldehydes is in accord with this view.

The mechanisms by which retinoid isomers can be catalytically interconverted are of considerably interest biologically. The initial event in vertebrate visual transduction involves the capture of a photon by rhodopsin, resulting in the isomerization of 11-*cis*-retinal, bound to opsin via a protonated Schiff base, to its all-*trans* congener.[1,2]. Subsequent hydrolysis of the *all-trans*-retinal–opsin Schiff base leads to the formation of *all-trans*-retinal and opsin. This overall process is called bleaching and is the only step in vision where light is directly involved. In order for rhodopsin regeneration to occur, *all-trans*-retinal, or a derivative thereof, must be thermally isomerized to its 11-*cis* congener. This bleaching/regeneration cycle is central to vision and dark adaptation and is an important component in the mechanisms which allow for maintainance of vision over a range of up to perhaps 10 log units in background light intensities.[3] Furthermore, in certain halophilic bacteria, a photochemical transformation of the *all-trans*- to the 13-*cis*-retinylidine Schiff base, probably in the protonated form, coupled with a thermal back reaction, is central to proton pumping and energy production in these organisms.[4] Finally, 13-*cis*-retionic acid is an important drug in the treatment of acne and other skin diseases and appears also to undergo thermal isomerization in vivo to its all-*trans* congener.[5] Of interest here are model systems through which the retinals can be thermally isomerized at ambient temperature which can serve as reference points for the in vivo isomerization mechanisms, whether the latter by enzymatic in nature or not.

To begin with, it is useful to consider the energetics of the isomerization reactions of 11-*cis*-retinal. It is noteworthy that 11-*cis*-retinaldehyde is thermally isomerized with an activation energy of approximately 25 kcal/mol.[6] A lowering of this ac-

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tivation energy by only approximately 5 kcal/mol would allow the isomerization reaction to proceed at room temperature in the absence of enzymatic catalysis. Thus, factors which could stabilize the transition-state complex to a relatively minor degree could allow for the facile chemical isomerization of the retinals. Simple Schiff base formation between the retinals and aliphatic amines has only a small effect on the rate of retinal isomerization.[7] For example, 11-*cis*-retinaldehyde is thermally isomerized with a first-order rate constant of $2.4 \times 10^{-6}$ $s^{-1}$ at 65 °C as opposed to $8.0 \times 10^{-6}$ $s^{-1}$ for the corresponding *n*-butylamine Schiff base under the same conditions.[7] The addition of base retards the isomerization of the imine.[7] Furthermore, it is shown here that aromatic amine retinal Schiff bases are not isomerized significantly more rapidly than the parent retinal itself ruling out possible electron delocalization over the aromatic ring as a possible driving force in the isomerization process. On the other hand, protonation of the Schiff bases might be expected to markedly enhance the rates of the double-bond isomerization. It is shown here that the isomerization of Schiff bases between several retinaldehydes and primary aliphatic and aromatic amines is markedly accelerated by acid and that retinal Schiff bases formed with secondary amines are readily isomerized in the absence of added acid since these retinal Schiff bases already bear a positive charge. The possible physiological significance of this work is discussed in terms of the possible in vivo mechanism of retinaldehyde isomerization.

## Experimental Section

Heptane and chloroform were products of the Aldrich Chemical Co. spectrophotometric grade, with stated purity 99+%. The chloroform was washed three times with distilled water, dried over $CaCl_2$ (protected from light) for 24 h, distilled over $P_2O_5$, and passed through an alumina and column (Fisher adsorption) and protected from light thereafter.

Triethylamine was distilled from calcium hydride, and *n*-butylamine (Aldrich) and piperidine (Aldrich) were distilled from BaO. HCl(g) was a product of Union Carbine (Linde Division) with purity >99%. 11-*cis*-, 13-*cis*-, and 9-*cis*-retinal were generous gifts of Hoffman-LaRoche Inc. All operations with retinal were conducted in dim red light.

**Chromatography.** High-pressure liquid chromatographic (HPLC) separation of the retinal oximes was accomplished by using a 5-μm particle size, 25-cm Hibar II LiChrosorb silica column (Merck, Inc.) with 10% ether/hexane as the eluant at a flow rate of 2 mL/min.[8] For the aldehydes, a flow rate of 1 mL/min was used. A Waters Model M-6000A HPLC pump was used with a Model 440 UV detector containing a 365-nm filter.

**Preparation of Retinal Schiff Bases.** To 1 equiv of retinal in a 5-mL round-bottom flask was added 1.1 equiv of butylamine or aniline in freshly distilled (from $LiAlH_4$) ether. The solution was stirred for 1 h at room temperature, and the solvent was removed on a rotary evaporator. The retinal Schiff base was then dissolved in the appropriate solvent and checked for stereochemical purity via its oxime and for Schiff base formation by protonation with excess trifluoroacetic acid (TFA).[9]

**Conversion of the Retinal Schiff Bases to Oximes.** A saturated solution of $H_2NOH \cdot HCl$ in methanol was adjusted to pH 7 with NaOH (in methanol) with the use of neutral red as the indicator. A sufficient quantity of ethanol was added so that the resulting solution was miscible with heptane. An aliquot (~0.15 mL) of the above solution was added to 1 mL of the retinal Schiff base solution (12 μM) in heptane. The mixture was shaken and washed with water, and the resulting heptane solution was injected onto the HPLC. Oxime formation under these conditions occurred with stereochemical retention, as had already been shown.[8]

**Kinetic Measurements.** For each reaction, and aliquot of the solution was removed at regular time intervals and the retinal Schiff bases were converted to oximes. The relative amount of the isomers present was determined by analysis of the syn oximes, taking into account extinction coefficients of the isomers and assuming a constant syn/anti ratio (8).

**HCl-Catalyzed Retinal Schiff Base Isomerization.** For 11-*cis*- and 13-*cis*-retinal Schiff bases, 0.6 mL of a 24 μM solution of the retinal Schiff base in hexane was added to 0.6 mL of a solution of 40 mM HCl in hexane at 25 °C. After a designated time period, the reaction was quenched with excess triethylamine and the retinal Schiff base converted to the corresponding oximes. For the 9-*cis*-retinal Schiff base, a single 25-mL volume of solution was used, and aliquots were removed therefrom for oxime conversion. The HCl/hexane solution was obtained by bubbling HCl gas into hexane for 10 min, and the concentration of HCl was determined in the aqueous extract of the hexane solution. The relatively high concentration of 20 mM HCl was chosen to produce a convenient rate of 9-*cis*-retinal Schiff base isomerization.

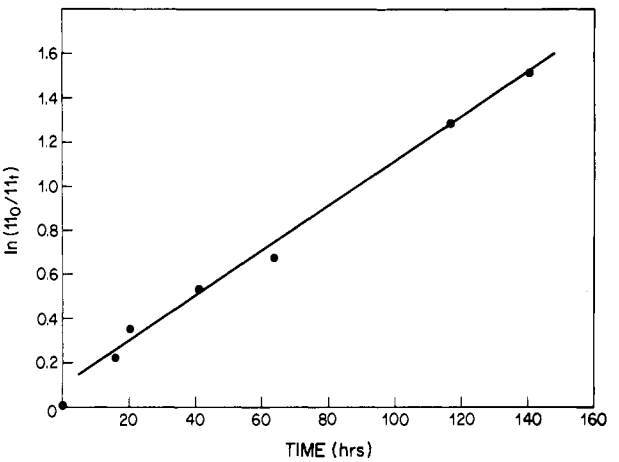


**Figure 1.** Thermal isomerization of the aniline Schiff base of 11-*cis*-retinal. The retinal Schiff base at 12 μM in *n*-heptane was heated at 65 °C. At various times aliquots were removed and the amount of isomerization was determined as recorded in the Experimental Section. In the above graph $11_0$ and $11_t$ refer to the amounts of 11-*cis*-retinal at 0 time and time *t*, respectively.

**Piperidine Perchlorate Catalyzed Isomerization of 11-*cis*-Retinal.** A solution containing 11-*cis*-retinal (12 μM) in 50 mL of chloroform at 37 °C was made 1.74 mM in piperdinium perchlorate and 0.35 mM in piperidine. At regular intervals, aliquots were removed for HPLC analysis and for UV spectra on a Carey 118 spectrphotometer. In all further experiments, 0.65 mL of piperdinium perchlorate and piperidine free base, at the appropriate concentration in chloroform, was added to 0.65 mL of 24 μM 11-*cis*-retinal and the increase in absorbance at 477 nm was monitored. The quantity $\ln (OD_\infty - OD_0)/(OD_\infty - OD_t))$ was plotted as a function of time to obtaine pseudo-first-order rate constants.

**Reaction of 11-*cis*-Retinal Schiff Base with Meerwein's Reagent.** To 5 mL of 14 μM 11-*cis*-retinal Schiff base in dry $CH_2Cl_2$ was added 4 μmol of triethyloxonium fluoroborate (Aldrich) in 0.4 mL of $CH_2Cl_2$. Addition of this reagent resulted in a red shift of the retinal Schiff base to 474 nm. Addition of methanolic $H_2NOH$ to the solution within 30 s after Meerwein's reagent resulted in an oxime mixture containing 15% of the 11-cis isomer, the remainder being primarily *all-trans*- and 13-*cis*-oximes.

## Results

**The Rate of Aromatic Retinal Schiff Base Isomerization.** The isomerization of 11-*cis*-retinal occurs in chloroform with an activation energy of approximately 25 kcal/mol.[6] At 65 °C the first-order rate constant for this isomerization is $2.4 \times 10^{-6}$ $s^{-1}$.[6,7] Under the same conditions the rate for the *n*-butylamine retinal Schiff base of 11-*cis*-retinaldehyde is $8.0 \times 10^{-6}$ $s^{-1}$.[7] Added amines actually decrease this rate. Therefore, the replacement of the carbonyl oxygen by a nitrogen per se has little effect on the measured isomerization rate. If a diradical intermediate or transition state were involved in the thermal isomerization process, it might be predicted that Schiff base formed from an aromatic amine and 11-*cis*-retinal would isomerize at a significantly increased rate because of the electron delocalization over the benzene ring. To test this, the Schiff base between purified aniline and 11-*cis*-retinaldehyde was prepared and thermally isomerized in *n*-heptane at 65 °C.

Surprisingly, this compound actually isomerized more slowly than the *n*-butylamine Schiff base, Figure 1. The first-order rate of isomerization proved to be $2.8 \times 10^{-6}$ $s^{-1}$ at 65 °C—a rate virtually identical with that for 11-*cis*-retinal.[6,7]

**Retinal Schiff Base Protonation and Catalysis of the Isomerization Reaction.** Since neither aliphatic nor aromatic amine Schiff bases led to an enhanced rate of thermal isomerization of the

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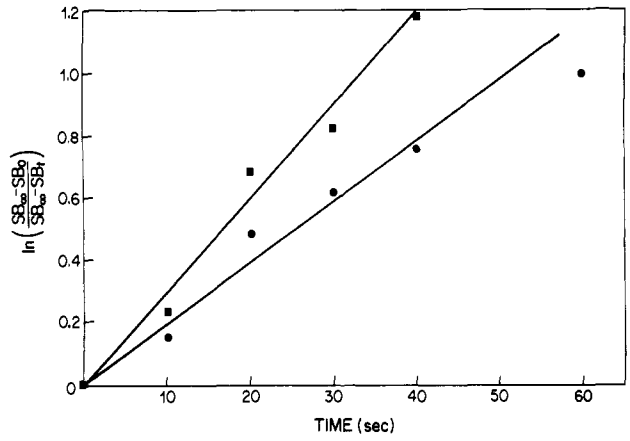


**Figure 2.** HCl-catalyzed isomerization of *n*-butylamine Schiff bases of 13-*cis*- and 11-*cis*-retinal. As recorded in the Experimental Section, 12 μM of each retinal Schiff base (13-cis, ■; 11-cis, ●) was treated with 20 mM HCl in *n*-heptane at 25 °C. At the various times indicated aliquots were removed and the extent of isomerization was determined.

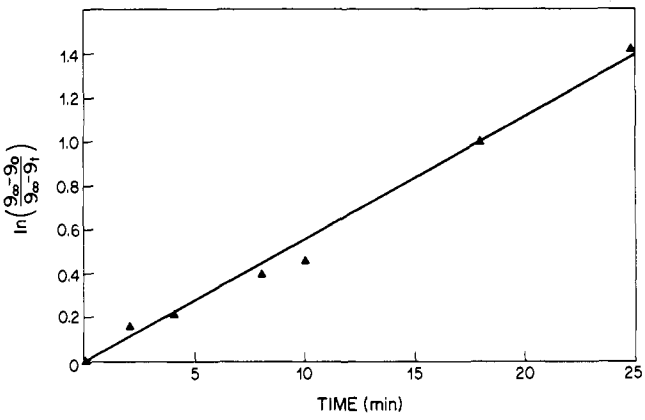


**Figure 3.** HCl-catalyzed isomerization of the *n*-butylamine Schiff base of 9-*cis*-retinal. As in the experiment described in Figure 2, the *n*-butylamine Schiff base of 9-*cis*-retinal (12 μM) was isomerized by 20 mM HCl in *n*-heptane.

retinaldehydes, the effect of protonation of the derivatives was studied. The preformed *n*-butylamine Schiff bases of 11-*cis*-, 13-*cis*-, and 9-*cis*-retinaldehyde were treated with 20 mM HCl in *n*-heptane, and the rates of isomerization of these retinoids were measured. Rather great rate enhancements were found here, with the 13-*cis*- and 11-*cis*-retinoids being isomerized with first-order rate constants of $3 \times 10^{-2}$ and $2 \times 10^{-2}$ $s^{-1}$, respectively, at 25 °C Figure 2. Under the conditions of these reactions, the 11-*cis*-retinal Schiff base was rapidly converted to a mixture of the *all-trans*- and 13-*cis*-retinal Schiff bases. Eventually the equilibrium distribution of the retinoids was reached.[10] The 9-*cis*-retinaldehyde Schiff base was isomerized rather more slowly, with a first-order rate constant of $9.4 \times 10^{-4}$ $s^{-1}$ at 25 °C Figure 3. The fact that the rate for 9-cis is relatively slower than the 11-cis or 13-cis rates probably reflects the fact that the double bond at the 9 position is further removed from the charged head group than either the 11 or 13 double bonds, and thus, has less of a dipole.[11]

The possible role of the conjugate base of the acid can be explored by studying the isomerization of retinoids by trifluoroacetic acid. 11-*cis*-Retinal, its *n*-butylamine Schiff base, and the aniline 11-*cis*-retinal Schiff base were treated with trifluoroacetic acid in chloroform (Table I). The concentrations of TFA added to the retinal Schiff bases are those which fully protonate them, as spectroscopically determined. The decreased rates of isomerization of the retinal Schiff bases by TFA vs. HCL probably

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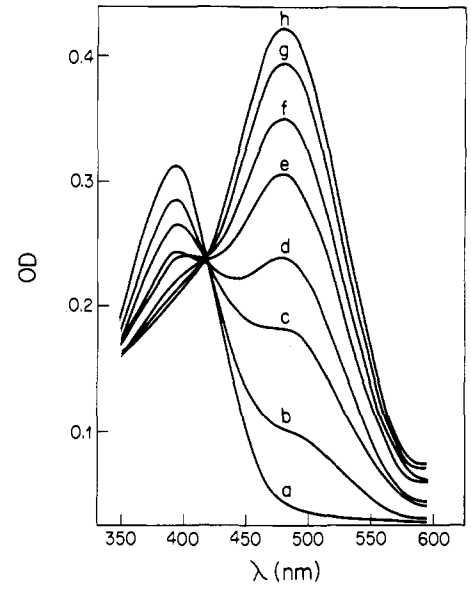


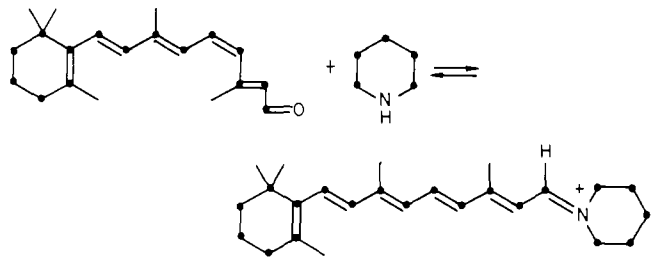

**Figure 4.** Reaction of 11-*cis*-retinal with piperidine. A solution of 11-*cis*-retinal (12 μM) in chloroform and containing piperidine hydrogen perchlorate (1.7 mM) and piperidine (0.348 mM) was monitored spectrophotometrically. Time after the additons of the amine was (a) 0, (b) 25, (c) 55, (d) 85, (e) 120, (f) 160, (g) 210, and (h) 245 min.

**Table I.** TFA-Catalyzed Isomerization of 11-*cis*-Retinal and Retinal Schiff Bases[a]

| retinoid | TFA concn | isomerization rate, $s^{-1}$ |
|---|---|---|
| 11-*cis*-retinal | 36 mM | >0.05 |
| 11-*cis*-retinal | 60 μM | $1.7 \times 10^{-5}$ |
| 11-*cis*-retinal aniline Schiff base | 36 mM | $7.9 \times 10^{-4}$ |
| 11-*cis*-retinal-*n*-butylamine Schiff base | 60 μM | $2.6 \times 10^{-6}$ |

[a] In chloroform, 12 μM 11-*cis*-retinal, the *n*-butylamine Schiff base of 11-*cis*-retinal, or the aniline Schiff base of 11-*cis*-retinal was treated with the indicated concentrations of TFA at 25 °C. The kinetics of isomerization of the retinoids were determined as described in the Experimental Section. The first-order rate constants are recorded above.

**Scheme I**



reflect the decreased nucleophilicity of trifluoroacetate vs. chloride.

**Secondary Amine Catalysis of the Isomerization of Vitamin A Aldehydes.** The experiments performed above suggest that secondary amines should be potent catalysts of the isomerization reactions of the Vitamin A aldehydes, since retinal Schiff base formation automatically yields a positively charged nitrogen (Scheme I). Initial studies suggested that secondary amines would be potent as catalysts of the isomerization reaction. Attempts to prepare the RSB between piperidine and 11-*cis*-retinaldehyde by the usual procedure for the preparation of primary amine retinal Schiff bases, involving the evaporation of equivalent amounts of the amine and the aldehydes, afforded the equilibrium mixture of retinoids.

When primary amines are mixed with vitamin A aldehydes, Schiff base formation occur.[7] This reaction can be followed by UV/vis spectral changes accompanying the reaction.[7] The vitamine A aldehydes absorb at approximately 380 nm and the un-

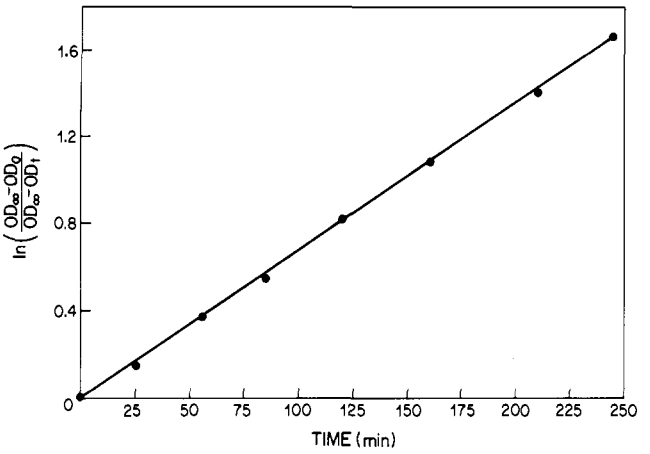



**Figure 5.** Rate of isomerization reaction of 11-*cis*-retinal by piperidine. The ultraviolet data shown in Figure 4 are plotted above to afford the isomerization rate.

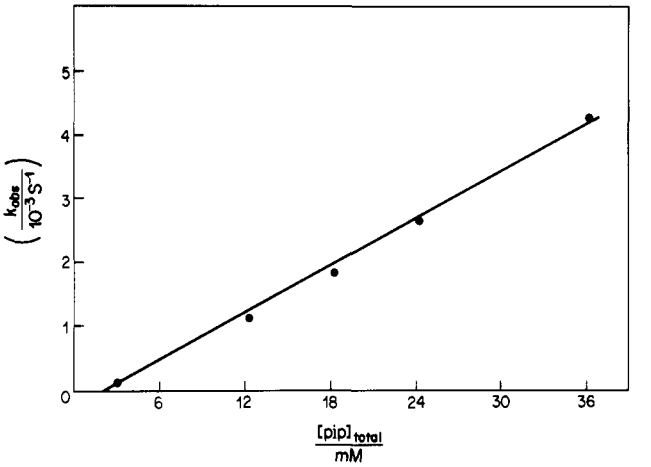
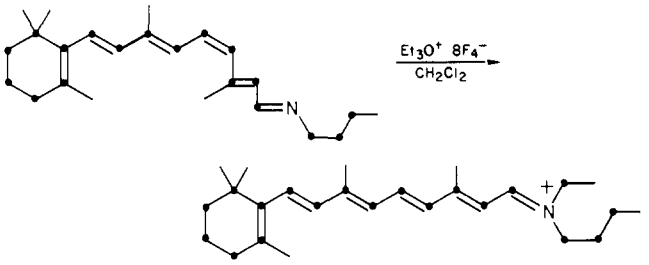



**Figure 6.** Rate of retinal Schiff base formation between 11-*cis*-retinal and piperidine. Pseudo-first-order rate constants were for retinal Schiff base formation between piperidine/piperidine hydrogen perchlorate at different concentrations and 11-*cis*-retinal at 12 μM in chloroform at 25 °C. The resulting $K_{obsd}$ are plotted as a function of total piperidine concentrations, i.e., piperidine $HClO_4$ and free piperidine where the ratio was maintained at 5:1.

protonated retinal Schiff bases at approximately 350 nm.[7] In the case of secondary amine, the RSB absorbs to the red of the aldehydes at 477 nm since the retinal Schiff base must bear a positve charge. As is well-known, positively charged Schiff bases of the vitamin A aldehydes show a bathochromic shift relative to the parent vitamin A aldehydes.[2] When 11-*cis*-retinal was mixed with an excess of piperidine plus piperidine perchlorate, the spectral changes shown in Figure 4 were observed. An increase in absorbance at 477 nm, indicating retinal Schiff base formation, was accompanied by a decline in absorbance at 380 nm due to 11-*cis*-retinal. The species absorbing at 477 nm is, however, not simply the Schiff base of 11-*cis*-retinal but an equilibrium mixture of the 11-*cis*-, 13-*cis*-, 9-cis-, and *all-trans*-retinal Schiff bases. At various times, aliquots of the solution were removed and the amount of remaining 11-*cis*-retinal or 11-*cis*-retinal Schiff base was determined by HPLC. The first-order rate of 11-*cis*-retinoid depletion was measured to be $7.1 \times 10^{-5}$ $s^{-1}$ by this method. Only an approximate rate can be measured from the spectroscopic data because the 477-nm peak represents a mixture of the various isomeric retinal Schiff bases which have different extinction coefficients. However, the approximate rate measured was $1.1 \times 10^{-4}$ $s^{-1}$ (Figure 5), which is in fairly good agreement with the values determined by HPLC (Figure 5). By varying the piperidine concentrations, with a constant 11-*cis*-retinal concentration of 12 μM under pseudo-first-order conditions, a second-order rate constant of 0.12 $M^{-1}$ $s^{-1}$ could be obtained for the rate of retinal Schiff base formation between 11-*cis*-retinal and piperidine (Figure 6). Again, in this instance the formation of the retinal Schiff base occurred concurrently with isomerization.

**Scheme II**




The above experiments suggest that the formation of the charged retinal Schiff bases is probably crucial to the overall isomerization process. This coupling is retinal Schiff base formation to isomerization was also evident from a separate experiment in which the concentration of piperidine was gradually increased from 120 μM to 13 mM at a constant 11-*cis*-retinal concentration. The progress of retinal Schiff base formation and isomerization was determined prior to each successive addition of amine. This experiment showed a one-to-one correspondence between retinal Schiff base formation and the progress of isomerization.

Since retinal Schiff base formation between 11-*cis*-retinal and piperidine appears to be rate limiting in the isomerization process, an effort was made to separate the reactions and measure the isomerization rate directly. Of course, a very rapid rate was expected here. To test this, the *n*-butylamine Schiff base of 11-*cis*-retinal was prepared and treated with Meerwein's reagent (triethyloxonium fluoroborate) at room temperature in methylene chloride.[13] An immediate spectral peak occurred at 474 nm. The latter absorption is for the charged retinal Schiff bases (Scheme II). Interestingly, treatment of the mixture with hydroxylamine, to form the retinal oximes and HPLC analysis, showed that greater than 80% isomerization of the starting material had occurred after only a 30-s incubation. This suggests that the isomerization process is virtually instantaneous. This experiment is thus consistent with what had been found earlier with piperidine.

## Dicussion

The studies reported here were motivated by the possibility of designing model systems which would allow for the isomerization of the retinals at ambient temperature. A simple physiologically relevant way to achieve rate enhancement for the isomerization reaction if by retinal Schiff base formation. It is well-known that in vivo the retinals are often found as retinal Schiff bases, and indeed a relatively recent report in the literature claimed to have observed striking rate enhancement for retinal isomerization by simply forming neutral retinal Schiff bases.[14] However, we have found that simple Schiff base formation with a primary amine did not lead to a very large enhancement of the rate for the isomerization reaction in freshly distilled chloroform.[7] The first-order rate of isomerization of 11-*cis*-retinal proved to be $2.4 \times 10^{-6}$ $s^{-1}$ at 65 °C and that for the *n*-butylamine retinal Schiff base was $8 \times 10^{-6}$ $s^{-1}$ under the same conditions.[7] Added bases such as triethylamine or *n*-butylamine actually decreased the rate of this isomerization process, suggesting the possibility that the observed rate enhancement could have been due to trace amounts of acid present in the chloroform.[7] The observation that Schiff base formation in and of itself does not lead to substantial isomerization rate enhancements is perhaps not unexpected in light

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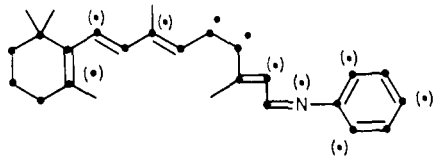
**Scheme III**

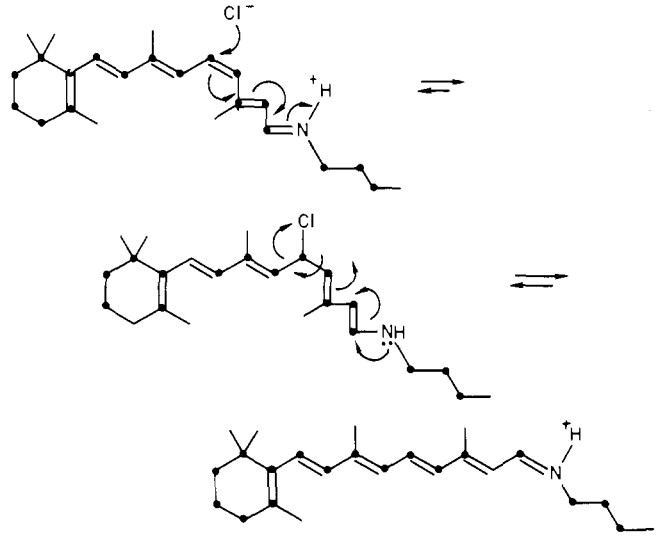


of the fact that the transition state for the thermal isomerization, perhaps possessing a diradical character, cannot be stabilized in an obvious way by the imine nitrogen.

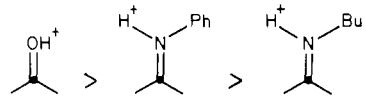
A putative diradical intermediate must already be substantially stabilized by the conjugated double-bond system, because the activation energy for the isomerization is some 40 kcal/mol lower than that for the isomerization about the unconjugated double bond of ethylene.[15] A possible strategy for the stabilization of this intermediate would, of course, be to utilize an R group which would itself delocalize and hence stabilize the putative transition-state diradical. An obvious possibility here would be to use aromatic amines such as aniline to form retinal Schiff bases, Scheme III. Here the diradical would be further delocalized over the benzene ring. Interestingly, however, the aniline Schiff base of 11-*cis*-retinal actually isomerized more slowly than the *n*-butylamine Schiff base and at about the same rate as 11-*cis*-retinal itself, Figure 1. A possible explanation of this observation is that the aromatic ring stabilizes the ground state of the retinal Schiff base approximately the same as the transition state, nullifying the possiblity of a lowering of the activation energy. This observation probably eliminates an in vivo mechanism for the catalysis of the isomerization process, requiring Schiff base formation between a retinal and any of the many physiologically occurring aromatic amines, such as adenine, guanine, or folic acid.

The experiments discussed above unequivocally demonstrate that Schiff base formation in and of itself will not promote the isomerization of retinal either in the presence or in the absence of added nucleophiles. The situation is dramatically different with protonated retinal Schiff bases, however. That this might be the case could be gleaned from reports on ultraviolet and visible spectral studies on protonated retinal Schiff bases, where low temperatures were required to obtain signals from a pure isomer.[16] The addition of HCl to a *n*-heptane (a solvent chosen for its closeness to the hydrocarbon matrix of cell membranes) solution of *all-trans*-, 11-*cis*-, and 13-*cis*-*n*-butylamine retinal Schiff bases leads to the ready isomerization to these compounds. The rates of isomerization of the 13-cis and 11-cis congeners were similar and approximately 50-fold faster than that for the 9-cis isomers. This result is a likely consequence of the decreased carbonium ion character exhibited by carbon atoms as they are further removed from the positively charged nitrogen.[11] The fact that the 9-cis isomer isomerizes relatively slowly appears to be matched in its slow rate of formation from the acid-catalyzed isomerization of *all-trans*- or 13-*cis*-retinal *n*-butylamine retinal Schiff bases.[10,17] Starting from either of these molecules, a kinetically controlled steady-state mixture including 11-*cis*-retinal is generated prior to the formation of 9-*cis*-retinal Schiff base.[10,17] A plausible mechanism for the isomerization process taking into account the facts just described is shown in Scheme IV. Another possible mechanism could involve a tautomerization scheme involving the 13- and 9-methyl groups. This kind of mechanism if considered less likely since chloride would not be expected to be a powerful enough base to abstract an H from a methyl group. Furthermore, when *all-trans*-retinal was treated with excess trifluoroacetic acid-*d*, no deuterium incorporation into the retinoids was recorded even though isomerization had occurred.[11] An enolization mechanism would necessitate deuterium incorporation concomitant with isomerization.

**Scheme IV**



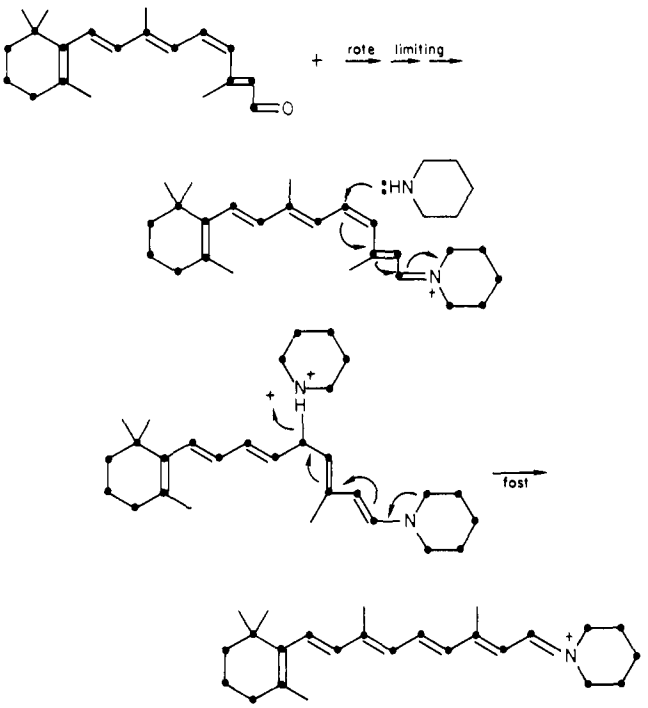
The notion that the isomerization mechanism involves a nucleophilic attack is supported by the trifluoroacetic acid catalyzed isomerization of 11-*cis*-retinal and the *n*-butylamine and aniline Schiff bases derived from it. The experiments were performed in chloroform under conditions where the retinoid was fully protonated. This was easy to determine with the Schiff bases because of the large bathochromic shifts in absorption spectra found concomitant with protonation.[9,12] However, with 11-*cis*-retinal the determination was not easily made, but nevertheless at 36 mM TFA a new peak at 410 nm was observed which would represent at least hydrogen bonding between the aldehyde and TFA. In any event, the isomerization of the 11-*cis*-retinal at 36 mM TFA was quite rapid; after 15 s less than 5% of the 11-*cis*-isomer was present, the remainder being primarily *all*-trans- and 13-*cis*-retinal. From this a rate constant of $\gg 0.05\ s^{-1}$ could be surmised. Under conditions of "complete" protonation (5 equiv of TFA) the isomerization of the *n*-butylamine retinal Schiff base was exceedingly slow. Only about 30% isomerization was observed over the course of 36 h. Although steps were taken to remove residual HCl from the $CHCl_3$, the possibility of HCl formation over the course of the reaction could not be ruled out. HCl has been found to be an effective catalyst and may be a contributing factor. Hence the rate of TFA isomerization must be viewed as an upper limit. It should be noted that even at identical concentrations of TFA (60 $\mu$M), the rate of 11-*cis*-retinal isomerization is faster than that of the *n*-butylamine retinal Schiff base ($1.7 \times 10^{-5}$ vs. $2.6 \times 10^{-6}\ s^{-1}$), elimimating the possibility that the observed differences in rate are simply related to the concentrations of TFA. The rate of isomerization of aniline retinal Schiff base at full protonation (36 mM) was more than two orders of magnitude more rapid ($7.9 \times 10^{-4}$ vs. $2.6 \times 10^{-6}\ s^{-1}$) than that for the saturated butylamine retinal Schiff base. It was also much slower than the rate of 11-*cis*-retinal isomerization at the same concentration of acid. The rank order of isomerization reactivity observed here, namely



is as expected and goes as the electronegativity of the electronegative atom (O or N). Briefly stated, if the ground state for the isomerization reactions is more charged than the transition state, then the greater the difference between the two, the greater the basicity of the substrate. For example, it has been found that protonated ketones enolize approximately three orders of magnitude more rapidly than the analogous protonated saturated Schiff bases.[18]

(15) Morrison, R. T.; Boyd, R. N. "Organic Chemistry"; 2nd ed.; Allyn and Bacon, Inc.: Boston, MA, 1967; p 146.
(16) Schriver, J. W.; Mateescu, G. D.; Abrahamson, E. W. *Biochemistry* **1979**, *18*, 4785–4792.
(17) Rando, R. R.; Chang, A., unpublished experiment.
(18) Bender, M. ..; Williams, A. *J. Am. Chem. Soc.* **1966**, *88*, 2502–2508.

**Scheme V**

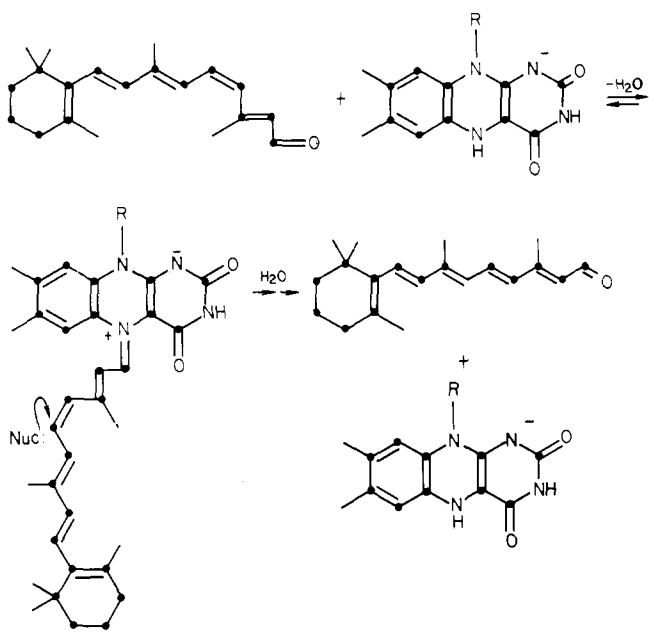



It is important to note that the isomerization rate of the *n*-butylamine retinal Schiff base was much slower with TFA as a catalyst than with HCl, even though the TFA reaction was done in the more polar solvent chloroform to achieve a measurable rate. This result is consistent with the notion that nucleophilic attack by the conjugate base on the protonated retinal Schiff base is required for effective catalysis to occur. Chloride ion is, of course, a much more potent nucleophile than trifluoroacetate. It might be expected that as nucleophilicity of the base was increased, the rate of the isomerization reaction would increase. This mode of catalysis could be the correct one for an enzyme but not for the aaforementioned RSB model system, because a strongly basic nucleophile would simply deprotonate the Schiff base and the possibility of catalysis would be eliminated. In fact, the rates of the acid-catalyzed *n*-butylamine retinal Schiff base isomerization were effectively quenched by the addition of triethylamine. Use of secondary amines to form the retinal Schiff bases would remove this obstacle, however, since the nitrogen would be rendered permanently positively charged. This suggests that secondary amines would be exceedingly potent catalysts of the isomerization reaction, and this supposition was borne out in the experiments reported here.

In the case of the primary amine retinal Schiff bases, nucleophilic catalysis was limited to relatively weak bases ($Cl^-$, $CF_3CO_2^-$). Highly nucleophilic amines could not be added because they would be preferentially protonated, being approximately 2 $pK_A$ units more basic than the Schiff bases derived from them.[19] On the other hand, a Schiff base formed from a secondary amine constitutets an exceedingly electrophilic system, which should undergo nucleophilic catalysis quite readily. This could be gleaned from the fact that it was not possible to prepare the retinal Schiff base of 11-*cis*-retinal and piperidine by the method used to prepare the *n*-butylamine retinal Schiff base—namely mxing the amine together with 11-*cis*-retinal in a solvent and evaporating the solvent to dryness. Under these conditions, 11-*cis*-retinal has been isomerized to yield an approximate equilibrium mixture of retinoids. Upon mixing excess piperidine with 11-*cis*-retinal in chloroform, the retinal Schiff base was smoothly formed. The charged retinal Schiff base had a $\lambda_{max}$ = 477 nm, which showed a marked bathochromic shift when compared to the *n*-butylamine retinal Schiff base, which had a $\lambda_{max}$ of approximately 360 nm.[7]

(19) Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 832–837.

**Scheme VI**




As is well-known, however, protonation of uncharged retinal Schiff base shifts its absorption spectrum to approximately 480 nm.[2] It was also found that formation of the retinal Schiff base between excess piperidine and 11-*cis*-retinal occurred concurrently with isomerization. Thus it is apparent that one of the reactions in retinal Schiff base formation itself is actually rate limiting, probably carbinolamine dehydration, a situation quite unlike that found in the case of the primary amine retinal Schiff bases. A plausible mechanism for the isomerization reaction is shown in Scheme V.

Since Schiff base formation appeared to be rate limiting in the isomerization process, an independent method of accessing the isomerization rate was sought. The method chosen involved the reaction of Meerwein's reagent (triethyloxonium fluoroborate) with the *n*-butylamine Schiff base of 11-*cis*-retinal. The addition of Meerwein's reagent to the retinal Schiff base led to an instantaneous alkylation, as demonstrated by the immediate spectral shift to 474 nm (charged Schiff base). After 30 s, over 80% of the retinoid had been isomerized. This result is consistent with what was expected from the studies in the piperidine-catalyzed isomerization of 11-*cis*-retinal.

The overall major conclusions to be drawn from this work are (1) that simple Schiff base formation between retinal and an aliphatic amine or aromatic amine does not substantially increase the rate of the thermal isomerization over that for 11-*cis*-retinal, (2) protonation of these retinal Schiff bases leads to a much larger rate enhancement for the isomerization reaction, and nucleophilic catalysis is partly required for the increased rate, and (3) secondary amines are superb catalysts for the isomerization process because they allow the use of strongly nucleophilic catalysts. With primary amines only weak nucleophilic catalysts would be used, because as they become stronger, they deprotonate the retinal Schiff base and thus eliminate the possibility of catalysis. Nucleophilic amine bases do not themselves promote the isomerization of the retinals.[7] Of course, an enzyme could manage to use a strong nucleophile to attack the 9 and 11 or 13 positions of retinal Schiff base while maintaining the latter in the protonated state. An effective way of modeling this process is to use secondary amines for retinal Schiff base formation, as already reported.

It is of interest to consider the possible relevance of the studies reported here to biological situations. It is fairly clear that any biological isomerization of the retinals will probably make use of Schiff base formation between the retinal and an amino-containing biomolecule. The retinaldehydes themselves are too weakly basic to be effectively protonated under physiological conditions. It is also probable that the retinal Schiff bases will be charged

and that nucleophilic catalysis at one of the retinoid carbons will be important. Two biological molecules have been reported capable of isomerizing the retinals, and both almost certainly do so by mechanisms very similar to, if not identical with, those described here. Although no mechanism was suggested, it has been found that reduced flavins are potent catalysts of the isomerization of *all-trans*-retinal to 13-*cis*- and 9-*cis*-retinal.[20] As this work was completed before the advent of HPLC and before it was appreciated that 11-*cis*-retinal was only represented to the extent of 0.1% at equilibrium, it now seems clear that reduced flavins rapidly bring the retinals to equilibrium. Based on what is now known about secondary amine catalysts, the mechanism shown in Scheme VI is suggested for the catalytic potency of the reduced flavins. Interestingly, when this reaction was carried out in tritium water, no tritium incorporation was detected in the retinals.[20] This again argues against the possibility of an enolization mechanism in the isomerization reaction. A second biomolecular system which can effect the catalyzed isomerization of the retinoids is phosphatidylethanolamine (PE). In an aqueous environment PE has the added advantage of sequestering the retinal and bringing it into apposition with the amino group of the lipid, thus ensuring rapid and specific Schiff base formation.[21] It has been suggested that the phosphate oxygen could act as a nucleophile in these isomerization reactions.[21] These observations open up the possibility that small biological molecules could be the endogeneous catalysts for vitamin A aldehyde isomerization in vivo and that the mechanism of isomerization could be quite similar to that reported here.

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**Registry No.** 11-*cis*-retinal, 564-87-4; 13-*cis*-retinal, 472-86-6; 9-*cis*-retinal, 514-85-2; 11-*cis*-retinal aniline Schiff base, 90696-42-7; 11-*cis*-retinal butylamine Schiff base, 52647-48-0; 13-*cis*-retinal butylamine Schiff base, 51847-39-3; 9-*cis*-retinal butylamine Schiff base, 51804-54-7; 11-*cis*-retinal piperidine perchlorate Schiff base, 90696-44-9; $H_2NO$-H·HCl, 5470-11-1; $C_5H_{11}N$·$HClO_4$, 57367-18-7; $BuNH_2$, 109-73-9; $PhNH_2$, 62-53-3; $C_5H_{11}N$, 110-89-4.

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# Photosensitive Monolayers. Photochemistry of Long-Chain Diazo and Azide Compounds at the Air–Water Interface

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**Abstract:** The photochemistry of a number of surface-active diazo and azide compounds was investigated in monolayers at the air–water boundary. Irradiation of long-chain α-diazo ketones with ultraviolet light leads to rapid loss of nitrogen. The resulting ketenes react with the subphase to generate carboxylic acids (photochemical Arndt–Eistert reaction) and dimerize to give β-lactones as side products. A long-chain diester of 2-diazopropanedioic acid loses nitrogen and adds water, yielding the diester of 2-hydroxypropanedioic acid. α-Azido ketones split off nitrogen and the resulting isocyanates react with water (photochemical Curtius reaction) and undergo further degradation to give complex product mixtures. These reactions lead to pronounced changes in the spreading behavior of the monolayers. Depending on functional group, chain length, substrate pH, and temperature it is possible to achieve changes in compressibility and collapse pressure, disappearance of expanded phases, collapse of monolayers to give oily films, or disappearance of monolayers by dissolution in the subphase.

## Introduction

Photoreactions in thin films are the basis of important industrial processes, including photolithography and photoresist technology.[1] In an effort to achieve still higher resolution, and possibly even to store binary information at the molecular level, the extension of these photoreactions to Langmuir–Blodgett monolayers has been suggested.[2] Other reasons for the present worldwide interest in the chemistry of surface-active molecules arise from the links to the processes of vision and photosynthesis. In addition, membranes whose properties are affected by photochemical reactions may find applications in photography and solar energy conversion.[3]

In general several types of chemical reactions might be expected to create pronounced changes in monolayer or membrane properties[4]: cis–trans isomerization within the hydrophobic chains of an amphiphilic molecule,[5] splitting of the hydrophilic head group away from the hydrocarbon chain,[6] reactions which cause large changes in the polarity of the head group,[7] dimerization,[8] or polymerization.[9] In a recent publication dealing with photochemistry in monolayers, surface-active spiropyrans were described

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