K. Hirao, and O. Yonemitsu, *J. Am. Chem. Soc.*, **96**, 2570 (1974); (f) H. D. Becker, B. Ruge, and T. Weslöf, *Tetrahedron Lett.*, 253 (1975).
(3) This reaction has also been interpreted in terms of the orbital interaction rule: S. Inagaki, H. Fujimoto, and K. Fukui, *J. Am. Chem. Soc.*, **98**, 4693 (1976).
(4) K. Hirao, M. Taniguchi, T. Iwakuma, O. Yonemitsu, J. L. Flippen, I. L. Karle, and B. Witkop, *J. Am. Chem. Soc.*, **97**, 3249 (1975).
(5) J. L. Flippen, *Acta Crystallogr., Sect. B*, **32**, 1269 (1976).
(6) C. A. Grob and P. W. Schiess, *Angew. Chem., Int. Ed. Engl.*, **6**, 1 (1967).
(7) E. Adler and K. Holmberg, *Acta Chem. Scand., Ser. B*, **28**, 465 (1974).
(8) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).
(9) K. Alder, F. H. Flock, and H. Lessenich, *Chem. Ber.*, **90**, 1709 (1957).
(10) E. Ōsawa, K. Aigami, and Y. Inamoto, *J. Chem. Soc., Perkin Trans. 2*, in press.
(11) Otherwise, the rearranged ketone **3d** may be formed directly from **3a** through a concerted 1,2 shift with deprotonation.
(12) For a review see R. L. Cargill, T. E. Jackson, N. P. Peet, and D. M. Pond, *Acc. Chem. Res.*, **7**, 106 (1974).
(13) B. P. Mundy and R. D. Otzenberger, *J. Org. Chem.*, **38**, 2109 (1973).
(14) Cf. E. N. Marvell and E. Magoon, *J. Am. Chem. Soc.*, **77**, 2542 (1955); B. Miller, *ibid.*, **92**, 6252 (1970).
(15) I. F. Halverstadt and W. D. Kumler, *J. Am. Chem. Soc.*, **64**, 2988 (1942).

Mechanistic Aspects of the Thermal Equilibration of Retinylidene Imines and Immonium Salts

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Abstract: The reactions of isomeric retinylidene *n*-butylimines and protonated imines in the dark were examined by proton nuclear magnetic resonance and ultraviolet absorption spectroscopy and by high-performance liquid chromatography. The immonium hydrochlorides were shown to decompose by a complex system of pathways. However, 13-*cis*-retinylidene-*n*-butylamine was shown to undergo thermodynamic equilibration with the trans isomer by general base catalysis, whereas the 9-cis isomer was thermodynamically stable. The specificity of the reaction for the 13-cis isomer suggests that it occurs via the 13-methylene enamine intermediate. Protonated retinylidene imines have been shown to be important as chromophores in the visual cycle and in a light-driven proton pump in certain halophilic bacteria. The mechanisms of action for these systems are briefly contrasted in the light of the result reported here.

Introduction

Retinal imines have long served an important role as models of the visual chromophore.[1] Interest in their study has been further augmented by the recent discovery of a light-driven proton pump in certain halophilic bacteria which catalyzes the photophosphorylation of ADP.[2] The pump has been isolated, and has been shown to consist of 25% lipid and 75% of a single protein called bacteriorhodopsin of molecular weight 25 000. Furthermore, its intense purple color is due to a retinyl moiety attached to an ϵ amino group of a lysine.[3] It exists in two forms: (1) the dark-adapted form, characterizable by a maximum visible spectral transition at 558 nm, which is believed to contain a 1:1 mixture of the chromophore as 13-*cis* and *all-trans*-ϵ-retinylidene-L-lysine;[4] and (2) a light-adapted state with a transition at 568 nm, in which it is believed to be essentially all trans.[4,5]

It is unclear whether a photolytic all-trans to 13-cis isomerization is important for a proton pumping,[6] although there is some evidence from chromophore extraction data that an intermediate exists with a 13-*cis*-retinyl chromophore.[7] If this is so, the 13-cis intermediate thermally reisomerizes to trans, since there must be a cyclic mechanism for the bacteriorhodopsin proton pump.[8]

The purpose of this paper is to demonstrate that at least one mechanism exists for the nonphotolytic rearrangement of the 13-cis imine to the all-trans form. The immonium hydrochloride, on the other hand, decomposes by several reaction pathways. Investigators are therefore cautioned to use care in the preparation and study of these compounds. Finally, the relevance of these findings to the visual cycle and to the bacteriorhodopsin system is briefly discussed.

Materials and Methods

Materials. *all-trans*-, 13-*cis*-, and 9-*cis*-retinal (Sigma) were checked for purity on a Waters ALC/GPC 204 liquid chromatograph outfitted with a dual-wavelength (254 and 365 nm) detector and a μ-Porasil column, as described by Pettei et al.[7] The eluent was 2% ether in hexane with a flow rate of 2.0 mL/min. Proton magnetic resonance spectra were taken on a Perkin-Elmer R12b spectrometer, outfitted with a temperature control, at 37 °C. Absorption spectra were recorded on a Cary 14 spectrometer. *N*-Butylamine (Mallinckrodt) was distilled prior to use and stored over molecular sieves. Retinals were used without purification for the NMR experiments, but were purified by high-performance liquid chromatography before studies involving electronic spectra were made.

Preparation of Imines. All operations were conducted in semidarkness at 0 °C. Retinal was allowed to react with a 0.05 molar excess of *n*-butylamine in anhydrous ether for 30 min. The solvent was removed on a rotary evaporator, and the excess amine was driven off by passing dry nitrogen over the residue for 30 min. The protonated imine was prepared by washing the residue with ether saturated with anhydrous hydrogen chloride and removing the solvent on the rotary evaporator. Excess acid was further removed by a nitrogen purge. Samples were dissolved in chloroform or (for NMR experiments) chloroform-*d* containing 1% tetramethylsilane.

Results

NMR Studies. Our interest in this study originated when we encountered difficulties in obtaining ^{13}C NMR spectra of 13-*cis*-retinylidene-*n*-butylamine, owing to its conversion to the trans isomer at ambient temperatures. We were able to conveniently follow the isomerization by ^1H NMR (Figure 1), and noted that the half-life was roughly 1 h. In a separate experiment, we followed the decay of the 13-cis imine at 37 °C by LC, using 2% ether in hexane as the eluent, and found a decay in the 13-cis isomer from 85 to 45% in 80 min for a 0.1 M solution.

In NMR experiments, the immonium hydrochloride was

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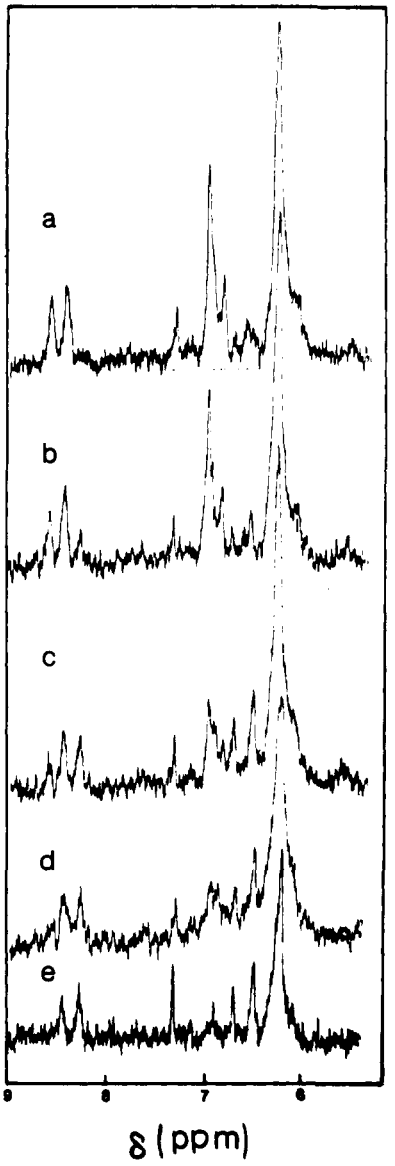

Figure 1. Vinyl proton NMR spectra of 0.14 M retinyl imines at 37 °C in chloroform-*d* containing 1% Me_4Si (ppm from Me_4Si): (a) 13-*cis*-retinylidene-*n*-butylamine; after (b) 37 min, (c) 3 h, (d) 6 h, (e) freshly prepared all-trans imine.

studied as well. The complex vinyl region of the protonated imines is unresolved at 60 MHz. However, it is qualitatively clear from Figure 2 that a 0.15 M solution of the 13-cis isomer decomposes by a variety of competing pathways at 35 °C and that a simple cis-trans isomerization is not immediately evident as the major route.

Electronic Spectral Studies. The molecularity of the isomerization was examined by preparing a 10 mM stock solution of the 13-cis imine in chloroform, and then preparing cuvettes containing a 1:25 (1-mm cuvette) and a 1:250 (1-cm cuvette) dilution. The three containers were kept at 32 °C, and their UV spectra were monitored periodically. The rearrangement was essentially complete after 3 h in the stock solution, whereas the dilute solutions were essentially unchanged. However, when the 40-μM solution was made 100 mM in *n*-butylamine, it quickly isomerized to the trans imine. In identical experiments, 13-*cis*-retinylidene-*n*-butylimmonium hydrochloride did not isomerize, although the stock solution did begin to decompose, as was witnessed by line broadening of its major (460 nm) spectral transition.

The rate of *n*-butylamine-catalyzed isomerization of the 13-cis imine was measured at 25 °C, as shown in Figure 3.

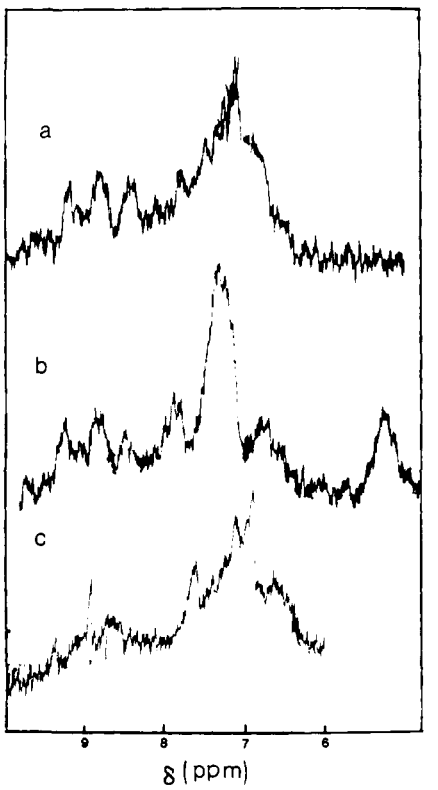

Figure 2. Vinyl proton NMR spectra of 0.14 M protonated retinyl imines at 37 °C in chloroform-*d* (ppm from Me_4Si): (a) 13-*cis*-retinylidene-*n*-butylimmonium chloride; (b) after 5 h; (c) freshly prepared *all-trans*-immonium chloride.

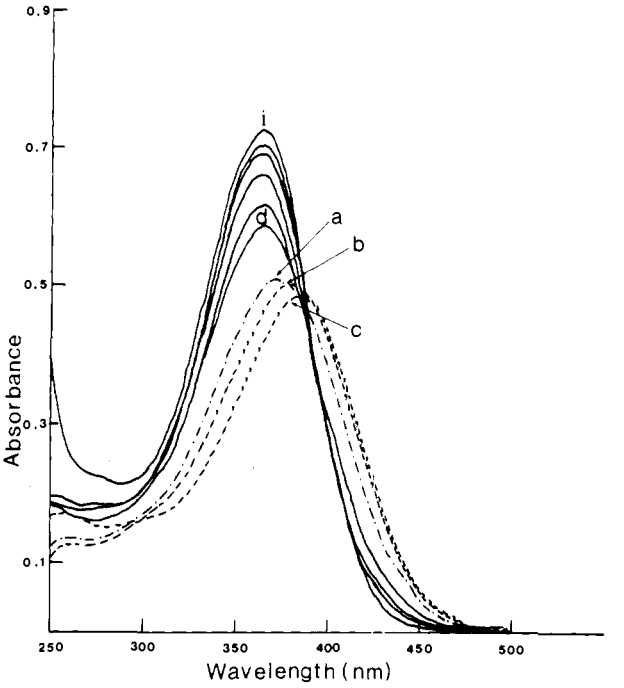

Figure 3. UV spectra showing the reaction between 13-*cis*-retinal (14 μM) and *n*-butylamine (100 mM) at 25 °C in chloroform: 13-*cis*-retinal (a); formation of 13-cis imine (b, c); formation of all-trans imine (d-i). Time after adding amine (min): (b) 5; (c) 11; (d) 22; (e) 47; (f) 65; (g) 100; (h) 130; (i) 211.

Butylamine (0.2 μmol) was added to a solution of 13-cis retinal (0.028 μmol) in chloroform (2.0 mL), and the solution was monitored spectroscopically. The cis imine was formed in 10 min, and then smoothly isomerized to the trans isomer. The spectral changes as the 13-cis and all-trans imines are formed

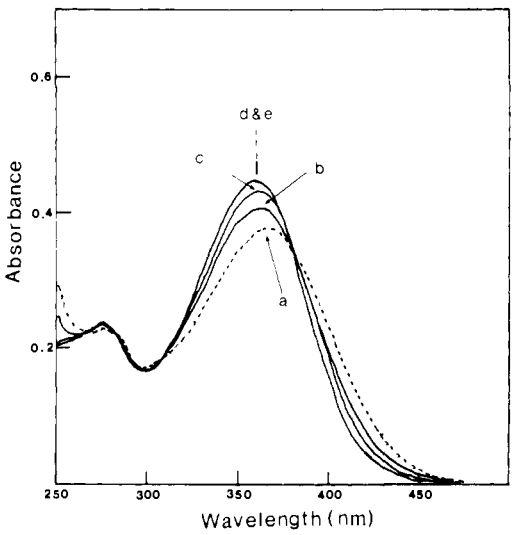

Figure 4. Electronic spectra showing the reaction between 9-*cis*-retinal (9.7 μM) and *n*-butylamine (100 mM) at 25 °C in chloroform: (a) 9-*cis*-retinal; formation of 9-cis imine. Hours after adding amine: (b) 0.5; (c) 1.0; (d) 6.0; (e) 30.

are qualitatively consistent with the spectral data for the isomeric retinals and imines published by Schaffer et al.[9] The pseudo-first-order rate could be conveniently monitored by adding *n*-butylamine to a solution of the imine and measuring the decrease in absorbance at 450 nm, giving a half-life of 26 min for a 0.1 mM amine and 15 μM imine at 25 °C (correlation coefficient for a linear regression analysis: 0.998), and a second-order rate constant of 4.4×10^{-3} M^{-1} s^{-1}.

All of the bases used here could potentially react by a transiminization mechanism, and not by simple general base catalysis. The 13-cis imine was therefore prepared, and triethylamine was added under conditions in which the concentrations of amine (70 μM) and imine (20 μM) were similar to those used for *n*-butylamine. The rearrangement was much faster ($t_{1/2} \sim 7$ s at 25 °C), establishing that the mechanism of action was by general base catalysis.

As a final control, solvent effects on the course of the isomerization were monitored, using ethanol, isopropyl alcohol, and acetonitrile at 25 °C. The rates relative to chloroform follow: ethanol (>100) ≫ isopropyl alcohol (55.3) ≫ acetonitrile (1.6).

The 9-cis isomer was investigated in the same fashion, in order to ascertain whether other *cis*-retinylidene imines isomerize. A 9.7 μM solution of 9-*cis*-retinal in chloroform was allowed to react with *n*-butylamine (100 mM). As shown in Figure 4, the imine quickly formed, but did not noticeably isomerize over a 2-day period at room temperature. From the quantitative data of Schaffer et al.,[9] one would expect a 9-nm blue shift of λ_{max} and a 20% increase in the extinction coefficient as the imine is formed from 9-*cis*-retinal, and followed by a 1-nm red shift in the major spectral transition accompanied by a 13% increase in ϵ_{max} as the trans imine is formed. The observed spectral changes (a 9-nm blue shift with an ϵ_{max} increase of 18%) are only in accord with the formation of the 9-cis imine. Note too that the characteristic cis peak is retained throughout.

Discussion

It is clear that 13-*cis*-retinylidene-*n*-butylamine easily isomerizes to the trans imine by a mechanism involving general base catalysis. Although the exact mechanism is unknown, the thermal stability of the 9-cis imine militates against mechanisms involving extensive charge delocalizations or a retro form. The simplest hypothesis would therefore consist of general-base-catalyzed thermodynamic equilibration between

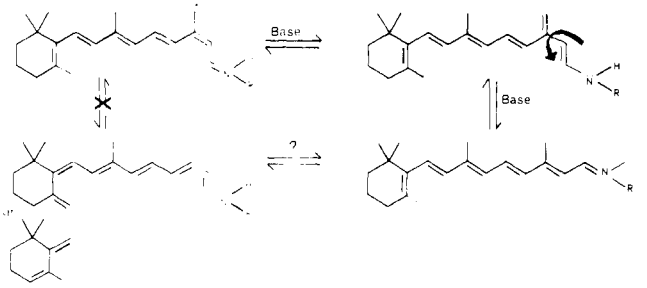

Figure 5. Proposed scheme for the thermal equilibration of 13-*cis*- and *all-trans*-retinylidene-*n*-butylamine.

imines via a 13-methylene enamine intermediate (Figure 5). Such intermediates are usually not encountered in high concentrations, because C=N bonds are more stable than C=C bonds when primary amines are the reactants,[10] and because enamines of aldehydes are generally not stable.[11] It is noteworthy in this respect that the trans imine showed signs of decomposition at 130 min (Figure 2).

The relative rates of isomerization in ethanol, 2-propanol, acetonitrile, and chloroform are in the order one might expect:[12] the reaction is faster in protic than in aprotic solvents and, within these groups, is faster in the solvent with the higher dielectric constant. We would conclude from this that nonpolar solvents such as 3-methylpentane are the most suitable for studying Schiff bases.

The manifestation of a 13-methylene enamine intermediate and not the 9-methylene or the thermodynamically more stable retroretinyl enamine may be expected under conditions of kinetic control. The 13-methyl hydrogens of the imine are relatively acidic, because they are homoallylic to the nitrogen, whereas the C4, C18, or C19 hydrogens are less so, because the electron-withdrawing nitrogen substituent effect is attenuated as it is broadcast down the chain.

In the light of the base-catalyzed isomerization mechanism discussed here, it is interesting to contrast the evolution of the mechanism of vision with that of the proton pump in halobacteria. As Lewis has pointed out,[13] the mechanism for vision involves a signal transducer, in which the sensitivity to a single photon event must be maximized. Natural selection has taken advantage of the thermodynamically favorable path of 11-cis to all-trans isomerization of the chromophore and has discriminated against a reversible cycle and specifically against the involvement of a 13-cis isomer. The proton pump, on the other hand, requires light energy to maintain a proton gradient and has opted for a cycle at the expense of sensitivity to the single-photon event. Reversibility is therefore allowed, and the 13-cis isomer is found in dark-adapted systems and may be present in the photocycle as well.

This mechanism may play an important role in the light-dark adaptation cycle in bacteriorhodopsin, since the mixture of isomers may be a thermodynamic distribution reflecting an equilibration which is catalyzed by a weak base. Although the mechanism may be important to the photocycle as well, it is unlikely, because the cycle would then involve a kinetically unfavorable protonation of a soft carbon base (the 13-methylene) by a hard acid (an oxygen or nitrogen acid).[12] This supposition is based on the fact that carbon acids and their conjugate bases do not form hydrogen bonds with the oxygen and nitrogen acids and bases one would expect to be involved in enzyme catalysis, thereby vitiating the formation of favorable encounter complexes. Yet, both of these hypotheses can be tested.

The protonated imines react by more than one path, with isomerization competing with Michael additions and the like. However, like their conjugate bases, they are stable at low temperature or at low concentrations. Retinylidene immonium

salts do not undergo thermodynamic equilibration as readily as do retinals in the presence of acids.[14] However, an enzyme-catalyzed route is possible. At any rate, care must be exercised in the preparation and handling of these compounds.

Acknowledgment. This work was supported by NHLI Program Project Grant HL06285.

References and Notes

(1) Honig, B.; Ebrey, T. G. *Annu. Rev. Biophys. Bioeng.* **1974**, *3*, 151–177.
(2) Henderson, R. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 87–109.
(3) Oesterhelt, D.; Stoeckenius, W. *Nature (London), New Biol.* **1971**, *233*, 149–152.
(4) Oesterhelt, D.; Meentzen, M.; Schuhman, L. *Eur. J. Biochem.* **1973**, *40*, 453–463.
(5) Jan, L.-Y. *Vision Res.* **1975**, *15*, 1081–1086.
(6) Aton, B.; Doukas, A. G.; Callender, R. H.; Becher, B.; Ebrey, T. G. *Biochemistry* **1977**, *16*, 2995–3000.
(7) Pettei, M. J.; Yudd, A. P.; Nakanishi, K.; Henselman, R.; Stoeckenius, W. *Biochemistry* **1977**, *16*, 1955–1958.
(8) Lozier, R. H.; Bogomolni, R. A.; Stoeckenius, W. *Biophys. J.* **1975**, *15*, 955–962.
(9) Schaffer, A. M.; Waddell, W. H.; Becker, R. S. *J. Am. Chem. Soc.* **1974**, *96*, 2063–2068.
(10) Franklin, J. L. *Ind. Eng. Chem.* **1949**, *41*, 1070–1076.
(11) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207–216.
(12) Szwarc, M.; Streitwieser, A.; Mowery, P. C. "Ions and Ion Pairs in Organic Reactions", Vol. 2; Szwarc, M., Ed.; Wiley: New York, N.Y., 1974; Chapter 2.
(13) Lewis, A. *Biophys. Struct. Mech.* **1977**, *3*, 97–100.
(14) Patel, D. J. *Nature (London)* **1969**, *264*, 799–800.

Reactivity in Methyl Transfer Reactions. 3. Equilibria and Rates in Transfers between Substituted Thiophenoxides

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Abstract: Methyl phenyl sulfide methylates substituted thiophenoxide ions reversibly at elevated temperatures. Using a GC analysis, the rates and equilibria for five substituted cases have been measured at several temperatures in the range ca. 110–210 °C, and the identity reaction with unsubstituted thiophenoxide has also been measured using a ^{35}S tracer. The Hammett ρ for the forward reaction is close to half that for the equilibrium, suggesting a transition state with a half-transferred methyl group. This represents the first measurement of substituent effect on both rate and equilibrium for a simple organic substitution reaction.

In the first two papers of this series,[1,2] substituent effects in an attacking nucleophile were compared for leaving groups of greatly differing reactivity in some virtually quantitative reactions. Surprisingly, substituent effects were large and remarkably similar over a wide range of rate, although the reactivity-selectivity principle[3] leads to an expectation of reduced substituent effects with the most reactive leaving groups, such as trifluoromethanesulfonate. The term "large" substituent effect implies, however, a standard of comparison, and the only ones available were those of one leaving group with another. A more reasonable comparison is between the substituent effect on the reaction rate and that on the equilibrium position in the same reaction, perhaps expressable as ρ_+ and ρ_{eq}, the Hammett reaction constants for the forward rate constants and the equilibrium constants, respectively (similarly ρ_- for the reverse reaction can be defined, but it is not independent, for $\rho_{eq} = \rho_+ - \rho_-$). The ratio ρ_+/ρ_{eq} can then be used to measure the extent of proton transfer with the same utility and limitations as using the Brønsted α or β (which are also comparisons of effect of structural changes on rate and equilibrium) to measure the extent of proton transfer.[4]

Equilibria have been measured for very few organic reactions, except acidities and oxidation-reduction processes. One, of complex mechanism, is listed in Hammett's tabulation,[5] Jaffe[6] lists six more, and most of these do not have rates measured also. More recently, both rates and equilibria have been measured for some diazonium[7] and carbonium[8] ion reactions with nucleophiles. There appears to be only one published report of equilibria in methyl transfers,[9] and rates were not reported. A very relevant study of methyl iodide with pyridine is not yet published.[10]

We report the study of the reaction

$$ArS^- + CH_3SPh \underset{k_-}{\overset{k_+}{\rightleftharpoons}} ArSCH_3 + {}^-SPh \qquad (1)$$

This reaction is, of course, too slow to study near room temperature, but is fast enough to measure between about 110 and 210 °C in ethanol. Rate constants, measured as initial rates by a gas chromatographic procedure comparing $PhSCH_3$ and $ArSCH_3$ peak areas, fit the Arrhenius equation with the results expressed as $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, presented in Table I. The case of Ar = Ph was followed using ^{35}S-labeled PhS^-. The kinetic law, assumed to be first order in each reagent, was essentially confirmed in one case illustrated in Figure 1, in which experimental points of mole fraction of $ArSCH_3$ as a function of time in experiments at 144 °C are shown together with the curve calculated from the equation

$$\frac{K}{2A_0} \ln \left[\frac{C\sqrt{K} - C + A_0}{C\sqrt{K} + C - A_0}\right] = k_+ t \qquad (2)$$

for a reversible second-order reaction with equal starting concentrations, A_0 of the two reagents. In this equation C is the product concentration, and values were $k_+ = 8.0 \times 10^{-7}$ M^{-1} s^{-1}, $K = 2.0$, and $A_0 = 0.90$ M. The plot also shows experimental points for the reverse reaction, starting with PhS^- and $CH_3SC_6H_4CH_3$; the calculated curve uses $k_- = 4 \times 10^{-7}$ M^{-1} s^{-1} and an initial concentration of 0.80 M. The time scale on Figure 1 shows why this was not done routinely. A further demonstration of the kinetic law is that no reaction was detected when thiol was used instead of the salt.

Equilibrium constants in Table I are calculated as k_+/k_-, but several were measured directly after long time periods.

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