(16) V. G. Levich, "Physicochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962, p. 7.
(17) F. Langenbucher, *Pharm. Acta Helv.*, **49**, 187(1974).
(18) M. J. Groves, M. H. Alkan, and M. A. Deer, *J. Pharm. Pharmacol.*, **27**, 400(1975).
(19) S. Ergun, *Chem. Eng. Progr.*, **48**, 227(1952).
(20) J. W. Mullen and T. P. Cook, *J. Appl. Chem.*, **15**, 145(1965).
(21) F. Puisieux and J. T. Carstensen, *Ann. Pharm. Fr.*, in press.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 4, 1975, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*
Accepted for publication January 21, 1976.
[x] To whom inquiries should be directed.

Microenvironmental Kinetic Effects within a Lyotropic Smectic Biophase Model: Conformational Restrictions in Fischer Indole Cyclization

HUSSEIN G. IBRAHIM [*] and EDWARD G. RIPPIE [x]

Abstract □ The microenvironmental orientation effects, arising from an ordered solvent structure, were studied in a model liquid crystalline biophase for the cyclization of a series of 2-substituted cyclohexanone phenylhydrazones. The magnitude of such solvent-induced intramolecular conformational constraints was determined from a comparison of the kinetics of the Fischer indole rearrangement in a lyotropic smectic liquid crystal *versus* those in an isotropic liquid of similar chemical composition but lacking the structured nature of the mesophase. Solutions consisting of 50% (w/w) polyoxyethylene 6 tridecyl ether or 44% (w/v) polyethylene glycol in aqueous buffers comprised the smectic or isotropic media, respectively. The apparent dissociation constants of the conjugate acids of the phenylhydrazones were determined kinetically, as were their partition coefficients between lipid and polar isotropic phases approximating the compositions of the smectic lamellae. Intrinsic first-order rate constants, corrected for partitioning within the lamellar mesophase, were used to compute the enthalpies and entropies of activation. The somewhat slower intrinsic rates of cyclization and the accompanying less negative entropies of activation generally observed in the liquid crystalline medium, as opposed to the isotropic system, are attributed to the orienting effects of the lamellar lyotropic mesophase.

Keyphrases □ Cyclohexanone phenylhydrazones, 2-substituted—cyclization reaction kinetics, effect of ordered solvent structure on microenvironmental orientation and intramolecular conformational constraints □ Phenylhydrazones, 2-substituted cyclohexanone—cyclization reaction kinetics, effect of ordered solvent structure on microenvironmental orientation and intramolecular conformational constraints □ Cyclization reaction kinetics—2-substituted cyclohexanone phenylhydrazones, effect of ordered solvent structure □ Solvent structure, ordered—effect on cyclization reaction kinetics of 2-substituted cyclohexanone phenylhydrazones □ Conformational constraints, intramolecular—2-substituted cyclohexanone phenylhydrazones, effect of ordered solvent structure □ Orientation, microenvironmental—2-substituted cyclohexanone phenylhydrazones, effect of ordered solvent structure

Mobile liquid crystalline phases composed of nonrandomly oriented molecules constitute the media for many, if not most, catalytic processes at the cellular level (1–3). In such cases, it is likely that they modify the rates and extent of biochemical reactions and receptor site interactions as well as the processes of active and passive transport. Investigation of the influences of such structured environments on the kinetics and thermodynamics of chemical reactions may explain the nature of their control of the biological processes occurring within them.

BACKGROUND

It is extremely difficult to assess the functions and effects of ordered fluids in a biological system by direct experimentation on living matter. Results from kinetic and thermodynamic solution studies in structured lyotropic solvents, such as the liquid crystals used in this work, can reveal significant information concerning both intra- and intermolecular orientation effects arising solely from the medium structure. Effects of solvent-hindered molecular mobility and the influence of various liquid crystalline structural features on reaction rates also indicate the relative importance of molecular grouping and interaction factors in mesophasic solvent systems and provide a basis for an understanding of the effects of the structured environment on the behavior of drugs in living organisms.

The principal structural feature characteristic of the liquid crystalline state is the parallel arrangement of the component molecules, with rotation primarily being permitted only about the long axis. Because of increasing interest in the mesomorphic states, the characteristic features and properties associated with these systems have been extensively reviewed (4, 5). However, studies of reaction kinetics in such novel solvents are relatively few (6–10).

Since solute species (reactants) dissolved in liquid crystalline media lose freedom of molecular motion to varying degrees due to microenvironmental effects arising from solvent structure, orientation-dependent rearrangement or cyclization reactions can be expected to undergo substantial entropic changes. Also, because of the specific orientations that reactant molecules experience in such ordered fluids, solvent-induced changes in conformation populations can influence significantly any stereospecificity characteristic of the reaction.

Preliminary studies of certain polymerization processes indicated that the molecular order of a nematic solvent often directs the reaction, generally forming isotactic polymers in preference to atactic ones (6). It is also known that molecular diffusion fluxes are functions of molecular orientation as well as the usual driving forces of diffusion. Thus, a reactant molecule dissolved in an ordered fluid experiences anisotropic or directed diffusion, which can significantly affect the kinetics of certain diffusion-controlled reactions.

Fendler and Fendler (11) stressed the importance of studies of chemical reactions in micellar systems because of the recognized analogies between micellar and solvated protein structures and between micellar and enzymatic catalysis. Most of these studies have been conducted in dilute micellar solutions. However, since lyotropic liquid crystals can be regarded as bulk micellar media, they probably would be more representative and better suited for such an analogy.

The effect of solvent anisotropicity on ester hydrolysis in lyotropic smectic mesophases was studied (7). Also, the hydrolysis of *p*-nitrophenyl laurate was studied in a similar system (8), and a catalytic effect comparable to that of micelles was found. These results gave evidence of changes in the catalytic effect with changes in medium structure. The present investigation is concerned with the effects of such solvent-modified intramolecular mobility on the kinetics of an orientation-sensitive reaction.

The cyclization of a series of 2-substituted cyclohexanone phenylhydrazones was conducted in a lamellar lyotropic liquid crystal composed of 50% (w/w) polyoxyethylene 6 tridecyl ether in aqueous buffer. The neat phase of this system exists over a broad range of surfactant concentration, as shown by the composition–temperature phase diagram (12, 13), and maintains its ordered structure in the presence of relatively high concentrations of added solutes (7). Reaction rates were determined in such a medium and in a medium simulating the chemical environment of the substrate in the liquid crystal but lacking the structured nature of the mesophase. The isotropic medium fulfilling these requirements was provided by a solution of 44% (w/v) polyethylene glycol 400 in aqueous buffer.

EXPERIMENTAL[1]

Materials—Triple-distilled water and reagent grade potassium chloride and hydrochloric acid were used in preparing the buffer solutions. Polyoxyethylene 6 tridecyl ether[2], polyethylene glycol 400[3], phenylhydrazine[4], cyclohexanone[5], 2-methylcyclohexanone[4], 2-*n*-propylcyclohexanone[6], and *n*-heptane[5] were used as received without further purification.

The cyclohexanone phenylhydrazones were prepared by condensing the hydrazine with the corresponding cyclohexanone and were purified by crystallization or separation from aqueous ethanol immediately before use (14). Identity and purity of the compounds were checked by melting or boiling points and UV and IR spectra: cyclohexanone phenylhydrazone (I), $C_{12}H_{16}N_2$, mol. wt. 194, mp 77°; 2-methylcyclohexanone phenylhydrazone (II), $C_{13}H_{18}N_2$, mol. wt. 208, bp 220°/35–40 mm; and 2-*n*-propylcyclohexanone phenylhydrazone (III), $C_{15}H_{22}N_2$, mol. wt. 236.

Medium Preparation—Initial hydrazone concentrations of 2×10^{-3} *M* were used in the various isotropic and anisotropic systems; they were adequate for UV assay and did not disrupt the liquid crystalline phases. Anisotropic reaction mixtures for the kinetic experiments were prepared by adding aqueous solutions, containing the calculated amounts of the hydrazones and buffer ingredients, to previously weighed quantities of surfactant in small, wide-mouth, erlenmeyer flasks.

To ensure the homogeneity of the media, the mixtures were stirred with a glass rod, rapidly heated in a water bath to 70°, and then rapidly cooled with stirring to the desired reaction temperature. Thus, the resultant very viscous liquid crystalline gels contained the required concentration of surfactant and were at the proper pH and ionic strength.

When examined under a polarizing microscope with crossed polars at a magnification of 100×, they showed the characteristics typical of a smectic-type structure. The 50% (w/w) polyoxyethylene 6 tridecyl ether aqueous systems had a smectic–isotropic transition temperature of 69°, in agreement with the previously reported value (7). In the presence of buffer ingredients and the reactant compounds, a lowering of 1° of the transition temperature was noted.

For the preparation of the isotropic reaction media, accurately weighed quantities of polyethylene glycol 400 in volumetric flasks were diluted to volume with aqueous solutions containing the calculated amounts of the hydrazones and buffer ingredients. Clark and Lub's potassium chloride–hydrochloric acid buffers were used to maintain a constant pH (±0.05) in the media. Whenever necessary, the ionic strength was adjusted to 0.08 using potassium chloride.

Analytical and Kinetic Procedures—Reactions in the liquid crystalline gels were followed by means of samples withdrawn at appropriate time intervals and quickly cooled. Approximately 1 g of each sample was accurately weighed in a 10-ml volumetric flask, dissolved and diluted to volume with 95% ethanol, and assayed. For reactions carried out in the isotropic media, 2-ml samples were withdrawn and cooled and a 1-ml aliquot was pipetted into a 10-ml volumetric flask and diluted to volume with 95% ethanol.

Absorbance measurements were taken immediately at 275 nm, using an appropriately treated blank in all instances. All measurements were made in alcoholic solutions because of the cloudiness characteristic of dilute aqueous solutions of the surfactant. In all cases, the flasks containing the reaction mixtures were maintained at constant temperature ±0.05° in thermostated water baths. The temperature dependence of the reaction was determined from the rates of cyclization at three temperatures under conditions of constant pH and ionic strength. Duplicate runs were made for each kinetic experiment.

Partition Studies—Partitioning of the hydrazones between the polar and hydrocarbon regions of the lamellar liquid crystals was simulated by studying the distribution of the reactants between 44% (w/v) polyethylene glycol in an aqueous buffer and *n*-heptane. The immiscible solvents were first equilibrated by shaking together for 3 hr at the desired temperature. The mixture was allowed to stand for 24 hr to permit complete separation. Equal volumes of the equilibrated solvents containing the calculated amount of the reactants were placed in an erlenmeyer flask and shaken in a constant-temperature water bath.

The reaction in the two-phase system was followed kinetically by withdrawing an aliquot of the aqueous phase and simultaneously removing and discarding an equal volume of the *n*-heptane, so that the ratio between the volumes of the phases remained constant. Duplicate runs were made for each experiment, and the samples were assayed in the normal manner.

NMR and UV Studies—Comparisons of the NMR shifts, as well as the band shapes and peak positions in the UV spectrum, of a solubilizate in a micellar system to those in polar and nonpolar solvents have been used to furnish information on the nature of the environment of the solubilizate (15–20). In the current investigation, the recorded spectra of the reactant compounds in the liquid crystalline solvent were compared with those in *n*-heptane, aqueous buffer, and a series of aqueous polyethylene glycol 400 solutions of various concentration to obtain information concerning the location of the reactant species within the lamellar mesophase.

The NMR spectra were recorded at room temperature, with tetramethylsilane as the external standard. In preparing the liquid crystalline system for the study, a syringe was used to introduce the viscous gel into the NMR tube or the Beckman cell (7). Except for *n*-heptane, all media were buffered to pH 1.0 and adjusted to an ionic strength of 0.08. For the UV study, spectra were recorded between wavelengths of 210 and 350 nm at room temperature, using appropriate blanks in all instances.

RESULTS AND DISCUSSION

Fischer Indole Reaction Kinetics—Aside from its general importance and wide scope of application, the Fischer indole synthesis, involving the intramolecular cyclization of the arylhydrazones of aldehydes or ketones, is known to involve a substantial entropy change during ring closure and can be expected to be sensitive to an ordered microenvironment. The reaction mechanism (Scheme I), as originally proposed by Robinson and Robinson in 1918, has been subsequently well supported by experimental observations (21).

While it has been reported that the phenylhydrazones of 2-substituted cylohexanones may yield both a tetrahydrocarbazolenine and the expected indole, the latter product is strongly favored in the presence of mineral acids such as were used in the present study. The concentration of the reactive protonated form of the enehydrazine depends upon the pH of the medium, so the rate of cyclization is a function of the hydrogen-ion concentration. Cyclohexanone phenylhydrazone and its 2-methyl and 2-*n*-propyl derivatives, I, II, and III, respectively, were selected for this study.

The determination of liquid crystal medium effects necessitates the adjustment of the kinetic rate constants to account for both the degree of protonation and the extent of partitioning of the reactant within the smectic phase itself. These calculated intrinsic first-order

[1] UV absorption readings were taken with a Hitachi Perkin-Elmer 139 visible-UV spectrophotometer and a Cary 14 recording spectrophotometer. NMR measurements were made with Varian A-60D and XL-100 NMR spectrometers. A Bausch and Lomb polarizing microscope fitted with a Kofler hot stage was used to determine phase transition temperatures.

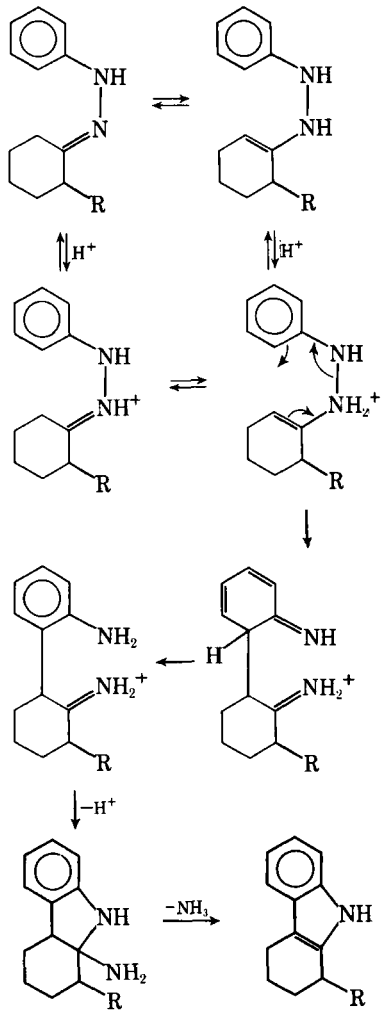
[2] Renex 36, Atlas Chemical Industries, Wilmington, Del.

[3] Ruger Chemical Co., Irvington, N.Y.

[4] Aldrich Chemical Co., Milwaukee, Wis.

[5] Mallinckrodt Chemical Works, St. Louis, Mo.

[6] Pfaltz and Bauer, Flushing, N.Y.



Scheme I

rate constants thus correspond to the reaction of the protonated enehydrazine within the polar polyoxyethylene aqueous lamellae. The data required for making these necessary rate constant adjustments were obtained in isotropic systems, and the procedures and calculations involved are discussed subsequently.

The measured pseudo-first-order rate constants of cyclization, both in the isotropic and anisotropic media, were determined by monitoring the disappearance of the reactants spectrophotometrically at 275 nm with subsequent treatment of data by the Guggenheim (22) method. For simplicity, they will be referred to subsequently as observed, k_{obs}, and apparent, k_{app}, rate constants. Equation 1 was used for these calculations:

$$\ln(A_t - A_{t+\tau}) = -kt + C \qquad \text{(Eq. 1)}$$

where A_t and $A_{t+\tau}$ represent the absorbance at time t and $t + \tau$, respectively, where τ is an appropriate constant time interval. This method of data handling was chosen to avoid the necessity of knowing with accuracy the initial and final concentrations of the reactants. Also, interference from other species in the various media could be avoided by this technique.

The slopes of plots corresponding to Eq. 1 were calculated by the method of least squares and are reported, together with their estimated standard deviations, in Table I.

Apparent Acid Dissociation Constants and Intrinsic First-Order Rate Constants in Isotropic Media—The apparent dissociation constants of the conjugate acids of I–III were determined kinetically from the pH dependency of the measured pseudo-first-order rate constants of cyclization. The study was conducted in the isotropic solvent at various hydrogen-ion concentrations and under conditions of constant temperature and ionic strength. The functional relationship between the hydrogen-ion concentration and the observed rate constants, k_{obs}, as derived in *Appendix I*, can be represented by:

$$\frac{1}{k_{obs}} = \frac{1}{k^*} + \frac{K_a}{k^*[H^+]} \qquad \text{(Eq. 2)}$$

Graphs of $1/k_{obs}$ *versus* $1/[H^+]$ yield linear plots such as that shown in Fig. 1, with slopes equal to K_a/k^* and intercepts of $1/k^*$, where K_a and k^* are the apparent acid dissociation constants and intrinsic first-order rate constants, respectively. Data from these measurements and calculations are presented in Tables I and II, together with the estimated standard deviations of the dissociation constants and their corresponding correlation coefficients. For reasons discussed later, no attempt was made to account for the temperature dependency of K_a in computing intrinsic first-order rate constants.

Kinetic Determination of Intrinsic Partition Coefficients—As previously indicated, the lyotropic smectic solvent used has a structure characterized by lamellae of alternating hydrocarbon and polyoxyethylene–water composition. Since, in the reaction under investigation, only the protonated form of the reactant can cyclize at a measurable rate and its concentration in the hydrocarbon lamellae is expected to be negligible, it is reasonable to assume that the cyclization reaction occurs solely in the polyoxyethylene–water regions of the liquid crystal.

Table I—Pseudo and Intrinsic First-Order Rate Constants[a] of Cyclization of 2-Substituted Cyclohexanone Phenylhydrazones

Medium	pH	Temperature	k_{obs} or k_{app} × 10^3, min^{-1}	k^* × 10^3, min^{-1}
Cyclohexanone Phenylhydrazone				
Aqueous polyethylene glycol	1.0	35.0°	5.38 ± 0.21	7.2
	1.0	40.0°	7.26 ± 0.42	9.7
	1.0	49.5°	14.54 ± 0.48	20.5
	1.4	49.5°	12.31 ± 0.39	20.5
	1.8	49.5°	6.77 ± 0.14	20.5
	2.0	49.5°	4.63 ± 0.11	20.5
	2.2	49.5°	3.43 ± 0.11	20.5
Liquid crystal	1.0	40.0°	1.57 ± 0.11	2.2
	1.0	49.5°	3.57 ± 0.10	5.1
	1.0	60.0°	8.80 ± 0.24	12.5
Biphase	1.0	49.5°	12.68 ± 0.53	
2-Methylcyclohexanone Phenylhydrazone				
Aqueous polyethylene glycol	1.0	35.0°	7.67 ± 0.45	10.4
	1.0	40.0°	9.38 ± 0.97	12.7
	1.0	49.5°	15.36 ± 0.91	22.3
	1.8	49.5°	7.56 ± 0.14	22.3
	2.2	49.5°	3.55 ± 0.08	22.3
Liquid crystal	1.0	40.0°	3.06 ± 0.11	5.1
	1.0	49.5°	5.48 ± 0.21	9.2
	1.0	60.0°	11.20 ± 0.90	18.8
Biphase	1.0	49.5°	8.98 ± 0.77	
2-*n*-Propylcyclohexanone Phenylhydrazone				
Aqueous polyethylene glycol	1.0	35.0°	5.84 ± 0.45	8.4
	1.0	40.0°	8.08 ± 0.24	11.5
	1.0	49.5°	16.67 ± 0.65	29.3
	1.8	49.5°	9.55 ± 0.11	29.3
	2.2	49.5°	3.86 ± 0.06	29.3
Liquid crystal	1.0	40.0°	2.18 ± 0.09	6.1
	1.0	49.5°	5.71 ± 0.07	15.9
	1.0	60.0°	14.23 ± 0.92	39.6
Biphase	1.0	49.5°	4.74 ± 0.76	

[a] The ± indicates 1 estimated standard deviation.

Table II—Apparent Conjugate Acid Dissociation Constants[a]

Compound	K_a × 10^2, M	r
I	3.40 ± 0.13	0.997
II	3.51 ± 0.17	0.999
III	4.30 ± 0.65	0.990

[a] The ± indicates 1 estimated standard deviation. All measurements were made at 49.5°.

Table III—Intrinsic Partition Coefficients[a] in Biphase Systems

Compound	K_p*[b]
I	0.81 ± 0.03
II	3.23 ± 0.22
III	11.05 ± 1.72

[a] The ± indicates 1 estimated standard deviation. [b] Determined in systems of *n*-heptane–44% (w/v) polyethylene glycol–aqueous buffer at 49.5°, pH 1.0, and ionic strength of 0.08.

Because of the reactivity of the phenylhydrazones under the conditions of interest, partition coefficients cannot be determined directly but were calculated from kinetic data gathered in two-phase systems. Data were obtained from *n*-heptane–aqueous polyethylene glycol systems under the same conditions of temperature, pH, and ionic strength as those used in determining the rates of reaction in the liquid crystals. All determinations were made in duplicate on systems containing initial overall hydrazone concentrations of $2 \times 10^{-3} M$.

Semilog plots of residual hydrazone concentration *versus* time exhibited linear correlation coefficients in excess of 0.99. The relationship between the intrinsic partition coefficients, K_p*, and the observed first-order rate constants, k_{obs}, as determined in these biphase systems is derived in *Appendix II* and represented by:

$$K_p^* = \frac{1}{r}\left(\frac{k^*[H^+]}{k_{obs}K_a} - 1 - \frac{[H^+]}{K_a}\right) \qquad \text{(Eq. 3)}$$

The intrinsic partition coefficients reported in Table III were calculated by substituting the experimentally measured values of k_{obs}, together with the previously determined values of K_a and k*, into Eq. 3. The ratio, r, of the hexane volume to that of aqueous polyethylene glycol was held at unity by withdrawing equal volumes of both phases when obtaining samples for assay.

NMR and UV Spectral Evidence of Solute Environment—Fendler and Fendler (11) and Winsor (15) observed that comparisons of the NMR shifts of solubilizates in micellar systems with those in polar and nonpolar solvents can be used to provide information on the nature of the environment of the solubilizate. In the current investigation, the recorded spectra of the phenylhydrazones in the media employed for the kinetic studies were used to furnish evidence concerning the location of the reactant species in the mesophase. Line positions of the aromatic protons of I–III in *n*-heptane, liquid crystals, aqueous polyethylene glycol, and aqueous buffer are listed in Table IV. If small allowances are made for differences in bulk susceptibility, comparisons are readily made between phases.

Spectra in the liquid crystal solvent resembled those in the isotropic solvents, except for an expected line broadening (23) due to decreased thermal mobility. In the present study, both the high microviscosity and the hindered rotational mobility experienced by solubilizates in the lamellar structure contributed to the observed line broadening. The strong shifts accompanying the change of solvent from *n*-heptane to the polar media can be attributed to field effects resulting from these media.

The correspondence of the line positions of I and II in the liquid crystal to those in aqueous polyethylene glycol indicates that the microenvironment of these compounds lies within the palisade-like polyoxyethylene layers of the mesophase. In contrast, III showed a line position intermediate to those in *n*-heptane and aqueous polyethylene glycol. Thus, its immediate environment is not purely hydrocarbon nor typically polar but is within the transition regions of lamellae and predominantly in the polar layers.

Table IV—NMR Line Positions[a] of Aromatic Protons of the Phenylhydrazones in Several Media, Isotropic and Anisotropic

Compound	*n*-Heptane	Liquid Crystal	Aqueous Polyethylene Glycol	Aqueous Buffer
I	366	309	309	310
II	398	345	345	348
III	400	333	310	310

[a] Line positions are in hertz relative to tetramethylsilane as the external standard. All measurements in the aqueous systems were made at pH 1.0.

Table V—UV Absorption Wavelength Maxima of the Phenylhydrazones in Several Media, Isotropic and Anisotropic

Medium[a]	Compound I, λ_{max}, nm	Compound II, λ_{max}, nm	Compound III, λ_{max}, nm
Liquid crystal[b]	276	270	260
n-Heptane	268	269	268
Buffer[b]	280	273	285
Polyethylene[b] glycol:			
30% (w/v)	277	274	278
44% (w/v)	276	274	276
50% (w/v)	278	276	276

[a] The temperature was held at 23° for all measurements. [b] Spectra were recorded at pH 1.0.

No NMR spectral differences were found between spectra of the pure liquid crystal and of the liquid crystal containing dissolved reactants. This finding can be attributed to the low concentrations of reactants used and to the marked stability and tolerance of the lyotropic lamellar neat phase both to water content and to added third components (24).

UV spectra are sensitive to solute characteristics (19, 20, 25, 26). Table V shows UV absorption spectra maxima of the three hydrazones in several media. The effects observed in the present studies are consistent with the expected behavior based on dielectric and hydrogen bonding properties of the media (27, 28).

Compound I exhibited the same λ_{max} in both 44% (w/v) aqueous polyethylene glycol and the liquid crystal. This fact, together with the NMR data, indicates the existence of the compound within the palisade layer of the lamellar structure.

Compound III exhibited a λ_{max} in the liquid crystal below that in *n*-heptane. A similar effect due to steric crowding was reported to cause comparable line shifts of stilbene and azobenzene (29). An increased energy for the $n \rightarrow \pi$ transition, with a resultant shorter absorption wavelength, is consistent with the existence of the compound in a strained configuration close to the plane of separation of the hydrocarbon and polar layers. This interpretation is in agreement with that reached from NMR results.

The spectrum of II can be readily explained on the basis of the behavior of the other hydrazones. The lesser steric crowding effect for the methyl derivative accounts for the similarity of λ_{max} in the liquid crystal and *n*-heptane. Since the hydrophobic character of II is between those of I and III, it is reasonable to expect it to locate within the polyoxyethylene layers in a region intermediate to those proposed for the other compounds.

Intrinsic First-Order Rate Constants in Anisotropic Media—The apparent first-order rate constants of cyclization in the liquid crystal system were adjusted for partitioning between polar and hydrocarbon layers and for dissociation of the conjugate acids. The resultant intrinsic rate constants, k*, were calculated from a rearranged form of Eq. 3:

$$k^* = \frac{k_{app}K_a}{[H^+]}\left(1 + \frac{[H^+]}{K_a} + rK_p^*\right) \qquad \text{(Eq. 4)}$$

The experimental values of K_a and K_p*, as determined previously, and k_{app} were substituted into Eq. 4 to give the corresponding values of k* presented in Table I.

Kinetic Activation Parameters—The enthalpies, $\Delta H^{\pm}$, and entropies, $\Delta S^{\pm}$, of activation (Table VI) were calculated from the Eyring equation using the calculated intrinsic first-order rate constants of cyclization. The primary significance of these data resides in the differences that can be observed between the kinetic activation parameters in the liquid crystalline media and the corresponding isotropic media. For this reason, no attempt was made to account for the relatively small contributions to $\Delta H^{\pm}$ and $\Delta S^{\pm}$ arising from the temperature dependence of the intrinsic partition coefficients and the apparent conjugate acid dissociation constants.

Plots of pKa for the dissociation of most weak electrolytes pass

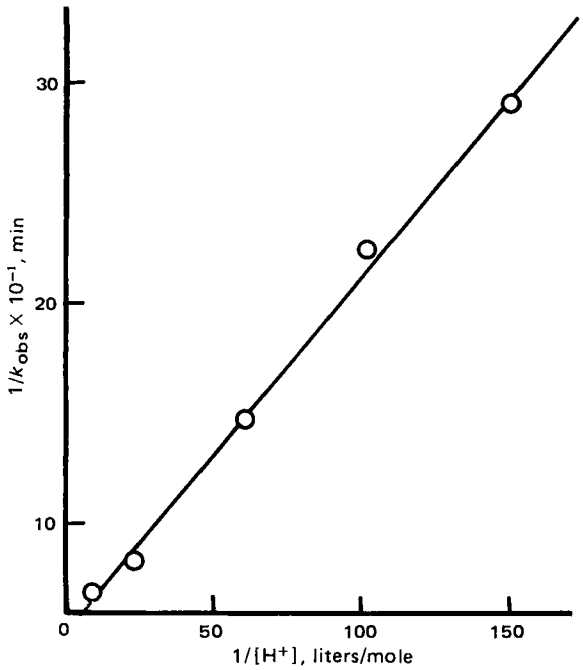


Figure 1—*Double reciprocal plot of* $[H^+]$ versus k_{obs} *for I at 49.5°.*

through a minimum in the 0–60° range (30). Consequently, the absolute value of the observed change is expected to be small; in the present study, it was zero within experimental error. Furthermore, no statistically significant change in K_p* with temperature could be found.

Similar trends in $\Delta H^{\ddagger}$ were evident in both isotropic and anisotropic media, although values of $\Delta H^{\ddagger}$ were generally higher in the latter solvent. Cyclization involves simultaneous nitrogen–nitrogen bond cleavage and carbon–carbon bond formation at the rate-determining step. In accordance with the accepted mechanism, carbon–carbon bond formation results from partial charges on the α-carbon atoms in the cyclohexane and benzene rings. The activation enthalpy would thus be expected to increase with an increase in the average distance separating the two partially charged centers. The experimentally observed increase in $\Delta H^{\ddagger}$ in the liquid crystal systems can likely be related, therefore, to solvent-restricted molecular bending.

Relatively large negative entropies of activation were found in both solvent systems. However, less negative values were observed in the anisotropic solvent, suggesting that the reactants in this system are less randomly disposed since their corresponding activated complexes are not likely to change significantly with solvent.

The combined effects of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were manifested in the reaction in such a manner that the reaction rates were only moderately lower in the ordered system as opposed to the structureless isotropic liquid. The existence of a linear relationship between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ was seen in both solvent systems. This latter behavior is well known as the thermodynamic compensation law or more commonly as the

Table VI—Kinetic Activation Parameters of Cyclization[a]

Compound	$\Delta H^{\ddagger}$, kcal/mole	$\Delta S^{\ddagger}$, cal/deg mole
	44% (w/v) Aqueous Polyethylene Glycol	
I	13.8 ± 1.1	−31.9 ± 3.5
II	10.0 ± 1.1	−43.5 ± 3.5
III	16.8 ± 1.9	−21.9 ± 6.0
	Lyotropic Liquid Crystal	
I	17.3 ± 0.3	−23.6 ± 1.0
II	12.9 ± 0.7	−36.2 ± 2.1
III	18.9 ± 0.5	−16.6 ± 1.5

[a] The ± indicates 1 estimated standard deviation. The pH was held at 1.0 and the ionic strength at 0.08 in all measurements.

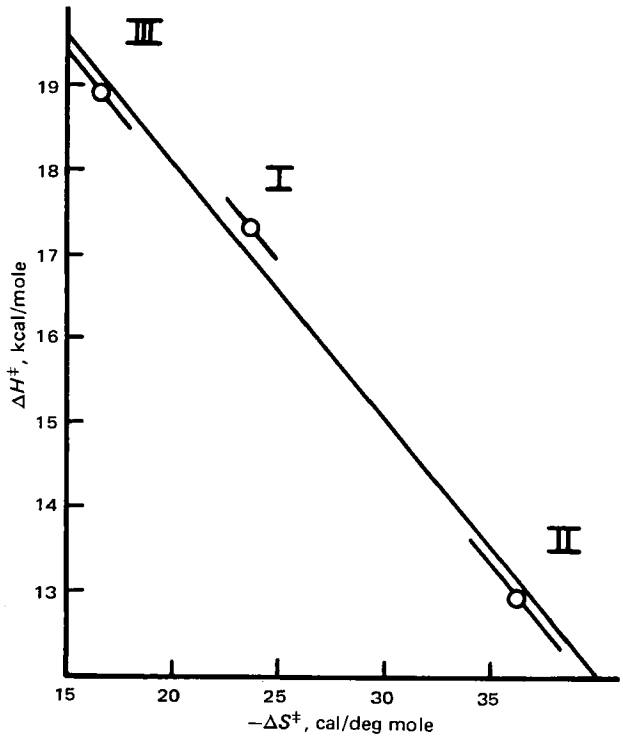


Figure 2—*Entropy–enthalpy correlation for cyclization of 2-substituted cyclohexanone phenylhydrazones in the lyotropic liquid crystalline phase. Points correspond to Compounds I, II, and III.*

isokinetic relationship (31). It takes the form:

$$\Delta H^{\ddagger} = \alpha + \beta \Delta S^{\ddagger} \qquad \text{(Eq. 5)}$$

where α is a constant, and β corresponds to the isokinetic temperature of the reaction. At this temperature, the measured rate constants for a series of structurally related compounds undergoing a well-defined chemical reaction will be the same irrespective of the effects arising from the structural changes. Since such an apparent relationship can result from experimental error in measuring the temperature coefficient of reaction, care must be exercised in its interpretation.

Leffler (32) outlined the criteria that must be satisfied before a valid isokinetic relationship can be claimed to exist. Only those data obtained in the liquid crystal meet these requirements and are illustrated in Fig. 2 as a plot of $\Delta H^{\ddagger}$ *versus* $\Delta S^{\ddagger}$. Slopes equal to 312 and 315 in the anisotropic and isotropic media, respectively, with correlation coefficients greater than 0.99 were found; however, the precision of measurement was insufficient to support strongly isokinetic behavior in the latter case.

SUMMARY AND CONCLUSIONS

The lyotropic smectic liquid crystalline medium provided by an aqueous solution of polyoxyethylene 6 tridecyl ether was found to possess sufficient thermal, chemical, and compositional stability to serve as a useful structured biophase model. While this medium is not chemically similar to a proteinaceous liquid crystal, its ability to orient and hinder the rotation and bending of solutes within it may be presumed to be comparable. The Fischer indole synthesis is sensitive to such a medium, and similar kinetic manifestations of liquid crystalline structure can be expected of biochemical processes. The fact that an increasing number and variety of tissues, including muscles, tendons, nerve fibers, erythrocytes, ovaries, and bone, have been shown to contain a wide variety of liquid crystalline media serves to underline their importance in living matter.

APPENDIX I

For a first-order reaction in which only the conjugate acid, BH^+, of a weak base, B, is able to react, the rate is given by:

$$d[B]_T/dt = -k^*[BH^+] \qquad \text{(Eq. A1)}$$

where $[B]_T$ is the stoichiometric concentration of the base, and k^* is the intrinsic first-order rate constant. The concentration of conjugate acid is given by:

$$[BH^+] = \left(\frac{[H^+]}{K_a + [H^+]}\right)[B]_T \qquad \text{(Eq. A2)}$$

where K_a is the apparent dissociation constant of the conjugate acid. By combining Eqs. A1 and A2, the rate equation takes the form:

$$\frac{d[B]_T}{dt} = -k^* \left(\frac{[H^+]}{K_a + [H^+]}\right)[B]_T \qquad \text{(Eq. A3)}$$

However, the expression $k^*[H^+]/(K_a + [H^+])$ is defined as the observed pseudo-first-order rate constant, k_{obs}, and can be rearranged to give the linear form in Eq. 2.

APPENDIX II

In a biphase system, the hydrazone compounds used in this study distribute between the hydrocarbon phase and the aqueous polyethylene glycol phase in a manner such that the total quantity of the compound, Q, will be given by:

$$Q = [B]_w V_w + [B]_o V_o + [BH^+]_w V_w \qquad \text{(Eq. A4)}$$

where the subscripts w and o denote aqueous and hydrocarbon phases, respectively; and V denotes volume. Equation A4 can be expressed in terms of K_a and $[H^+]$ in the form:

$$Q = [BH^+]_w V_w \left(1 + \frac{K_a}{[H^+]} + \frac{K_a[B]_o V_o}{[H^+][B]_w V_w}\right) \qquad \text{(Eq. A5)}$$

By setting $r = V_o/V_w$ and $K_p^* = [B]_o/[B]_w$, Eq. A5 can be written as:

$$Q = \frac{[BH^+]_w K_a V_w}{[H^+]} \left(1 + \frac{[H^+]}{K_a} + rK_p^*\right) \qquad \text{(Eq. A6)}$$

where r is the phase volume ratio and K_p^* is the intrinsic partition coefficient. The overall mass rate equation:

$$dQ/dt = -k_{obs}Q = -k^*[BH^+]_w V_w \qquad \text{(Eq. A7)}$$

can be combined with Eq. A6, rearranged in the form of Eq. 3, and used to calculate K_p^*.

REFERENCES

(1) J. D. Bernal, *Trans. Faraday Soc.*, **29,** 1082(1933).

(2) D. B. DuPré, E. T. Samulski, and A. V. Tobolsky, "Polymer Sciences and Materials," Wiley, New York, N.Y., 1971.

(3) C. L. Khetrapal, "Lyotropic Liquid Crystals," Springer-Verlag, New York, N.Y., 1975.

(4) G. Gray, "Molecular Structure and the Properties of Liquid Crystals," Academic, New York, N.Y., 1962.

(5) G. H. Brown, J. W. Doan, and V. D. Neff, "A Review of the Structure and Physical Properties of Liquid Crystals," CRC Press, Cleveland, Ohio, 1971.

(6) G. H. Brown, *Am. Sci.*, **60,** 64(1972).

(7) E. G. Rippie and K. S. Murthy, *J. Pharm. Sci.*, **59,** 459(1970).

(8) S. I. Ahmad and S. Friberg, *J. Am. Chem. Soc.*, **94,** 5196(1972).

(9) W. E. Bacon and G. H. Brown, *Mol. Cryst. Liq. Cryst.*, **6,** 155 (1969).

(10) *Ibid.*, **12,** 229(1971).

(11) E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, **8,** 271(1970).

(12) K. S. Murthy, Ph.D. thesis, University of Minnesota, Minneapolis, Minn., 1968.

(13) A. R. Morad, Ph.D. thesis, University of Minnesota, Minneapolis, Minn., 1973.

(14) K. H. Pausacker and C. I. Schubert, *J. Chem. Soc.*, **1950,** 1814.

(15) P. A. Winsor, *Chem. Rev.*, **68,** 1(1968).

(16) J. C. Eriksson and G. Gilberg, *Surf. Chem.*, **1965,** 148.

(17) J. C. Eriksson and G. Gilberg, *Acta Chem. Scand.*, **20,** 2019(1966).

(18) J. C. Eriksson, *ibid.*, **17,** 1478(1963).

(19) S. Riegelman, N. A. Allawalla, M. K. Hrenoff, and L. A. Strait, *J. Colloid Sci.*, **13,** 208(1958).

(20) S. J. Rehfeld, *J. Phys. Chem.*, **74,** 117(1970).

(21) B. Rolinson, *Chem. Rev.*, **63,** 373(1963).

(22) E. A. Guggenheim, *Phil. Mag.*, **2,** 538(1926).

(23) L. M. Jackman, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, England, 1959.

(24) P. Ekwall, L. Mandell, and K. Fontell, *Acta Chem. Scand.*, **22,** 1543(1968).

(25) C. F. Hisky and T. A. Downey, *J. Phys. Chem.*, **58,** 853(1954).

(26) B. A. Mully and A. D. Metcalf, *J. Pharm. Pharmacol.*, **8,** 774(1956).

(27) L. G. S. Brooker and R. H. Sprague, *J. Chem. Soc.*, **63,** 3214(1941).

(28) S. Nagakura, *J. Am. Chem. Soc.*, **76,** 3070(1954).

(29) H. H. Jaffé and M. Orchin, *J. Chem. Soc.*, **1960,** 1078.

(30) R. G. Bates, "Determination of pH; Theory and Practice," Wiley, New York, N.Y., 1964, p. 114.

(31) L. P. Hammett, "Physical Organic Chemistry," 2nd ed., McGraw-Hill, New York, N.Y., 1970, pp. 397–401.

(32) J. E. Leffler, *J. Org. Chem.*, **20,** 1202(1955).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 12, 1975, from the *Department of Pharmaceutics, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455*

Accepted for publication February 4, 1976.

* Present address: Pharmacy Research and Development, Pharmaceuticals Division, Ciba-Geigy Corp., Suffern, NY 10901

x To whom inquiries should be directed.