Solvent Effects in the Cyclization of Thiol Amides and Hydroxy Acids

G. Alan Dafforn and Daniel E. Koshland, Jr.*

Contribution from the Department of Chemistry, Bowling Green State University, Bowling Green, Ohio 43403, and Department of Biochemistry, University of California, Berkeley, Berkeley, California 94720. *Received December 15, 1976*

Abstract: Solvent effects on the ring closure of some thiol amides and hydroxy acids have been examined using sulfolane as a solvent. In general, the relative reactivities in the dipolar aprotic solvent parallel those in water, but there are some deviations. Solvent effects on rates of the individual reactions studied here as well as on related reactions are generally modest, but examples of changes in rate as large as 10^6 with changing solvent are noted if catalyst activity is considered. The role of solvation as a factor in reactions at an enzyme surface is strongly dependent on the specific reaction; solvation cannot be a universal factor in enzyme catalysis.

I. Introduction

Among the effects which may contribute to the high catalytic efficiency of enzymes are possible solvation and microenvironmental effects. Both effects could presumably accelerate a chemical reaction by taking advantage of the sensitivity of many reactions to the nature of the surrounding medium. For example, a reaction which proceeds faster in a solvent of low dielectric constant might be accelerated by binding in a hydrophobic active site. More subtly, the structure of an enzyme active site might contain regions of very different solvating properties arranged in such a way as to desolvate the bound substrate but not the transition state for a reaction. Like many other effects contributing to enzymatic rates, this one is difficult to measure directly and is best approached through model systems.

The simplest analogy to enzymatic solvation effects is the effect of a change in solvent on the rate of a model reaction such as ester hydrolysis.[1] It is widely appreciated that such solvent effects vary from moderate to many orders of magnitude, particularly when dipolar aprotic solvents are involved or a change in charge type occurs.[2] Only limited efforts have been made to extrapolate these results to enzymatic reactions, leading to the conclusion that solvent effects certainly affect enzymatic reactions, but probably are not a dominant factor.[1] The question of microenvironmental effects is more difficult to deal with quantitatively. Although microheterogeneous environments can be created with micelles or polyelectrolytes,[3] the specificity of placement of charges, etc., in an enzymatic active site is difficult to mimic. Insofar as interpretation is possible, the generally modest catalysis by micelles (1–2 orders of magnitude) would argue against large microenvironmental effects.[3] Related observations such as strong electrostatic catalysis by $LiClO_4$ in ether demonstrate the powerful catalytic effects of charge embedded in an apolar environment.[4] Even in this case, very high concentrations of salt are required to equal the solvating power of water. Thus the available evidence would seem to indicate a limited role for solvation and microenvironment effects; nevertheless, a number of reactions show quite spectacular solvent effects and the possibility of important solvation catalysis by enzymes cannot be dismissed.

A related problem is the possible effect of solvation on the intramolecular reactions often used as models of enzymatic processes. The high rates of intramolecular reactions relative to bimolecular counterparts can be taken as evidence that placing two functional groups next to each other in a specific alignment, as at an enzyme active site, enhances their rate of reaction. In both enzymatic and intramolecular reactions, this effect could arise because the groups are desolvated relative to each other. In both situations, the groups may be held so close that no solvent molecule can be interposed between them; compared to a bimolecular reaction, no solvent molecules must be removed from a solvation shell for reaction to occur. Such an effect would be of interest because observed rate enhancements of intramolecular over bimolecular reactions have been used as evidence for other theories of enzyme action.[5] In particular, a large range of relative rates for acid-catalyzed lactonization or esterification in water of compounds IV–VI shown in Table I is observed.[6] This and other examples of sensitivity of rate to structure have been interpreted in terms of orientation of functional groups. Although good evidence was presented that these relative rates were not a consequence of solvation, the possibility could not be totally ruled out.

Structural and solvation effects on such a series of reactions could be completely separated by repeating the reactions in the gas phase or by gas-phase quantum mechanical calculations, but at least two kinds of problems arise in either case. Most reactions of interest as biochemical model systems do not occur in the gas phase, though such techniques as ion cyclotron resonance offer promise along these lines. Similarly, quantum mechanical calculations sophisticated enough to be believable remain extremely expensive and time consuming. In addition, the significance of such results is questionable since stripping away the solvent could substantially alter the mechanism.[7]

A more feasible approach to the observation of solvation effects is to measure relative rates in two solvents of radically different character. This approach has been used by Bruice and co-workers[8] to examine relative rates for intramolecular vs. bimolecular anhydride formation in water and Me_2SO–water. The individual reactions both involved nucleophilic attack by carboxylate on aryl esters and showed quite large solvent effects on going from water to 1 M H_2O in Me_2SO. The ratio of the two rates was only slightly affected, indicating that solvation is probably not responsible for the high rate of the intramolecular reaction.

We have undertaken a study of the effects of changing solvent on series of intramolecular reactions for two reasons. First, the extensive series of reactions used earlier as support for an orientational theory of high enzymatic rates were mostly acid catalyzed. Consequently, exclusion of solvation effects in our systems based on a single example of a reaction proceeding by a different mechanism was not proven. Second, the effect of large changes in solvation on rates of individual reactions might be useful as models for solvation effects in enzymatic reactions.

The reactions chosen for reinvestigation in nonaqueous media are shown in Table I. Lactonization or esterification of the series IV–VI was an obvious choice as the hydroxy acids

were the most thoroughly studied series earlier. Thiolactone formation from the series I–III was particularly interesting because of the unusual relative rates observed in water with I closing more rapidly than II. Thus this series seemed a promising starting point for investigation of solvation. It also offered two potential experimental advantages: the thiolactone products absorb strongly in the UV region, making rates easy to measure, and the amide group might be completely protonated by an acid catalyst, simplifying the interpretation.

Trifluoromethanesulfonic acid (HOTF) in sulfolane was chosen as the nonaqueous solvent system. The dipolar aprotic solvent sulfolane is quite similar to Me_2SO in most solvent properties such as dielectric constant and sufficiently different from water to expose any solvation effects. Sulfolane was preferable to Me_2SO for our purposes because it apparently could be obtained transparent down to at least 240 nm,[9] the wavelength where thiolactones absorb,[6] because it lacks the ability of Me_2SO to oxidize thiols,[10] and because of its general high stability. Trifluoromethanesulfonic acid is an extremely strong acid[11] but is unlikely to oxidize thiols. A strong acid catalyst offered the possibility of complete protonation of the amide starting materials, eliminating changes in pK_a of the amides as a possible factor in relative rates. In addition, complications due to partial dissociation of the catalyst could be avoided with a sufficiently strong acid.

II. Experimental Section

General. Kinetic measurements monitored at constant wavelength were made using a Gilford recording spectrophotometer Model 2000 with a cell compartment thermostated by a K-2/R Lauda/Brinkmann circulating bath or a Zeiss PMQ II. UV spectra were determined using a Cary Model 14 with cell compartment thermostated at 30 °C. Infrared spectra were taken with a Perkin-Elmer Model 257 grating infrared spectrometer using KBr pellets. NMR spectra were recorded on a Varian Associates A-60 spectrometer. Melting points were determined with a Thomas capillary melting point apparatus and were uncorrected. Elemental analysis and Karl-Fischer determinations were done by the Microchemical Analytical Laboratory, University of California, Berkeley.

Lactones and Thiolactones. The preparation and purification of these compounds have been described previously.[6]

γ-Mercaptobutyramide (I). γ-Thiobutyrolactone was treated with anhydrous liquid ammonia in a Parr bomb for 24 h at room temperature. The product was contaminated by a significant amount of the disulfide dimer of γ-mercaptobutyramide. The monomer was separated from the oxidation product by silica gel chromatography using acetone as a solvent. The product was stored under nitrogen in a desiccator to prevent oxidation of the mercapto group: mp 91–93 °C; IR ($CHCl_3$) 1620, 1680 cm^{-1} (amide I and II bands).

Anal. (C_4H_9NOS) C, H, N, S.

2-*endo*-Mercaptomethylbicyclo[2.2.1]heptane-3-*endo*-amide (II). This amide was synthesized by treating 2-*endo*-mercaptomethylbicyclo[2.2.1]heptane-3-*endo*-carboxylic acid thiolactone with anhydrous liquid ammonia in a Parr bomb at room temperature for 24 h. The product was chromatographed on silica gel using acetone as a solvent and recrystallized from benzene–*n*-hexane: mp 80–83 °C; IR (KBr pellet) 1680, 1620 cm^{-1} (amide I and II bands).

Anal. ($C_9H_{15}NOS$) C, H, N, S.

2-*endo*-Carboxamido-6-*endo*-thiobicyclo[2.2.1]heptane (III). The corresponding thiolactone (0.5–1 g) was placed in a 20-mL glass vial inside a thick-wall stainless steel Parr bomb. The bomb was capped with a drying tube and cooled to liquid N_2 temperature. The vial was quickly filled with liquid NH_3, capped, and allowed to stand at room temperature for 2 days. The bomb was then recooled with liquid N_2, opened, and immediately placed in a lyophilizing flask. NH_3 was removed by pumping overnight after careful evacuation to minimize bumping. The resulting fluffy white powder was transferred to vials under N_2 in a dry bag and stored in the refrigerator.

The amide was identified by IR (KBr pellet), showing N–H bands at 3360 and 3190 cm^{-1} and C=O bands at 1700, 1660, and 1620 cm^{-1}. Conversion was always incomplete. Extent of conversion was determined by the increase in UV absorbance at 240 nm on complete thiolactonization in HCl/H_2O or sulfolane, and ranged from 10 to 75%. (More recent experience indicates that the product is most stable when stored at 0 °C over P_2O_5.)

2-*endo*-Hydroxymethylbicyclo[2.2.1]heptane-3-*endo*-carboxylic Acid (V). The lactone of the above acid was stirred overnight with a slight excess of 1 M NaOH and sufficient ethanol to solubilize lactone to give the sodium salt of the acid. The solution was cooled in an ice bath, and 1 equiv of acetic acid or concentrated HCl was added, giving an immediate precipitate. Extraction of the mixture with chloroform, separation, and evaporation of the chloroform layer gave the product as a white powder: IR O–H 3475, C=O 1705 cm^{-1} (no 1760 cm^{-1} lactone).

4-Hydroxybutanoic Acid (IV). This compound was too water soluble for isolation as described above. Instead, the free acid was obtained by potentiometric titration with methanolic HCl of sodium 4-hydroxybutanoate (obtained by hydrolysis of the lactone as above followed by lyophilization) in methanol. Addition of 5 volumes of ether and filtration removed NaCl, and the free acid was obtained as a yellowish oil by evaporation of solvent at low temperature: IR 3200–3450 (broad), 1720 cm^{-1} (broad).

Trifluoromethanesulfonic acid (HOTf) was obtained anhydrous from Minnesota Mining and Manufacturing Co. It was conveniently handled as an approximately 1 M stock solution in sulfolane, standardized by dilution in water and titration with standard NaOH solution.

Sodium Trifluoromethanesulfonate. CF_3SO_3H (1.91 g) was dissolved in about 20 mL of water, neutralized with dilute NaOH using a pH meter, and evaporated to dryness. The resulting white crystals were recrystallized from 50:50 acetone–benzene, mp 251.5–252.5 °C (lit. mp 248 °C).[12]

Ammonium Trifluoromethanesulfonate. CF_3SO_3H (2.54 g) in 15 mL of water was neutralized to pH 6.6 with aqueous ammonia, evaporated to dryness, and recrystallized from 5:1 acetone–benzene, mp 225.5–226.5 °C.

Hammett indicators were obtained as a set from Aldrich Chemical Co. All other compounds were of reagent grade.

Sulfolane (Tetrahydrothiophene 1,1-Dioxide). Sulfolane was obtained from Phillips Petroleum Co. or from Aldrich Chemical Co. Aldrich material showed some visible light absorption and a solvent cutoff ($A = 1$) at 300 nm. Several purification procedures were tried, all based generally on the procedure used by Coetzee, Simon, and Bertozzi.[13] The best of several procedures to remove material absorbing in the UV requires heating to 180–200 °C for 24 h under N_2 in the presence of NaOH pellets, vacuum distillation of the resulting black liquid, stirring overnight with 30% fuming sulfuric acid, and redistillation. After standing over NaOH flakes for several days to remove sulfuric acid, the solvent was rapidly vacuum distilled in small batches (<500 mL) from CaH_2 and stored under nitrogen until used. The resulting solvent had a UV cutoff ($A = 1$) at 200–203 nm and was transparent above about 210 nm. Karl-Fischer titration always showed <0.1% water.

The initial heat step (to decompose sulfolene) and treatment with concentrated H_2SO_4 or oleum were common to all batches prepared. In batches distilled from H_2SO_4, fractional distillation under vacuum or treatment with activated charcoal was usually necessary to remove residual impurities before final distillation from CaH_2. Slow distillation of large batches from CaH_2 generally results in a slight increase in UV absorption in the product and was avoided.

Slight decomposition of the solvent after heating overnight at 90 °C was usually observed either in the presence or the absence of CF_3SO_3H. Generally this resulted in an increase of <0.1 A at 240 nm, but occasionally decomposition was extensive, resulting in visible color or a new absorption peak at 255 nm. The tendency to produce absorption in the UV varied somewhat from batch to batch, sometimes increased as a particular batch got older, and could be retarded when necessary by degassing of the solvent before use.

Kinetics. Most reactions were monitored directly in the UV. The closure of thiol amides to thiolactones was generally followed by the appearance of characteristic UV absorption at 240 nm (except 234 nm for III). Thiol amide concentration was generally $<10^{-4}$ M and such that a substantial excess of acid catalyst over thiol amide was present. The ring closure of IV was routinely followed at 230 nm using 0.025–0.1 M substrate ($\Delta\epsilon = 2$). Closure of compound V was followed at 225 nm ($\Delta\epsilon = 4$). All rates for hydroxy acids were measured at 30 °C.

A stock solution of the reactant in sulfolane was diluted with sulfolane or sulfolane–water in a 3-mL capped cuvette and allowed to equilibrate thermally in the cell compartment. Reaction was initiated

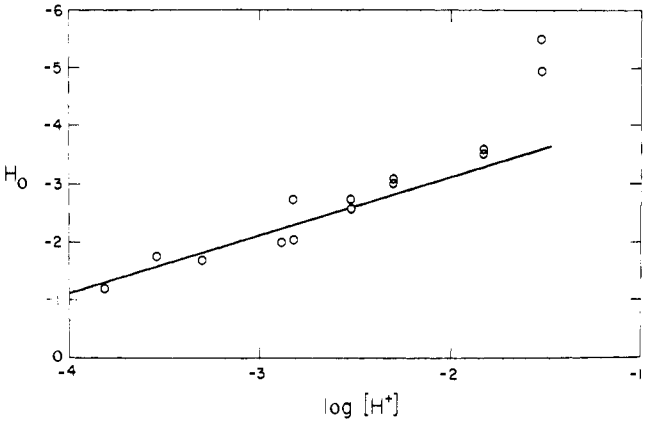


Figure 1. Hammett acidity of trifluoromethanesulfonic acid in sulfolane. Each point represents a separate measurement of H_0 at the acid concentration specified. Indicators used were 4-chloro-2-nitroaniline, 2,4-dichloro-6-nitroaniline, and 2,4-dinitroaniline.

by addition of a concentrated stock solution of trifluoromethanesulfonic acid in sulfolane; changes in UV absorbance were followed for at least 3 half-lives. A solvent–acid blank was included in all runs at 90.4 °C. Slight changes in solvent absorption were applied as a baseline correction to the kinetics; if extensive changes in absorption occurred, the run was discarded. The spectra of the products of representative runs were determined using a Cary 14. A calibration curve was established between the temperature of the circulating water bath and actual reaction temperature using a thermocouple.

First-order rate constants were obtained using a shortened version of the least-squares kinetic program LSKIN 1, provided by Professor Streitwieser. Individual runs generally gave excellent first-order kinetics, with standard deviations of 2–3%.

Controls. The product solution obtained from closure of the 2,3-thiol amide (II) appeared fairly stable to oxygen. At the end of a kinetic run at 30 °C and 0.3 M acid, the solution in the cuvette was saturated with oxygen for 20 min; its absorbance at 240 nm decreased only about 10%. The range of conditions over which usable data could be collected was limited by a shift in λ_{max} of the product absorption from 240 to 252 nm occurring at acid concentrations of 0.3–0.4 M. This shift was found to be reversible. The absorption maximum of a product solution could be changed repeatedly by dilution with solvent or addition of acid. Individual kinetic experiments above 0.3 M acid gave good first-order rates and the resulting rate constants fit satisfactorily a first-order dependence on acidity. Nevertheless, these rates have been omitted from consideration because of uncertainty in the nature of the product.

For closure of thiobutyramide I, degassing of the solvent appeared to have little effect on rates within the usual scatter. One run at 90.4 °C and 1.54×10^{-2} M acid was carried out in solvent saturated with oxygen; in this case, extensive decomposition of the product was noted in the later stages of the reaction. As with compound II, the useful range of acidities was limited at the high end by a wavelength shift, in this case from 234 to 244 nm.

The product was also identified by incubating a sulfolane solution 0.2 M in thiol amide I and 0.4 M in acid for 1.5 h at room temperature, diluting 1:1 with water and extracting with hexane. The residue obtained by evaporation of the extract had an IR spectrum identical with that of the authentic thiolactone.

Ring Closure of Thiobutyramide (I) by Thiol Determination. In order to ensure that the reaction being followed was direct conversion of thiol to thiolactone, two cuvettes were prepared as described above for monitoring rates by UV except containing 10^{-3} M thiol. After initiation of reaction by addition of acid at 90.4 °C, 0.1-mL aliquots were removed from one cuvette and diluted to 1 mL with 0.2 M phosphate buffer, pH 7.5, containing an excess of 5,5′-dithiobis(2-nitrobenzoic acid) or DTNB over compound I. The amount of free thiol remaining was determined by absorbance at 410 nm.[14] The rate of thiol disappearance and the rate of thiolactone formation (monitored simultaneously by UV) were essentially identical.

Formation of Ethyl Acetate from Acetic Acid and Ethanol in Sulfolane. Solutions of acetic acid and ethanol in sulfolane were thermally equilibrated in a small volumetric flask and reaction was initiated by addition of acid stock solutions. Aliquots were withdrawn and quenched in 0.02 M phosphate buffer, pH 7. Concentrations of ethyl acetate were determined by hydroxamate assay[15] and used to obtain pseudo-first-order rate constants.

Initial Velocity of Thiobutyramide (I) Closure. Solutions of compound I and acid in sulfolane were preheated separately to 90 °C and reaction was initiated by adding 0.5 mL of acid stock to 0.5 mL of compound I in a 1-mL stoppered volumetric flask. Aliquots were removed and rapidly quenched in 20 vol % aqueous ethanol at room temperature. Absorbance at 236 nm of the quenched solutions was plotted as a function of time. Linear plots were obtained with Δ_{OD} ranging from 0.07 to 0.6. Reactions were monitored from 5 min to 2 h depending on concentration of acid. Velocities were calculated based on a molar absorptivity of 4.5×10^3 for thiolactone in 20% ethanol.

Acidity Studies. Acidities of CF_3SO_3H–sulfolane solutions were determined by preparing $\sim 10^{-4}$ M solutions of the appropriate Hammett indicator in sulfolane or mixed solvent in a 3-mL capped cuvette and equilibrating to 30 °C in the cell compartment of a Cary 14. The visible spectrum was determined, an aliquot of acid stock solution was added, and the spectrum was redetermined. The observed changes in absorption were converted into H_0 in the normal manner. Indicators used and their pK_as in sulfolane follow: 4-chloro-2-nitroaniline (−1.03), 2,4-dichloro-6-nitroaniline (−3.37), 2,4-dinitroaniline (−4.36).[16] In general, the technique used was designed to duplicate the conditions of corresponding kinetic studies. No special efforts were made to exclude traces of atmospheric water.

Protonation of acetamide or isobutyramide could also be observed directly in the ultraviolet. In these studies, the absorbance at 212 nm of amide solutions was monitored as aliquots of concentrated acid were added. Plots of absorbance (corrected to constant volume) vs. log [H+] showed the expected drop in absorbance as amide was protonated. The lowest concentration of either amide for which a usable change in absorbance could be obtained was 2.5×10^{-3} M. Even at this concentration, addition of 1 equiv of acid resulted in about 50% protonation of amide. Combination of these data and information on acidity as a function of acid concentration (eq 2, Results) indicates a pK_a of about −2 for acetamide and isobutyramide in sulfolane, but an accurate pK_a could not be obtained because of scatter and the necessity of correcting for acid consumed in protonation.

III. Results

Ring Closure of Thiol Amides to Thiolactones. Kinetic Procedures. The observed first-order rate constants k_{obsd} for thiolactone formation in the presence of excess acid were fitted to the equation

$$k_{obsd} = k_0 + k_{HOTf}(HOTf) \quad (1)$$

The structures of the compounds examined and the resulting rate constants at 30 °C are given in Table I. More detailed discussions of individual compounds are given below.

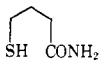
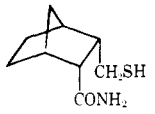
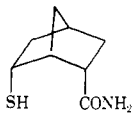
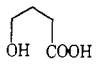
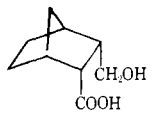
Acidity Studies. A rough estimate of the acidity of the medium was obtained by the use of Hammett indicators in the usual way. A plot of the function H_0 against acid concentration is given in Figure 1. Considerable scatter was observed even for different points obtained using the same indicator; similar scatter with low acid concentrations has been observed for H_2SO_4 in sulfolane[16] and is presumably a result of absorption of atmospheric water by solvent. With the exception of two points at high acidity, the data illustrate a first-order dependence of acidity on acid concentration over the region used for kinetic studies. A least-squares fit of points between 1.5×10^{-4} M and 1.5×10^{-2} M acid gives

$$H_0 = -\log [H^+] - 5.1 \quad (2)$$

Although acidity functions are subject to some uncertainty in general and protonation of amides by H_2SO_4 is known to deviate from the Hammett scale,[17] these results at least suffice to give a rough measure of the acidity of the medium.

It was also demonstrated directly that acetamide or isobutyramide in sulfolane are about half protonated at a free acid concentration of 10^{-3} M by observing a decrease in UV absorbance at 212 nm with increasing acid concentration. Ex-

Table I

Reactant	k_0, min^{-1}, in sulfolane[a]	k_{HOTf}, M^{-1} min^{-1}, in sulfolane[b]	$k_{H_3O^+}$ in H_2O[c]	k_{H_2O}, min^{-1}, in H_2O[d]	k_{H_0} in sulfolane[e]
I 		0.13	5×10^{-4}		1.0×10^{-6}
II 		0.09	2.9×10^{-4}		7×10^{-7}
III 	1.5×10^{-2}	5	0.25	0.11	4×10^{-5}
IV 		18.7	8.58×10^{-2}		0.10
V 		920	7.23		5.1
VI CH_3CH_2OH / CH_3COOH		0.95[f]	1.09×10^{-3}		2.7×10^{-3}

[a] Acid-independent first-order rate constant in sulfolane at 30 °C. [b] Second-order rate constant dependent on concentration of trifluoromethanesulfonic acid (HOTf) in sulfolane at 30 °C. [c] Second-order rate constant dependent on dilute acid in water. From ref 6 or unpublished work at 25 °C. [d] Acid-independent first-order rate constant in water. [e] Second-order rate constant dependent on acidity as measured by Hammett H_0 in sulfolane. [f] Pseudo-second-order rate constants corrected to 1 M ethanol–sulfolane.

perimental difficulties precluded determination of a pK_a.

Ring Closure of 2-*endo*-Carboxamido-6-*endo*-mercaptobicyclo[2.2.1]heptane (III). Observed first-order rate constants for thiolactone formation at 30 °C without added water and with 0.185 M added water were plotted against acid concentration over the range 10^{-4}–10^{-1} M acid on a log–log scale as shown in Figure 2. For both sets of data, clearly defined regions of zero and first-order dependence on acidity are evident. Considerable scatter among rate constants at similar acid concentrations is observed above about 10^{-3} M acid, but no systematic deviation in rates was noted for 65 runs in several batches of solvent. Stock solutions of thiol amide in the absence of acid were stable overnight or longer; thus the neutral reaction is slow at 30 °C. Two rate constants were determined in sulfolane containing 0.185 M D_2O; comparison with simultaneous runs with 0.185 M water gave an isotope effect of $k_{H_2O}/k_{D_2O} = 1.17$ at 1.08×10^{-3} M acid. An isotope effect of 2–3 is clearly outside the range of experimental error. Activation parameters based on the observed first-order rate constant over the temperature range 30–90 °C at 1.08×10^{-3} M acid without added water were $\Delta H^{\ddagger} = 15.0 \pm 0.3$ kcal/mol and $\Delta S^{\ddagger} = -25.3 \pm 1.7$ eu.

Ring Closure of 2-*endo*-Carboxamido-3-*endo*-mercaptomethylbicyclo[2.2.1]heptane (II). Rate constants were obtained over the range 0.05–0.3 M acid at 30 °C and 0.02–0.1 M acid at 90.4 °C, using thoroughly degassed solvent, and are shown in Figure 3. In some earlier kinetic runs in which the solvent was not degassed, individual rate constants showed a high degree of scatter and a second-order dependence on acid concentration. A 1.5-order dependence of rate on acid concentration observed at 90.4 °C may represent a vestige of the same effect. At 30 °C, a good first-order dependence of rate constants on acid concentration was observed.

Ring Closure of γ-Mercaptobutyramide (I). Rate constants determined at 90.4 °C over the acid range 2×10^{-3} to 2×10^{-1} M are shown in Figure 4. A good first-order dependence on acidity of 27 rate constants measured in three batches of solvent was obtained. Seven rate constants measured in the first batch of solvent purified were a factor of 2–3 slower than expected. At 30 °C, 18 rate constants over the range 0.03–0.15 M acid also showed a good first-order dependence. Above 0.15 M acid, the rate increased more slowly than expected with increases in acidity. Activation parameters calculated from these data were $\Delta H^{\ddagger} = 11.4$ kcal/mol and $\Delta S^{\ddagger} = -37.4$ eu based on observed first-order rate constants at 0.1 M acid. Stock solutions containing γ-mercaptobutyramide but no added acid were stable, even overnight at 90 °C. Rate constants could be obtained at less than 2×10^{-3} M acid, but the sharp absorption peak of the product at 234 nm had been replaced by a generally high but featureless absorption below about 330 nm.

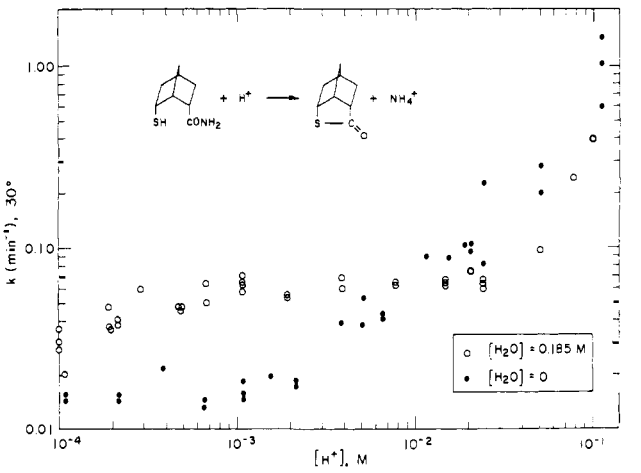


Figure 2. Observed first-order rate constants for thiolactone formation from compound III at 30 °C in sulfolane. On a log–log scale, $[H^+]$ represents the concentration of CF_3SO_3H added; acid was always present in large excess over III.

The possibility that the observed catalysis resulted from an electrostatic effect caused by the partially ionic character of the acid rather than true acid catalysis was rendered less likely by examining the effect of NH_4OTf and NaOTf on ring clo-

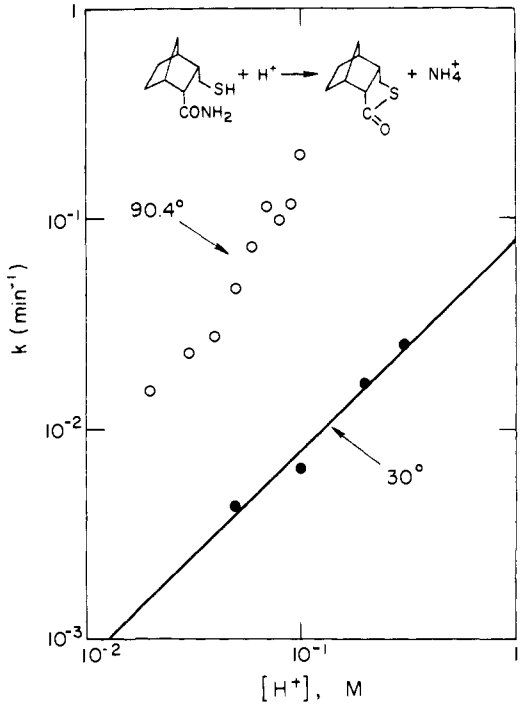


Figure 3. Observed first-order rate constants for thiolactone formation from II in sulfolane at 30 and 90.4 °C. On a log-log scale, line at 30 °C has slope +1.

Table II. Salt Effects on the Ring Closure of γ-Mercaptobutyramide (I) in Sulfolane[a]

Salt	[Salt], M	[HOTf], M	k, min^{-1}	No. of runs
NH_4OTf	0.045	0	0	1
NH_4OTf	0	0.045	0.142	2
NH_4OTf	0.045	0.045	0.087	2
NH_4OTf	0.09	0.045	0.074	1
NaOTf	0.045	0	0	1
NaOTf	0	0.045	0.115	2
NaOTf	0.045	0.045	0.085	2
NaOTf	0.09	0.045	0.058	1

[a] Average pseudo-first-order rate constants for ring closure at 90.4 °C with about 10^{-4} M reactant and the indicated amounts of trifluoromethanesulfonic acid and its ammonium or sodium salts.

sure rates. These effects are summarized in Table II. In both cases, salt alone is incapable of catalyzing the reaction and addition of salt to the acid-catalyzed reaction slows the reaction.

This and other controls described in the Experimental Section strongly indicate that the observed reaction is acid-catalyzed ring closure of the thiol amide I to the thiolactone.

Second-Order Rate Expression for Thiolactonization. The observed first-order dependence of the measured pseudo-first-order rate constants on acid concentration corresponds to the behavior observed in aqueous solution. Nevertheless, the high acidity of the medium compared to the pK_a of normal amides and the observation of a plateau region in the closure of compound III suggested that the amide substrates might be completely protonated in this medium. If S represents the starting material and P the product, the "normal" mechanism of ring closure in water is usually assumed to follow the rate law

$$-dS/dt = dP/dt = v = k_0[S] + k_{HOTf}[S][H^+] \quad (3)$$

whereas the following rate law must be considered in sulfolane:

$$-dS/dt = dP/dt = v = k_0[SH^+] + k_{HOTf}[SH^+][H^+] \quad (4)$$

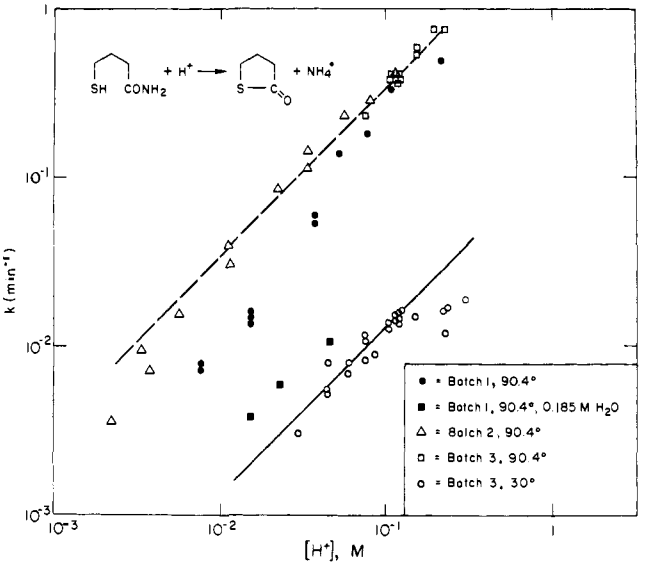


Figure 4. Observed first-order rate constants for thiolactone formation from I in sulfolane. On a log-log scale lines are of slope +1. Batch members refer to batches of solvent purified at different times.

If rates are always determined with a large excess of acid and S is completely protonated throughout the range of acidity studied, the two mechanisms are kinetically indistinguishable. The only kinetic observation described so far which favors eq 4 is that the rate of closure of compound III in the plateau region between 10^{-3} and 10^{-4} M acid is much faster than its rate of decomposition in neutral stock solutions. The simplest alternative explanation for this observation is that the equilibrium thiol amide ⇄ thiolactone + NH_3 lies to the left unless acid is present. This alternative was ruled out by showing that the UV spectrum of the thiolactone corresponding to compound III was unchanged after 2 h in a sulfolane solution saturated with anhydrous NH_3.

Another way of distinguishing between the two rate laws is raising the substrate concentration to a level comparable with the acid concentration added. Although the kinetics then become more complex because of the release of ammonia during the reaction, eq 3 or 4 should adequately describe the initial velocity of the reaction. The distinction arises because protonation of the amide consumes a substantial amount of acid, so that the amount of acid added is no longer a good measure of $[H^+]$.

The simplest examples are the limiting cases. If eq 3 holds, the initial velocity can of course be predicted using k_{HOTf} from pseudo-first-order experiments. If we define $S_t = (S + SH^+)$ and $H_S = (SH^+ + H^+)$, then the other extreme occurs at stoichiometric protonation when $S_t = SH^+$ for $H_t \geq S_t$. The initial velocity is then

$$v_i = k_0[S_t] + k_{HOTf}[S_t][H_t - S_t] \quad (5)$$

For compound I where k_0 is very small, eq 3 predicts that the first 10% reaction should require 0.63 min at 90.4 °C, $H_t = S_t = 0.05$ M. Equation 5 predicts that no reaction should occur. Solution for the general case of an amide with acid dissociation constant K_a (in terms of acid concentration) leads to more complex equations but the same general conclusion.

Attempts to determine initial rates for the butyro compound I at 90.4 °C with $H_t = S_t = 0.05$ M by following absorbance at long wavelengths on the shoulder of the product peak failed, apparently because the solution developed color. However, it

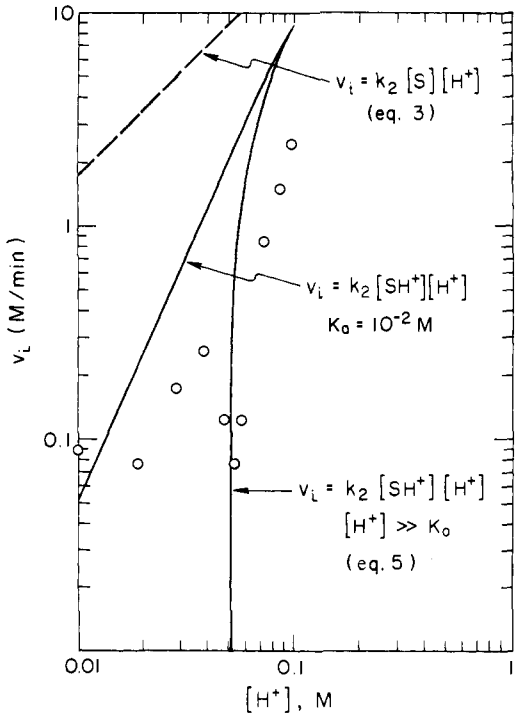


Figure 5. Initial velocity of formation of thiolactone from In in sulfolane at 90.4 °C (mol L^{-1} min^{-1}). Concentration of I = 0.05 M. o = experimental values. - - - - expected values for a normal second-order reaction with $k_{HOTf} = 3.5$ M^{-1} min^{-1}. ——= expected values from eq 5 and from eq 4 with $([S][H^+]/[SH^+]) = 10^{-2}$ M.

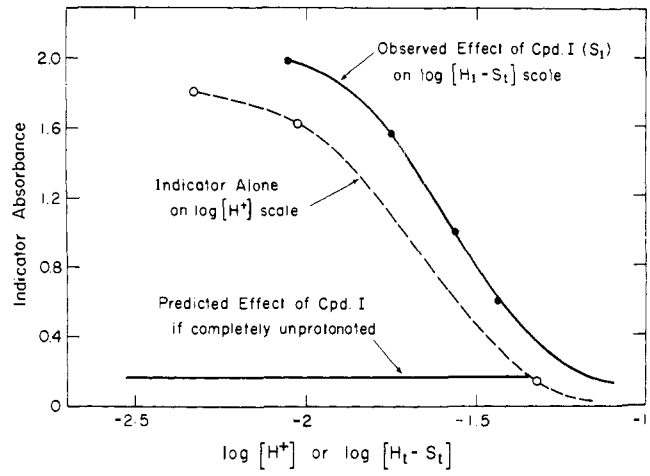


Figure 6. Titration of I with CF_3SO_3H using *p*-nitrodiphenylamine as indicator in sulfolane at 30 °C. For stoichiometric protonation of the amide I, all points should fall on the same curve. A constant low absorbance (Δ—Δ) would result if I were not protonated.

was found that linear initial rates could be obtained for up to 10–40 min at 90.4 °C and S = 0.05 M by removing aliquots, quenching them in aqueous buffer at room temperature, and measuring absorbance at 234 nm. Under these conditions, sharp product peaks were obtained. The measured initial velocities are shown in Figure 5 along with theoretical lines calculated using eq 3, eq 5, and equations using intermediate values of K_a.

The "normal" mechanism of eq 3 predicts too high a velocity by 2 orders of magnitude at 0.05 M acid and is thus ruled out. The stoichiometric protonation mechanism of eq 5 provides a much better fit above 0.05 M acid, but is still high by a factor of 3. The region below 0.05 M may represent a plateau reflecting the k_0 term, but the scatter was too high to justify calculating a rate constant from these data. The lack of quantitative agreement between experiment and either prediction may reflect either further subtleties of the mechanism or scatter of the kind obtained earlier between solvent batches for the pseudo-first-order rate constants; in either case, the stoichiometric protonation mechanism provides a much more nearly satisfactory description of the kinetics than does the normal mechanism observed in aqueous solution.

The amide group can also be titrated directly under suitable conditions using the Hammett indicator *p*-nitrodiphenylamine ($pK_a = -2.40$). The relationship between acid concentration and indicator protonation was first established by observing the absorbance of 10^{-4} M indicator at 397 nm in solutions containing 4.75×10^{-3} to 4.75×10^{-2} M acid. Aliquots of the butyro compound I were then added to a solution containing 4.54×10^{-2} M acid and indicator and the amount of free acid remaining was determined by indicator absorbance. Two limiting cases can again be distinguished. If no protonation occurs, then a plot of indicator absorbance against log $[H_t - S_t]$ should be a horizontal line since addition of amide should not affect the acid strength. If protonation is stoichiometric, then $[H_t - S_t]$ gives the concentration of acid actually present. A plot of absorbance against log $[H_t - S_t]$ should fall on the same sigmoid curve as a plot of absorbance against the log of the added acid in the absence of S (the protonation curve of the indicator).

The curves obtained are shown in Figure 6. The effect of adding substrate is to decrease the acidity to a level somewhat lower than predicted. This result is consistent with stoichiometric protonation and with the initial velocity experiments, in which the observed velocities were about three times slower than predicted. Finally, it suggests that the extra rate decrease seen in the kinetics may result from water or some minor impurity in the substrate rather than from some more complex kinetic scheme than those taken into account here.

Considered together, these results strongly support the application of eq 4 or 5 rather than eq 3 to the results obtained with thiol amides.

Ring Closure of Hydroxy Acids to Lactones. This reaction was initially followed in anhydrous sulfolane, but was found to give very complex, poorly reproducible kinetics. Since a large excess of substrate (0.05 M) over acid (10^{-3}–10^{-4} M) was used, these results probably reflect the effect of the substrate hydroxyl group on the acidity of the medium. The solution adopted was the addition of small amounts of water to minimize the effect of substrate hydroxyl groups without markedly affecting bulk solvent properties.

The rate constants obtained for hydroxybutyric acid IV with 0.37 M added water are shown in Figure 7. First-order rate constants were essentially independent of substrate concentration over the range 0.025–0.1 M and exhibited a first-order dependence on acid concentration. The titration curves obtained in the presence and absence of 0.025 M substrate (4-chloro-2-nitroaniline indicator) are also shown in Figure 8; no effect of the substrate on acidity was observed. The rate constants obtained for the 2,3 hydroxy acid V in 0.28 M water are also shown in Figure 7. The reaction had a slightly higher than first order dependence on acid concentration, but was found to be independent of substrate concentration over the range 0.0125–0.05 M. Acid concentrations were too low to allow a titration.

Bimolecular Reaction between Ethanol and Acetic Acid in Sulfolane. Formation of ethyl acetate was followed by removing aliquots, diluting them in pH 7 buffer, and determining the concentration of ethyl acetate present by the hydroxamate assay. Figure 9 shows the first-order rate constants obtained with 0.37 M ethanol, 0.029 M acetic acid and 1.14 M ethanol, 0.058 M acetic acid. A first-order dependence of rates on acid concentration was found at 1.14 M ethanol, but rates in 0.37

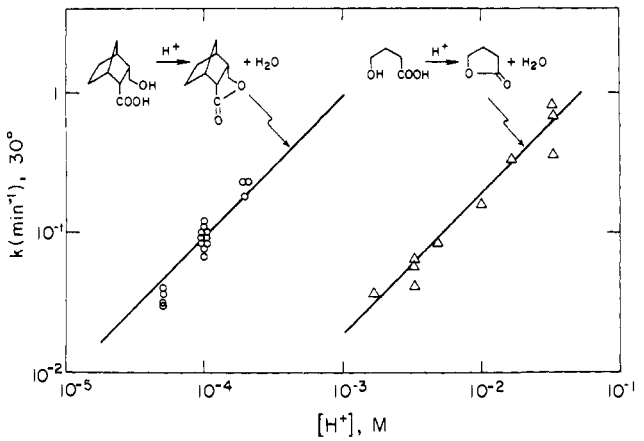


Figure 7. Observed first-order rate constants for lactone formation from hydroxy acids IV and V in sulfolane-water at 30 °C on a log-log scale: O = compound V in sulfolane containing 0.28 M water; Δ = compound IV in sulfolane containing 0.37 M water.

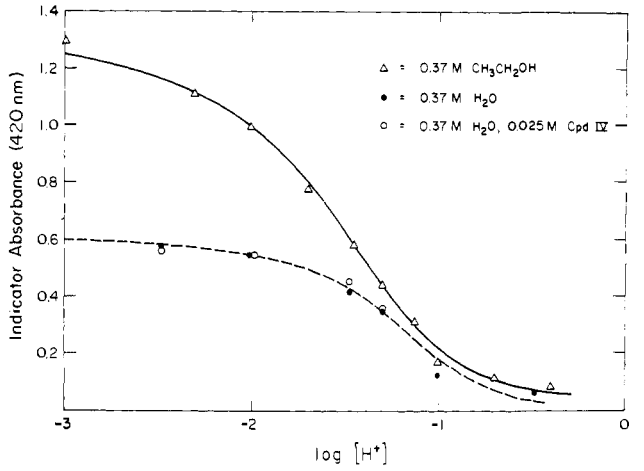


Figure 8. Hammett acidity of CF_3SO_3H in sulfolane containing ethanol, water, and hydroxy acid IV—absorbance of 4-chloro-2-nitroaniline as a function of log $[CF_3SO_3H]$ at 30 °C. Compound IV has no effect on acidity in sulfolane containing 0.37 M water, as compared to its rather large effect in anhydrous sulfolane.

M ethanol were slower than expected at high acid concentrations. If it is assumed that each equivalent of acid renders 1 equiv of ethanol unreactive by protonation, these rate constants may be roughly corrected to 0.37 M ethanol by multiplying by $0.37/[0.37 - H^+]$. The corrected rates shown in Figure 9 exhibited a first-order dependence on acid concentration and were used to calculate the rate constant in Table I.

It is interesting to note that rates were almost identical in 0.37 and 1.14 M ethanol. This presumably reflects opposing effects of increased ethanol concentration and lowered acidity rather than a deviation from second-order kinetics at a constant acidity. The acidity of 0.37 M ethanol was also determined with the Hammett indicator 4-chloro-2-nitroaniline as shown in Figure 8. The relationship between acid concentration and acidity is seen to be similar in sulfolane containing either 0.37 M water or 0.37 M ethanol.

Calculation of Rate Constants at Unit Acidity in Sulfolane. Since the acidity of the sulfolane solutions was much higher than that of aqueous acids, a comparison of rate constants based on acidity rather than acid concentration seemed desirable. The rate constant k_{H_0} was defined as the rate at $H_0 = 0$. For thiol amides, k_{H_0} was calculated using eq 2. For hydroxy acids, it was calculated from data on indicator protonation at 0.37 M water (or ethanol) given in Figure 8. These results are also given in Table I.

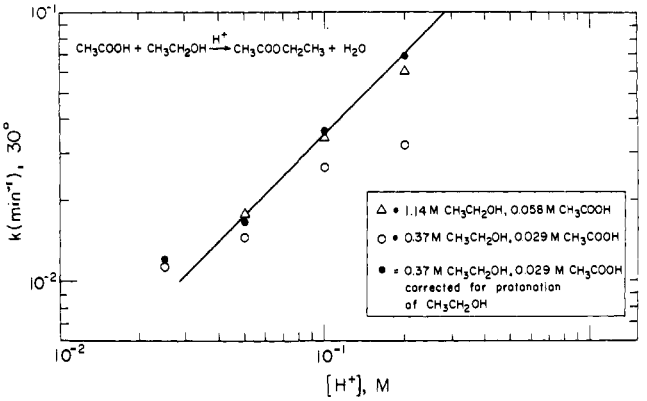
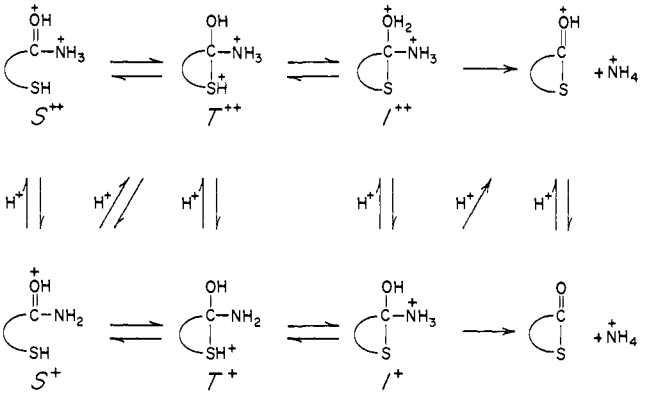


Figure 9. First-order rate constants for formation of ethyl acetate from acetic acid in sulfolane-ethanol mixtures at 30 °C on a log-log scale.

Scheme I



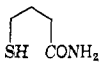
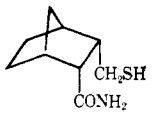
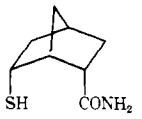
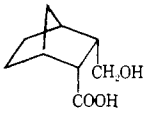
IV. Discussion

Mechanism of Thiolactone Formation from Thiol Amides. At low pH, the mechanism of ring closure of thiol amides to thiolactones in aqueous solution is a simple acid-catalyzed reaction involving rate-determining nucleophilic attack by sulfur on a small equilibrium concentration of protonated amide.[18]

The reaction in sulfolane must involve uncatalyzed and acid-catalyzed ring closure of the protonated amide. Mechanisms consistent with the observed kinetics are shown in Scheme I. Considering only the acid-catalyzed reaction shown by all three compounds in 0.1 M acid, it is clear that a diprotonated intermediate or transition state must be involved. Two general alternatives can be distinguished depending on whether formation or breakdown of the tetrahedral intermediate is rate determining. If concerted or stepwise formation of I^{++} through T^{++} is rate determining, then the transition state most probably resembles a diprotonated amide. Alternatively, the reaction could proceed through preequilibrium formation of I^+ from S^+, followed by rate-determining breakdown either through I^{++} or concerted acid catalysis. Neither of these pathways appears attractive; it is hardly surprising that no such kinetic term is observed in hydrolysis of benzamides (up to 8 M aqueous HCl or H_2SO_4).[19]

The kinetic data presented here cannot differentiate between these pathways. One interesting possibility is that the intermediate I^+ requires water or a general base to facilitate its breakdown to product through a zwitterion. In the absence of water, breakdown of this intermediate might be slow and thus subject to acid catalysis. Alternatively the observed acid dependence may reflect simply the high acidity of the system. For present purposes, the central point is that there are substantial differences between the mechanism in water and sulfolane.

Table III. Relative Rates of Ester, Lactone, or Thiolactone Formation

Compd	No.	Rel rate in water	Rel rate in sulfolane
	I	1.72	1.44
	II	1	1
	III	862	55.6
EtOH, HOAc	VI	1	1
	IV	79	20
	V	6.6×10^3	970

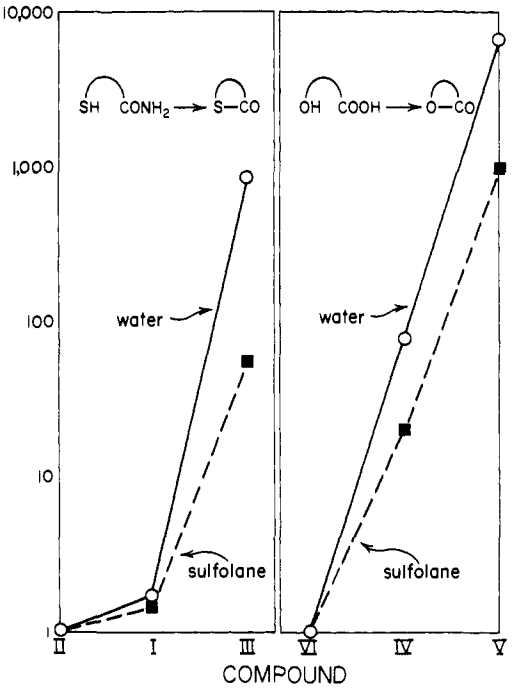


Figure 10. Relative rates of ring closure reactions on a logarithmic scale. Rates and compound numbers are from Table III.

Influence of Solvation on Relative Rates. The central question addressed in this work is whether relative rates in the series of compounds considered reflect structural or solvation effects. As suggested earlier, solvation effects should manifest themselves as a change in relative rates with changing solvent. Relative rates in water and sulfolane are given in Table III and are plotted on a logarithmic scale in Figure 10.

Two conclusions are evident from Figure 10. First, trends in relative rates are preserved in every case—the dominant factor influencing relative rates is clearly structure in spite of a dramatic change in solvent. Second, some solvent effect is evident: the range of rates observed in sulfolane is somewhat compressed for the hydroxy acids and considerably compressed for the thiol amides. However, even for compound III, which shows the most discrepancy, the largest contribution to the rate enhancement is structural (in terms of free energy).

Possible explanations for the observed solvent effects can be grouped into two rough categories. The first is a substantial change in mechanism caused by a change in solvent or conditions. Such an effect certainly must be considered for the closure of thiol amides which apparently involves acid catalysis acting on the protonated amide.

It is interesting to attempt a comparison between compound III and compound I in the "plateau" region where uncatalyzed closure of the protonated amide is rate determining. In very early work we observed such a plateau for compound I with a first-order rate constant $k \approx 5 \times 10^{-4}$ min^{-1} at 90.4 °C and 3.2×10^{-4} M acid concentration. As noted in the Results, these rates were excluded from consideration because the observed UV spectrum was not that of the product. Blanks containing pure product decreased in absorbance and the nature of the reaction was unclear. However, an alternative measure of the "uncatalyzed" rate k_0 is available from the initial velocity studies with compound I. In Figure 5, a plateau in initial velocities occurs with a value $\sim 10^{-4}$ M/min. From eq 5, $k_0 = 2 \times 10^{-3}$ min^{-1} at 90.4 °C. Finally an upper limit of $k_0 \leq 4 \times 10^{-3}$ min^{-1} is set by the fact that no plateau is observed in Figure 4. The rate constant for compound III in the plateau region at 90.4 °C is 1.3 min^{-1} as calculated using the activation parameters given in the Results. The relative rate for III/I is then 325–2600 at 90 °C in sulfolane. The corresponding relative rate in water is 500 at 30 °C.

The significance of this observation is limited by the data. Neither of the techniques used gave clean, highly reproducible results, although the initial velocity experiments probably give a somewhat better estimate of k_0. Insofar as these numbers are reliable, there is little or no solvent effect on compound III in the plateau region where the mechanism is probably similar to that observed in water.

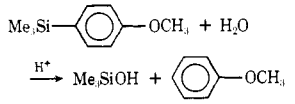
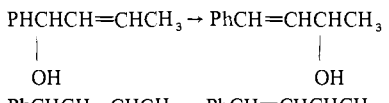
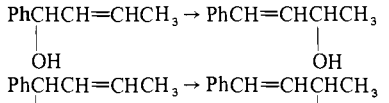
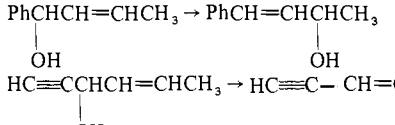
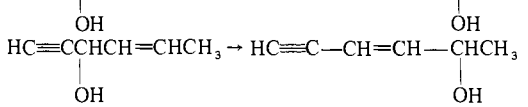
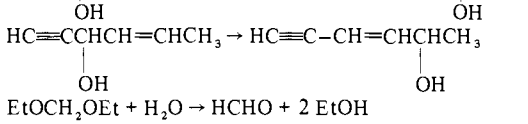
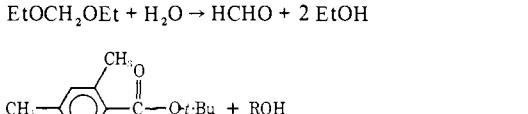
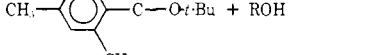
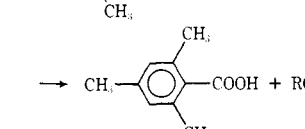
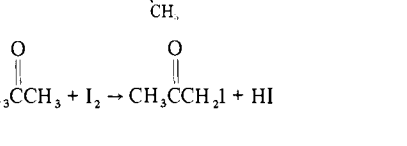
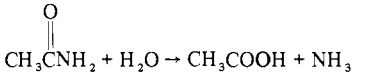
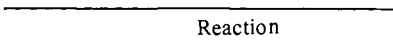
An equally plausible explanation for the observed effects is that they arise purely from differences in solvation rather than in mechanism. The decrease in the range of rates observed in sulfolane would certainly be in accord with a model in which partial desolvation of functional groups is a necessary prelude to reaction. In such a model, the separate solvation shells around each functional group must be replaced by a common solvation shell enclosing both groups before reaction can occur. If the structure of a compound held reactive functional groups together such that they always share a common solvation shell, they might be expected to react more rapidly. As the solvation ability of the solvent decreased, this effect might become less important and differences in rate would become smaller.

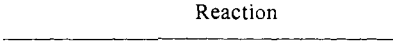
Regardless of the source of the observed solvent effect, the basic point is that it is small compared to the range of rates observed. The only compound for which a sizable solvent effect occurs is compound III, where there is good evidence for a change in mechanism. Consequently, the observed spread of rates is best interpreted in terms of purely structural features of the series of molecules. Bruice and Turner have reached a similar conclusion in a comparison of intramolecular and bimolecular anhydride formation in water and 1 M water in Me_2SO.[8]

Storm and Koshland have considered a number of possible factors which might account for the effect of structure on rate in the series of hydroxy acids studied here.[6] They concluded that the observed rates resulted largely from differences in orientation of the reacting functional groups with respect to each other. By eliminating solvation as a possible alternative explanation, the present results strongly reinforce that conclusion.

Effects of Solvent on Acid- and Base-Catalyzed Reactions. Table I illustrates both a considerable range of solvent effects

Table IV

Reaction	Range	k_s/k_w	k_{H_0}/k_w	$k_{a_{H^+}}/k_w$	Ref
	8–28.8 M H_2O in dioxane (HCl)	4.05	0.51		*a*
	20–100 vol % dioxane in H_2O (0.1 M HCl)	0.27	0.33		*b*
	40–100 vol % EtOH in H_2O (0.1 M HCl)	2.88	0.35	3.6×10^{-2}	*b*
	40–100 vol % acetone in H_2O (0.1 M HCl)	3.93	0.155		*b*
	20–100 vol % dioxane–H_2O (1 M HCl)	0.73	1.26		*b*
	20–100 vol % EtOH in H_2O (1 M HCl)	0.69	0.26		*b*
	20–100 vol % acetone in H_2O (1 M HCl)	152	5.75		*b*
	0–78 mol % EtOH in H_2O (0.1 M HCl)	0.15	2.14	0.35	*c*
	60–100 vol % EtOH–H_2O (0.01 M HCl)	2.28	0.1	8.3×10^{-3}	*d,e*
	60–95 vol % acetone in H_2O (0.01 M HCl)	0.42	0.4		*d*
	0–44.4 mol % EtOH in water (HCl)	1.56	14	8.05	*f*
	0–60 vol % dioxane in H_2O ($HClO_4$)	0.72	1.47		*g,h*
	0–50 wt % EtOH in H_2O ($PhSO_3H$)	0.347		1.32	*i*

Reaction	Range	k_s/k_w	k_{H_0}/k_w	Ref
Sucrose hydrolysis	0–60 vol % dioxane in H_2O ($HClO_4$, HCl)	1.34	13.4	*g,j*
	0–75.9 wt % acetone in H_2O (cor to 1 M HCl)	0.316	3.16	*b,k*

Base-Promoted Reactions

Reaction	Range	k_s/k_w	$k_H{}^q/k_w$	Ref
	0–90 wt % Me_2SO in water, 0.011 M ^-OH	9.0	9×10^{-7}	*l,m*
	0–49.3 mol % EtOH in water	0.217	1.1×10^{-2}	*n,o*

Table IV (*Continued*)

Reaction	Range	k_s/k_w	k_{H^q}/k_w	Ref
$CH_3\overset{O}{\overset{\|}{C}}NH_2 + {}^-OH \rightarrow CH_3COO^- + NH_3$	0–50 wt % EtOH in water	0.185	1.9×10^{-2}	*i,o*
$ClCH_2\overset{O}{\overset{\|}{C}}OEt + {}^-OH \rightarrow ClCH_2COO^- + EtOH$	0–91.8 wt % EtOH in water	0.076	2.5×10^{-3}	*o,p*

[a] C. Eaborn, *J. Chem. Soc.*, 3148 (1953). [b] E. R. Braude and E. S. Stern, *ibid.*, 1976, 1982 (1948). [c] P. Salomaa, *Acta Chem. Scand.*, 11, 461 (1957). [d] V. R. Stimson, *J. Chem. Soc.*, 2010 (1955). [e] V. R. Stimson and E. J. Watson, *ibid.*, 2848 (1954). [f] D. P. N. Satchell, *ibid.*, 2878 (1957). [g] C. A. Bunton, J. B. Ley, A. J. Rhind-Tutt, and C. A. Vernon, *ibid.*, 2327 (1957). [h] R. P. Bell, A. L. Dowding, and J. A. Noble, *ibid.*, 3106 (1955). [i] K. J. Laidler and P. A. Landskroener, *Trans. Faraday Soc.*, 52, 200 (1956). [j] P. M. Leininger and M. Kilpatrick, *J. Am. Chem. Soc.*, 60, 2891 (1938). [k] J. L. Hockersmith and E. S. Amis, *Anal. Chim. Acta*, 9, 101 (1953). [l] E. Tommila and M. -L. Murto, *Acta Chem. Scand.*, 17, 1947 (1963). [m] D. Dolman and R. Stewart, *Can. J. Chem.*, **45**, 911 (1967). [n] R. A. Fairclough and C. N. Hinshelwood, *J. Chem. Soc.*, 538 (1937). [o] K. Bowden, *Can. J. Chem.*, 43, 2624 (1965). [p] G. J. Nolan and E. S. Amis, *J. Phys. Chem.*, 65, 1556 (1961). [q] Calculated as pseudo-first-order rate constants: $k = k_1$ at $[{}^-OH] = 1$ M or $H_- = 14$. Assumes H_- linear in base concentration.

on rates and a surprising constancy of rate constants for hydroxy acid ring closure in either water or 0.37 M water in sulfolane when based on Hammett acidity. The latter observation was unexpected and raised the general question of whether many solvent effects in enzyme model systems reflect largely changes in "activity" of a catalyst.

The effect of solvation on organic reactions has of course been studied intensively; quite sophisticated empirically based treatments are available, with primary emphasis on solvolysis, nucleophilic displacements on alkyl and aryl systems, and proton transfer.[2] Because of our focus on reactions of more direct significance as enzymatic models, we undertook to find other examples of solvent effects on acid-catalyzed hydrolysis or related reactions involving a protonation preequilibrium and on base-promoted hydrolysis. A brief survey of the literature revealed 19 reactions for which sufficient data were available to compute rates at constant catalyst concentration and at constant Hammett acidity or basicity over a reasonably wide range of solvent composition. Rate ratios for these reactions are given in Table IV.

For each reaction, ordinary second-order rate constants based on concentrations were first compared at the extremes of the solvent composition by dividing the rate k_s in less aqueous solvent by the k_w in more aqueous solvent.

Somewhat surprisingly, the solvent effects k_s/k_w based on concentration are not large; of the 19 reactions cited, only 2 show an effect of more than a factor of 10. Of course, the changes in solvent character are less fundamental than in the familiar cases of spectacular solvent effects. For instance, both series investigated here in sulfolane all show rate increases greater than an order of magnitude. Closure of thiol amides shows quite a large solvent effect, but one which may plausibly be attributed to the change in mechanism discussed above. Nevertheless, the range of solvent properties covered in most of the reactions in Table IV is quite substantial. The small observed solvent effects suggest that these reactions are not highly sensitive to changes in solvation. Most of the reactions in which spectacular solvent effects are observed involve either the generation of charge (solvolysis or amine quaternization) or formation of delocalized anions (nucleophilic aromatic substitution), although this generalization is not universally true (e.g., nucleophilic displacement on methyl iodide). In all of the cases cited here, the catalyst and the transition state are of the same charge type.

Qualitatively, small observed solvent effects mean only that changes in solvation affect reactants and products in a similar manner, not that no changes in solvation have occurred. In order to separate solvent effects on individual species in a reaction, some measure of the change in activity of one species is necessary. The Hammett acidity or basicity of a solvent-catalyst mixture is often taken as a rough measure of acid or base strength. A number of solvent effect studies correlating Hammett acidity and kinetic data have been reported and data are available in the literature for a number of other systems. These data have been collected in Table IV under the heading k_{H_0}/k_w or k_{H_-}/k_w. In each case rate constants were computed at unit acidity ($H_0 = 0$) or "unit basicity" ($H_- = 14$) at both extremes of the solvent composition; the ratio of rate in less aqueous to rate in more aqueous solvent is given.

In general, the acid-catalyzed reaction rates in Table IV vary by little more than an order of magnitude with change in solvent: ester hydrolysis, which may be compared to the esterification reported here, is particularly insensitive to changes in solvent composition. Again, closure of thiol amides (Table I) is an exception, probably because of the change in mechanism.

These results still do not imply that the solvation of individual species is insensitive to changes in solvent. However, the results do indicate that solvation of Hammett indicators is similar to that of the reacting species.[22]

A rigorous determination of the size of solvent effects on the transition state for these reactions requires an absolute measurement of the activity of the hydrogen ion catalyst. No such absolute measurement is thermodynamically possible, but good approximate methods are becoming available.[24] An approach based on the assumption that tetraphenylarsonium and tetraphenylborate are equally solvated appears to be relatively reliable[25] and data for the activity of H^+ in ethanol–water mixtures are available based on this assumption.[26] Rate ratios at constant activity of H^+ are also given in Table IV, where the ratio $k_{a_{H^+}}/k_w$ is defined as before. For two of the five reactions for which data are available, these solvent effects are significantly larger than those based on Hammett acidity or acid concentration. This observation suggests that the expected compensation between solvation of catalyst and transition state does occur to some extent, with solvation of single ionic species in different ethanol–water mixtures affecting rates by as much as 10^2.

Considered as a whole, the acid-catalyzed reactions show only a limited sensitivity to solvation regardless of the measure of catalyst activity employed. The very limited results for base-promoted hydrolysis reactions present quite a different picture. Much more marked solvent effects are apparent if H_- is used as a criterion of base strength, including a rate some 10^6 slower in 91% Me_2SO than in water. In general, these large solvent effects result from marked increases in basicity of OH^- as measured by H_- in the less aqueous solvents with relatively little change in actual rate constants. Interpretation of these results is attended by the same difficulty encountered in interpreting solvent effects based on H_0; the observed solvent

effects depend both on solvation of the reaction itself and solvation of the Hammett indicator used to measure H_-. Nevertheless, the large observed effects clearly demonstrate that the reactant and transition state are solvated differently than the Hammett indicators. The qualitative interpretation of H_- values as an indication that the activity of OH^- is higher in nonaqueous solvents has received some experimental support.[23] The insensitivity of measured rates to solvent changes, or the decrease in k_{H_-}/k_w, most likely results then either from decreasing solvation of the transition state or increasing solvation of the reactant. Either effect is qualitatively reasonable though effects on the charged transition state seem more likely.

Solvation Effects on Enzymatic Rates. The specific results above regarding effects of solvent on the rates of acid- and base-catalyzed reactions suggest that solvent effects alone cannot account for enzymatic reaction rates—of 19 reactions examined using several measures of catalyst activity, only one example of a very large ($\gg 10^2$) solvent effect was observed. Before this suggestion can be accepted, further examination of this and other data is necessary.

Solvation effects on enzymatic rates can conveniently be divided into those affecting k_{cat} and those affecting k_{cat}/K_m. The second-order rate constant k_{cat}/K_m is easier to analyze since the enzyme can increase this rate only by increasing the stability of the transition state relative to the transition state in water, but not by decreasing the stability of the ES complex or other intermediate species. For ionic or very polar transition states, it is unlikely that charged groups in enzyme active sites could provide a far better solvating environment than water.[1] Additional recent evidence for this contention is provided by Pocker's investigations of ionic reactions in $LiClO_4$–ether solutions:[4] 6 M $LiClO_4$ in ether was necessary to give a solvent with the ionizing power of 60% ethanol–water, as measured by Winstein's Y value. The effect of charge in a more polar environment is seen in the generally modest effects of ionic strength on reaction rate.

Large second-order rate accelerations by solvation are possible in other cases. The most plausible examples are those in which the transition state itself is nonpolar and thus solvated far better by a hydrophobic active site than by water. For example, reactions involving conversion of NAD^+ to NADH might be subject to such acceleration. The observation that mono- and divalent metal ions are solvated better by dipolar aprotic solvents than by water[28] suggests that solvation effects might be important in metalloenzymes. Such effects are difficult to quantitate, however, since metal ions in active sites are normally bonded to several complex ligands.

The second, more widely considered class of solvent effects[29] are those which operate by desolvating the ES complex relative to the transition state. Accelerations of k_{cat} relative to a nonenzymatic model can be rationalized, but the necessary energy is obtained at the expense of binding energy. For example, a large acceleration might be obtained by binding a polar substrate in a hydrophobic environment.

In applying model systems to these effects, a further subdivision is useful. In one mechanism, the microscopic environment of the active site allows selective solvation of transition states but not substrates—a simple example is the oxyanion hole of subtilisin and chymotrypsin.[30] To determine the importance of this effect, information about the detailed solvent properties of the active site and about the sensitivity of individual species to solvation is necessary. This information is generally difficult to obtain and must be considered case by case based both on crystal structures and extensive model studies. There is no reason to believe that such effects are not both common and large, but little quantitative information is available.

In a second mechanism, the enzyme active site is uniformly different from water in solvating ability, but the substrate has a different sensitivity to solvation than does the transition state. An extreme example would be a charged substrate but a neutral transition state. Solvent effects of this type can always be detected in an appropriate model system—since the active site behaves as a homogeneous medium, transfer of an exact nonenzymatic model to a solvent with the same solvating properties should show a similar effect. The solvent effects on acid- and base-catalyzed reactions in Table IV can be considered in this context. With one exception, transfer of these reactions between solvents led to only modest solvent effects. This observation suggests that homogeneous solvent effects are unlikely to provide major rate accelerations of k_{cat}. Such a conclusion is doubtless true for many enzymatic reactions, in spite of the crude nature of the model reactions in Table IV. For example, the hydrolysis of *p*-nitrophenyl acetate by imidazole can serve as a better model for reactions catalyzed by serine proteases. This reaction proceeds 187 times faster in water[31] than in acetonitrile containing 1 M water.[32] The charge relay system can be mimicked by adding a carboxylate ion, but the rate in acetonitrile–water containing 1 M benzoate is only about twice as fast as imidazole-catalyzed hydrolysis in water. Thus model reactions for serine proteases suggest that homogeneous solvation effects are relatively small for these enzymes. Large accelerations of k_{cat} by solvation could plausibly arise only from specific effects such as the oxyanion hole.

The conclusion that homogeneous solvation effects are small certainly is not applicable to all enzymatic reactions for two reasons. First, none of the reactions discussed so far involve changes in charge or large changes in charge delocalization. Second, most of the changes in solvent properties do not involve dipolar aprotic solvents or extremes such as nonpolar solvents.

Quite large effects of solvation on k_{cat} might be expected for reactions involving changes in charge type. Reactions which develop charge are unlikely to be accelerated relative to their rate in water because water is such a good solvent for charged species, but large accelerations could be obtained by conducting a reaction which destroys or delocalizes charge in a nonpolar environment. Such solvation effects have now been observed in several cases, with moderate changes in solvent polarity leading to changes of 10^3–10^8 in rate.[33-35] The classes of reactions represented by these models could conceivably be markedly accelerated by transfer from water to a homogeneous medium of low polarity such as a hydrophobic active site. Other classes of reactions might be susceptible to the same kind of acceleration depending on the catalytic groups in the active site.

In conclusion, solvation effects may prove to be a significant factor in enzymatic catalysis in some types of reactions. The microscopic environment of the active site could certainly lead to rate accelerations in k_{cat}, although such accelerations are difficult to predict from simple models. Very high rates induced by solvation are plausible for both k_{cat} and k_{cat}/K_m for those reactions where the transition state is less polar than the reactants. These reactions probably constitute a major class of enzymatic reactions. Conversely, reactions with polar transition states are likely to be accelerated by solvation only to the extent that the active site is highly heterogeneous. Finally, the potential size of many of these effects is predictable from the organic chemistry of model reactions.

Acknowledgment. This work was supported by U.S. Public Health Service Grant AM 09765 and National Science Foundation Grant BMS 71-0133A03.

References and Notes

(1) D. E. Koshland, Jr., and K. E. Neet, *Annu. Rev. Biochem.*, **37**, 376–380 (1968).
(2) A. J. Parker, *Chem. Rev.*, **69**, 1 (1969); J. F. Coetzee and C. D. Ritchie, Ed.,

"Solute-Solvent Interactions", Marcel Dekker, New York, N.Y., 1969; M. H. Abraham, *Prog. Phys. Org. Chem.*, **11**, 1–87 (1974).
(3) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems", Academic Press, New York, N.Y., 1975.
(4) Y. Pocker and R. F. Buchholz, *J. Am. Chem. Soc.*, **92**, 2075, 4033 (1970); **93**, 2905 (1971).
(5) D. R. Storm and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U.S.A.*, **66**, 445 (1970); S. Milstein and L. A. Cohen, *ibid.*, **67**, 1143 (1970).
(6) D. R. Storm and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **94**, 5805 (1972).
(7) S. Scheiner, W. N. Lipscomb, and D. A. Kleier, *J. Am. Chem. Soc.*, **98**, 4770 (1976).
(8) T. C. Bruice and A. Turner, *J. Am. Chem. Soc.*, **92**, 3422 (1970).
(9) G. Choux and R. L. Benoit, *J. Am. Chem. Soc.*, **91**, 6221 (1969).
(10) C. N. Yiannios and J. V. Karabinos, *J. Org. Chem.*, **28**, 3246 (1963); T. J. Wallace, *J. Am. Chem. Soc.*, **86**, 2018 (1964).
(11) R. N. Haszeldine and J. M. Kidd, *J. Chem. Soc.*, 4228 (1954).
(12) T. Gramstad and R. N. Haszeldine, *J. Chem. Soc.*, 173 (1956).
(13) J. F. Coetzee, J. M. Simon, and R. J. Bertozzi, *Anal. Chem.*, **41**, 766 (1969).
(14) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(15) S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
(16) E. M. Arnett and C. F. Douty, *J. Am. Chem. Soc.*, **86**, 409 (1964).
(17) K. Yates, J. B. Stevens, and A. R. Katritzky, *Can. J. Chem.*, **42**, 1957 (1964).
(18) D. R. Storm, Ph.D. Thesis, University of California, Berkeley, 1971; P. G. Strange, A. Dafforn, and D. E. Koshland, Jr., unpublished observations.
(19) J. T. Edward and S. L. R. Meacock, *J. Chem. Soc.*, 2000 (1957).
(20) M. Liles, *J. Chem. Soc., Perkin Trans. 2*, 816 (1972).
(21) This observation does not rule out concerted proton transfer in the acid-catalyzed region around 0.1 M acid.
(22) R. H. Boyd, in J. F. Coetzee and C.D. Ritchie, "Solute-Solvent Interactions", Marcel Dekker, New York, N.Y., 1969, p 68.
(23) A. K. Das and K. K. Kundu, *J. Chem. Soc., Faraday Trans. 1*, **69**, 730 (1973).
(24) O. Popovych, *Crit. Rev. Anal. Chem.*, **1**, 73 (1970); A. J. Parker, *Chem. Rev.*, **69**, 1 (1969); Von H. Strehlow, W. Knoche, and H. Schneider, *Ber. Bunsenges. Phys. Chem.*, **77**, 760 (1973); R. G. Bates in ref 22, pp 46–97.
(25) B. J. Cox and A. J. Parker, *J. Am. Chem. Soc.*, **95**, 402 (1973); R. Alexander, A. J. Parker, J. H. Sharp, and W. E. Waghorne, *ibid.*, **94**, 1148 (1972).
(26) O. Popovych, A. Gibofsky, and D. H. Berne, *Anal. Chem.*, **44**, 811 (1972).
(27) O. Popovych, *Anal. Chem.*, **46**, 2009 (1974).
(28) G. R. Hedwig, D. A. Owensby, and A. J. Parker, *J. Am. Chem. Soc.*, **97**, 3888 (1975).
(29) W. P. Jencks, *Adv. Enzymol.*, **43**, 249–254 (1975).
(30) R. Henderson, *J. Mol. Biol.*, **54**, 341 (1970); J. D. Robertus, J. Kraut, R. A. Alden, and J. J. Birktoft, *Biochemistry*, **11**, 4293 (1972).
(31) J. F. Kirsch and W. P. Jencks, *J. Am. Chem. Soc.*, **86**, 837 (1964).
(32) G. Wallerburg, J. Boger, and P. Haake, *J. Am. Chem. Soc.*, **93**, 4938 (1971).
(33) J. Crosby, R. Stone, and G. E. Lienhard, *J. Am. Chem. Soc.*, **92**, 2891 (1970).
(34) D. S. Kemp and K. G. Paul., *J. Am. Chem. Soc.*, **97**, 7305 (1975); D. S. Kemp, D. D. Cox, and K. G. Paul, *ibid.*, **97**, 7312 (1975).
(35) P. van Eikeren and D. L. Grier, *J. Am. Chem. Soc.*, **98**, 4655 (1976).

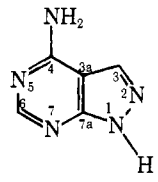
Tautomerism of Neutral and Cationic N-Substituted 4-Aminopyrazolo[3,4-*d*]pyrimidines

Guy Dodin, Marc Dreyfus, Olivier Bensaude, and Jacques-Emile Dubois*

Contribution from the Laboratoire de Chimie Organique Physique de l'Université Paris VII, associé au CNRS, 75005 Paris, France. *Received March 18, 1977*

Abstract: Neutral 4-aminopyrazolo[3,4-*d*]pyrimidine (4APP) exists in water in two tautomeric forms, 1-H4APP and 2-H-4APP (K = 2-H4APP/1-H4APP = 0.1 at 10 °C, $\Delta H_{tautomerization}$ = 0.9 kcal mol^{-1}). The interconversion of the two forms is catalyzed by H^+ and OH^- and proceeds through either an intermediate cation common to both neutral tautomers or through the intermediate anion. ^{13}C NMR spectroscopy shows that 1-*i*-Pr4APP (the nontautomerizable model compound for the 1-H4APP tautomer) protonates mainly at N(5). 2-*i*-Pr4APP (model for 2-H4APP) protonates to similar extents at N(5) and N(7). Temperature-jump relaxation confirms this scheme; cation exchanges are catalyzed by H^+, H_2O acting as a base, OH^-, and the corresponding neutral N-substituted 4APP. It is inferred from the corresponding methylated derivatives that together with the abundant species, 1-H and 2-H4APP, there are small proportions of 7-H4APP ($\simeq 10^{-3}$) and 5-H4APP (2×10^{-4}). 7-H4APP exists only as an amino tautomer, whereas 5-H4APP in water has a partial imino structure ([amine]/[imine] = 10); the interconversion of the tautomeric 5-H4APP is catalyzed by OH^-, cationic 5-H4APP, and H_2O as proved by the kinetic study of the model compound, 5-Me4APP. Biochemical implications of the location of the basic sites and of the presence of an imino tautomer are tentatively discussed.

The adenine analogue, 4-aminopyrazolo[3,4-*d*]pyrimidine (4APP), has proved to affect nucleotide synthesis in two different ways. It can either bind to adenine phosphoribosyltransferase, an enzyme responsible for the transfer of the phosphoribosyl group from PP-ribose-P to exogenous purine,[1,2]



or it can inhibit purine synthesis *de novo* (from nonpurine components).[3] In the latter case, the mechanism of the inhibition is not clearly understood, but it is suggested[3] that it may arise from the interaction of 4APP with some enzyme involved in the early steps of the reaction. Inhibition of purine synthesis de novo has been considered as the cause of the cytotoxic activity of 4APP. The fixation of adenine phosphoribosyltransferase would involve the electron donor property (the basicity) of the nitrogen atoms at positions 3 and 7 of adenine.[1] It can be thought that the reaction of adenine with the protein which catalyzes synthesis *de novo* will also be dependent on the basicity of the various nitrogen sites. This conclusion can be reconciled with the early empirical rule stating that the effectiveness of purine analogues as cytotoxic drugs is related to their basicities as measured by their pK_a (proton gained), i.e., the closer their values to those of natural purines, the higher the efficiency.[4]

Understanding 4APP activity at the molecular level necessitates, as a starting point, a reexamination of the basicity criterium, not in terms of the *overall basicity*, as evaluated from the pK_a, but in terms of the *partial basicity*[5] of the various atoms as determined by the protonation site(s) of the molecule.

Moreover, theoretical considerations suggest that the cytotoxic activity of neighbor compounds aminopyrazolo[4,3-*d*]pyrimidines might arise alternatively from the existence of rare imino tautomers capable of mispairing with cytosine when