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Near Infrared Investigation of Interamide Hydrogen Bonding in Aqueous Solution

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Various methods have been used by different investigators to estimate the stability of amide to amide hydrogen bonds in aqueous solution. Schellman (1) and Kauzmann (2) have discussed the thermodynamic properties of the C=O-HN bond in water with the use of aqueous urea solutions as a model system. From studies on heats of dilution of urea in water, Schellman concluded that this bond has an enthalpy of 1.5 kcal per mole in aqueous solution (1). Hibberd and Alexander (3) concluded from monolayer studies of octadecylacetamide that the energy of the C=O-HN hydrogen bond in the presence of water is close to 2 kcal per mole. Klotz and Franzen (4) studied *N*-methylacetamide in aqueous solution by near infrared methods and concluded that the enthalpy of amide to amide hydrogen bond formation in aqueous solution is close to zero. Némethy and Scheraga (5) have suggested that the possible presence of a hydrophobic bond furnishes an alternative explanation for the latter observation, because the positive enthalpy of formation of the hydrophobic bond may cancel a negative enthalpy contribution made by the hydrogen bond.

A considerable amount of near infrared work, mostly in the 3 μ spectral region, has been carried out in nonaqueous solutions. Tsuboi calculated an enthalpy of approximately -10 kcal per mole for the dimerization of δ -valerolactam in carbon tetrachloride solution (6), -3.5 kcal per mole for *N*-methylacetamide in carbon tetrachloride solution (7), and -1.6 kcal per mole in chloroform solution (7). The latter result agrees well with conclusions by Klemperer *et al.* (8). Mizushima *et al.* (9) have pointed out that in ether solution the stability of amide to amide hydrogen bonds is very low because of hydrogen bonding between the amide and ether. Lord and Porro, and Isaacson have reinvestigated the dimerization of lactams with more recent instrumentation (10, 11). Because of the absence of a tendency to form long chain polymers, lactams are particularly suited as model compounds for hydrogen bonding studies (6, 8, 10). Lord and Porro suggest (10) that earlier results (6) might be in error and report a somewhat lower ΔH value for the dimerization of ϵ -caprolactam in carbon tetrachloride solution.

The present communication describes and discusses results obtained in aqueous solution with δ -valerolactam as a model compound. Spectroscopic measurements were carried out in the spectral region around 1.5 μ , which offers experimental advantages over the 3 μ region.

EXPERIMENTAL PROCEDURE

Absorption spectra from 1.3 to 1.65 μ were obtained with a Cary model 14 recording spectrophotometer.¹ Cells of 1-mm and 2-mm path length were used. The cells were framed with copper tubing of 5-mm inner diameter, soldered to the quartz with an indium-tin alloy to assure good thermal contact. Water from a constant temperature bath was circulated through the frame and the temperature in the cell was measured by a thermocouple inserted in the solution outside the path of the radiation beam. The temperature difference between bath and solution was smaller than 1° at all temperatures. δ -Valerolactam (Aldrich Chemical Company, Inc.) was purified by distillation to remove traces of water. One sample was dried with acetic anhydride in pyridine. The spectrum of this sample was identical with the distilled samples.

The spectra of a solution with a stoichiometric composition of 0.419 mole fraction units of δ -valerolactam, recorded at three different temperatures, are shown in Fig. 1. The sharp band centering close to 1.5 μ is assigned to the first overtone of the NH stretching fundamental of monomeric lactam by comparison with spectra obtained in carbon tetrachloride, chloroform, and dioxane solution, and the spectrum of pure solid lactam.² The spectra obtained in noninterfering solvents, and the spectrum of the pure amide, suggest that absorption by dimeric or polymeric species is negligible between 1.46 and 1.56 μ . The very broad band between about 1.3 and 1.65 μ is the first overtone of the OH stretching fundamental of water (12). The absorbance of the amide band was estimated by the base-line procedure indicated in Fig. 1. It is easily shown that if a narrow band is located on the shoulder of a much broader band, the base-line absorbance of the narrow band is proportional to the concentration of the component that gives rise to the narrow band, independent of the concentration of the other components, and independent of the slope of the background absorption. This holds even if dimeric species (as well as water) should absorb in the defined region, provided that the curvature of the background absorption is small and that all species obey Beer's law. The latter assumption forms the basis of all measurements of this nature (6-11). Although the described procedure involves some uncertainties (because the curvature of the background cannot be determined

¹ Mention of commercial products and firms does not imply endorsement by the United States Department of Agriculture over others of a similar nature not named.

² Unpublished results from this laboratory.

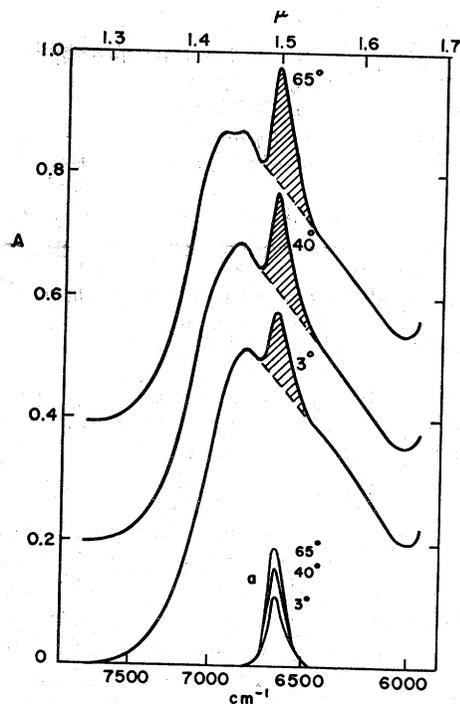


Fig. 1. Infrared spectra of an aqueous δ -valerolactam solution at various temperatures. (Consecutive spectra displaced by 0.2 absorbance unit.) a, estimated amide absorption. $X_{(solute)} = 0.419$.

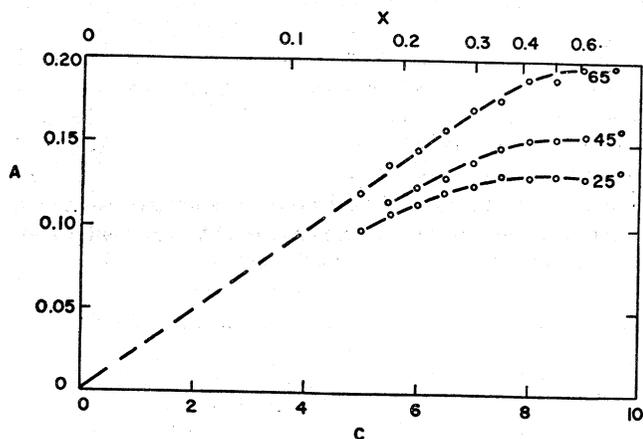


Fig. 2. Estimated absorbance of 1.5μ amide band as a function of temperature and concentration (path length, 2 mm).

exactly) we felt that it offers advantages over differential techniques (which, among other difficulties, involve the very same uncertainty). An examination of Fig. 1 suggests that a small curvature in the indicated base-line would not significantly alter the results.

RESULTS

Fig. 2 gives the base-line absorbance of the 1.5μ amide band as a function of concentration and temperature. If no higher polymers are formed, then the relationship between the dimerization constant K'_x (mf^{-1}),³ total solute concentration C , solvent

³ The abbreviation mf denotes mole fraction.

concentration C_s , monomer absorbance A , monomer absorptivity ϵ , and path length d is given by

$$K'_x = \frac{C - A/\epsilon d}{2(A/\epsilon d)^2} \left(\frac{C + A/\epsilon d}{2} + C_s \right) \quad (1)$$

Rearrangement leads to

$$\frac{C_s}{A} = \frac{\epsilon d}{A^2} \left(\frac{C^2}{2} + C_s C \right) - \frac{(4K'_x + 1)}{2\epsilon d} \quad (1a)$$

The reasoning leading to Equations 1 and 1a is quite analogous to the one described by Lord and Porrò (10). Because intense water absorption necessitates the use of relatively high concentrations, the dimerization constant is calculated in units of mole fraction.

For determination of the unknown quantities K'_x and ϵ we set

$$y = \frac{C_s}{A} \quad (2)$$

$$x = \left(\frac{1}{A^2} \right) \left(\frac{C^2}{2} + C_s C \right) \quad (2a)$$

A plot of y versus x yields a straight line if K'_x and ϵ are independent of concentration. ϵ is given by the slope, and K'_x is calculated from the intercept. If the absorbance values recorded in Fig. 2 are substituted into Equations 2 and 2a, Fig. 3 is obtained, leading to the following numerical values: $\epsilon = 0.120 \text{ M}^{-1} \text{ cm}^{-1}$ (independent of temperature); $K'_{x,298} = 0.75 \text{ mf}^{-1}$; $K'_{x,318} = 0.42 \text{ mf}^{-1}$; $K'_{x,338} < 0.1 \text{ mf}^{-1}$.

K'_x values calculated by applying Equation 1 directly to absorbance values did not show any systematic trends with concentration and agreed within ± 0.1 unit with the values listed above, as shown in Table I. This constancy of K'_x over the

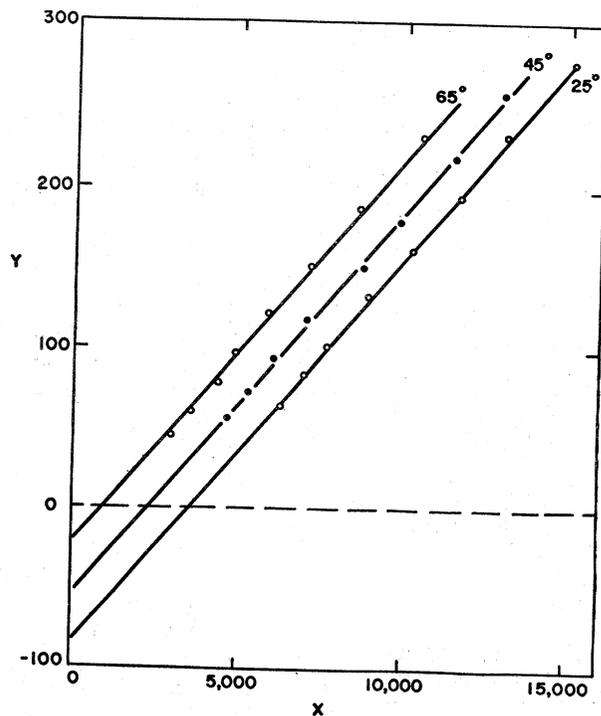


Fig. 3. Graphical estimation of monomer absorptivity and K'_x . $y = C_s/A$; $x = (1/A^2)(C^2/2 + C_s C)$; slope, $\epsilon \cdot d$; intercept, $-(4K'_x + 1)/2\epsilon d$.

TABLE I
 K'_x as a function of composition as calculated by Equation 1

$X_{(solute)}$	$K'_{x,298}$	$K'_{x,318}$
0.618	0.78	0.46
0.505	0.79	0.42
0.419	0.74	0.38
0.350	0.74	0.48
0.295	0.73	0.39
0.250	0.73	0.46
0.213	0.77	0.44
0.180	0.74	0.48
0.152	0.77	0.41

TABLE II
 K'_x as a function of temperature as calculated by Equation 1

Temperature °K	Monomer absorbance (A)	K'_x
276	0.107	1.54
278	0.110	1.40
282	0.113	1.31
288	0.122	1.00
293	0.125	0.91
298	0.133	0.72
303	0.137	0.63
308	0.144	0.50
313	0.150	0.41
318	0.157	0.31
323	0.165	0.22
328	0.173	0.14
333	0.180	0.08

studied concentration range appears to exclude the formation of higher polymers of δ -valerolactam.

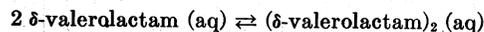
Table II gives the observed temperature dependence of the estimated absorbance of the 1.5μ amide band of a δ -valerolactam solution with $X_{(solute)} = 0.419$ in the range $3-60^\circ$, and lists corresponding K'_x values as calculated by Equation 1. If the logarithms of these values are plotted as a function of $1/T$, Fig. 4 results. In the temperature range from 0° to about 40° the quantity $[d(\log K'_x)/d(1/T)]$ appears to have a constant value and leads to $-R d(\ln K'_x)/d(1/T) = -5500 \pm 1000$ cal per mole, where R is the gas constant in calories, and K'_x is defined by Equation 1. Above 40° , a curvature appears in the plot, and this quantity appears to take on progressively more negative values with further increase in temperature.

DISCUSSION

Lactams offer certain advantages as model compounds for hydrogen bonding studies over *trans* secondary amides of the type $RCONHR'$. The results suggest that it is not necessary to consider multiple equilibria involving hydrogen-bonded chain polymers in order to estimate thermodynamic properties associated with hydrogen bond formation. This is in agreement with previous work in inert solvents (6, 8, 10). Dimerization of lactams through hydrogen bonding involves the formation of two bonds, facilitating numerical estimations. The spectra in the first overtone region (around 1.5μ) are easier to interpret

because overlapping between absorption bands arising from monomeric and polymeric species is greatly reduced.

The numerical results given in the previous section indicate that at moderate temperatures the obtained absorption data can be consistently interpreted in terms of a dimerization reaction,



Such a formulation is analogous to the one given by Schellman (1) and by Kauzmann (2) for urea solutions, with the distinction that our results are based on measurements of a specific property of the NH groups. Thus, if dimers involving only one hydrogen bond were formed in significant amounts, the results would differ from the observed data. A slightly different way of presentation which would formally lead to the same observed results would be in terms of functional groups.

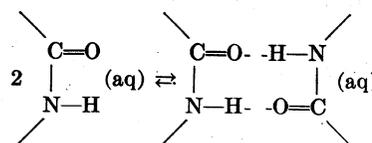


Fig. 3 indicates that the values of K'_x and ϵ (as defined) are independent of concentration in the temperature range where both can be estimated with reasonable accuracy, and that ϵ is independent of temperature within experimental error. At 65° ϵ can still be estimated reasonably accurately (because the extent of dimerization is very small) but the value of K'_x becomes obscure. Fig. 4 suggests that above about 40° the nature of the reaction undergoes a significant change. It is not easy to ascribe the value of $-R d(\ln K'_x)/d(1/T) = -5500 \pm 1000$ cal per mole, obtained in the last section, to the formation of a discrete number of hydrogen bonds. The formation of interamide hydrogen bonds is accompanied by the disruption of an unknown number of amide-water bonds, as well as a change in the total number of water-water hydrogen bonds. If the experimental data are interpreted in terms of a dimerization of solvated *cis* amide groups, the consistent results which are obtained lead to an over-all enthalpy decrease of several kilocalories per mole.

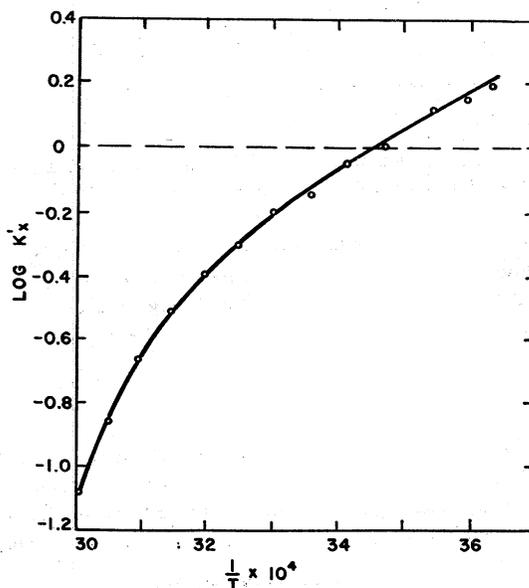


Fig. 4. Temperature dependence of $\log K'_x$

SUMMARY

The interaction of amide groups through hydrogen bonding in aqueous solution was investigated by means of near infrared absorption spectroscopy. δ -Valerolactam was used as a model compound. Absorption measurements were carried out as a function of temperature and concentration in the wave length region from 1.3 to 1.7 μ . Data obtained between 0° and about 40° can be consistently interpreted in terms of dimerization of *cis* amide groups. Such a dimerization necessarily involves changes in solute-solvent interactions, solvent-solvent interactions, and possibly nonpolar solute-solute interactions. An over-all enthalpy change of -5.5 ± 1 kcal per mole was obtained.

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