The Effect of a Dipolar Solvent System on Interamide Hydrogen Bonds*

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The association of *N*-methylacetamide and ε-caprolactam has been studied in a solvent system in which the dielectric constant can be adjusted between 2.25 and 9.13. The solvent system is composed of varying ratios of *cis*- and *trans*-dichloroethylene. The studies reveal that the free energy and enthalpy of association of the amides can be expressed as a linear function of the reciprocal of the dielectric constant. An alternative explanation in terms of solvent dipole competition for the interamide complexes is also presented. The results support the viewpoint that interamide hydrogen bonds of proteins are stable only in the well-shielded, nonpolar, hydrocarbon-rich regions of the protein molecule.

A variety of studies on polypeptides and proteins in aqueous solutions give evidence for the existence of structures which contain hydrogen bonds (Applequist and Doty, 1962; Riddiford and Scheraga, 1962; Rosenheck and Doty, 1961; Hermans and Scheraga, 1960). However, it has been demonstrated that the instability of the N-H · · · O interamide hydrogen bond in water solution is very great (Klotz and Franzen, 1962). It appears, therefore, that this hydrogen bond by itself is not a significant stabilizing force in proteins in aqueous solution; nevertheless, its presence must be inferred in certain cases, e.g., where the helical conformation is present. It has been suggested that the clustering of hydrocarbon residues in certain regions of the protein molecule would exclude water molecules, and, consequently, greater stability of amide-amide hydrogen bonds in this region of low dielectric constant and relative freedom from competing solvent molecules would result (Klotz, 1960). In such a cooperative fashion the interpeptide hydrogen bond could help to stabilize a unique protein conformation.

In order to evaluate the magnitude of the effect of the polar nature of the surrounding medium on hydrogen bonding, we have chosen two model compounds: *N*-methylacetamide, which resembles the peptide unit found in proteins, and ε-caprolactam, which forms dimers only upon aggregation, thus simplifying the treatment of the data (Lord and Porro, 1960). The variation of the dielectric constant (or of the dipolar nature of the environment) was accomplished by using different ratios of *trans*-dichloroethylene, (D = 2.25 at 25°), and *cis*-dichloroethylene, (D = 9.13 at 25°). Employing this solvent system it is possible to alter the dielectric constant between 2.25 and 9.13 without changing the hydrogen-bonding properties of the solvent itself. Since *cis*-dichloroethylene has a dipole moment of 1.89 Debye units while *trans*-dichloroethylene has no dipole moment, the number of solvent dipoles increases as the solvent composition approaches unit mole fraction *cis*-dichloroethylene (Wesson, 1948). Any effect on the amide association equilibrium would be expected to result from both the competition of solvent dipoles and the weakening of electrostatic interaction between solute molecules by a medium of relatively high dielectric constant.

The association equilibrium constants were determined from the variation of the extinction coefficient of the first overtone of the free N-H absorption according to established methods (Klotz and Franzen, 1962; Davies and Thomas, 1956; and Lord and Porro, 1960).

Materials and Methods

N-Methylacetamide.—*N*-methylacetamide (Eastman) was distilled at atmospheric pressure through a 90-cm electrically heated Vigreaux column protected from moisture by a $CaCl_2$ drying tube. The fraction boiling from 204.5 to 205.5° was collected and stored in a dry box in which all subsequent transfers were made.

N,N-Dimethylacetamide.—*N,N*-Dimethylacetamide (Eastman) was distilled at atmospheric pressure through a 90-cm Vigreaux column; boiling range of main fraction, 164–165°.

ε-Caprolactam.—ε-Caprolactam (K and K Laboratories) was dried *in vacuo* overnight at room temperature; melting point in open capillary, 68.5–70.0°.

N-Methylcaprolactam.—*N*-Methylcaprolactam was furnished as a sample from Dupont and was used without further purification.

cis- and trans-Dichloroethylene.—*cis*- and *trans*-Dichloroethylene (Eastman) were contaminated severely with each other as revealed by gas chromatography and infrared spectra. They were, therefore, dried over CaO and distilled at atmospheric pressure from CaO through a 90-cm electrically heated column packed with Berl saddles and equipped with a variable-reflux head. Employing a reflux ratio of 10:1, fractions boiling at 60.0–60.2° in the case of the *cis* isomer and 48.2–49.0° for the *trans* isomer were collected in light-protected flasks. Thymol was added to make a final concentration of 0.5%. These precautions were found necessary in order to prevent light-induced polymerization of the highly purified isomers. Immediately before use, the solvents were distilled rapidly from CaO to remove any trace of moisture and thymol inhibitors.

Dichloroethane, Dioxane, Tetrahydrofuran, Dimethoxyethane, and Carbon Tetrachloride.—These solvents from Fisher Scientific Co. were not purified further.

Chloroform.—Chloroform (Fisher) was dried over CaO and distilled through the Berl saddle column at a reflux ratio of 5:1. The fraction boiling at 60.0–61.0° was collected and used immediately.

Near-Infrared Spectra.—Measurements in the 6600–7200 cm^{-1} region were made with the Cary Model 14 spectrophotometer. The cell compartments were flushed with dry nitrogen to avoid condensation of water on the cell faces at low temperatures and to eliminate solvent vapor at high temperatures. The reference path cell contained either pure solvent or solvent plus carbon tetrachloride. The carbon tetrachloride was added to compensate for the volume of

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TABLE I

VALUES OF THERMODYNAMIC FUNCTIONS FOR *N*-METHYL ACETAMIDE IN *trans*- AND *cis*-DICHLOROETHYLENE

Solvent	T (°K)	D	k_{12}^{α}[a] (liter mole^{-1})	k_{12}[b] (liter mole^{-1})	ΔF[c] (kcal mole^{-1})	ΔH[c] (kcal mole^{-1})	$\Delta H'$[d] (kcal mole^{-1})	ΔS (Gibbs mole^{-1})
trans-Dichloro-	283	2.29	1.90	1.88	−0.36		−3.2	
ethylene	298	2.25	1.52	1.54	−0.26	−3.3	−3.2	−10.0
	314	2.21	1.09	1.08	−0.05		−3.2	
8:1 *trans/cis*	298	3.08	1.32	1.35	−0.18	−2.6	—	—
2:1 *trans/cis*	298	4.21	1.22	1.23	−0.12	−2.2	—	—
cis-Dichloro-	283	9.75	1.10	1.12	−0.06		−1.6	
ethylene	298	9.13	1.00	1.00	0.00	−1.5	−1.6	−5.0
	314	8.40	0.91	0.88	+0.08		−1.6	

[a] k_{12}^{α} = association constant determined according to the relation

$$\lim_{\alpha \to 0}\left[\frac{\alpha}{1-\alpha}\right]\frac{1}{C_f} = k_{12}^{\alpha}$$

where α = the fraction of hydrogen-bonded NH groups and C_f = the molar concentration of free NH groups (Klotz and Franzen, 1962). [b] k_{12} = association constant determined according to the relation

$$\frac{C_b^{1/2}}{C_f - C_b} = k_{12}^{1/2} + \bar{k}\frac{C_f C_b^{1/2}}{C_f - C_b}$$

where C_b = the molar concentration of hydrogen bonded NH groups and $\bar{k} = k_{23} = k_{34} \ldots$ (Klotz and Franzen, 1962). [c] ΔF and ΔH: Obtained from k_{12} and the temperature variation of k_{12}. ΔH is corrected for the change in D^{-1} with temperature as described in the text. [d] $\Delta H'$—the enthalpy obtained from a plot of α vs. ln (NH_f) (Klotz and Franzen, 1962). $\Delta H'$ is not corrected for dD^{-1}/dT. This is a minor correction.

TABLE II

VALUES OF THERMODYNAMIC FUNCTIONS FOR ϵ-CAPROLACTAM ASSOCIATION IN *trans*- AND *cis*-DICHLOROETHYLENE AND OTHER SOLVENTS

Solvent	T (°K)	D	k_2[a] (liter mole^{-1})	ΔF (kcal mole^{-1})	ΔH[b] (kcal mole^{-1})	ΔS (Gibbs mole^{-1})
trans-Dichloroethylene	283	2.29	20.44	−1.70		
	298	2.25	11.60	−1.45	−6.8	−18.3
	314	2.21	6.68	−1.19		
8:1 *trans/cis*	298	2.81	7.26	−1.18	−6.5	—
2:1 *trans/cis*	298	4.15	4.14	−0.84	−6.0	—
cis-Dichloroethylene	283	9.75	3.40	−0.69		
	298	9.13	2.17	−0.46	−5.3	−16.2
	314	8.40	1.44	−0.23		
Carbon tetrachloride	298	2.23	78.8	−2.59		
	314	2.20	43.9	−2.36	−6.8[c]	−14.2
Chloroform	298	4.73	1.55	−0.26	—	—
Dichloroethane	298	10.2	1.43	−0.21	—	—
Dioxane	298	2.21	0.48	+0.43	—	—
Tetrahydrofuran	298	8.20	1.96	−0.40	—	—
Dimethoxyethane	298	7.04	0.70	+0.19	—	—

[a] k_2 = association constant determined according to the equation

$$\mathrm{OD} = \left[\frac{k_2}{2}\right]\epsilon_m^2 l^2\left[\frac{C_0}{\mathrm{OD}}\right] - \left[\frac{k_2}{2}\right]\epsilon_m^2 l$$

where ϵ_m = the extinction coefficient of the monomer; l = the optical path length; C_0 = the molar concentration of ϵ-caprolactam (Lord and Porro, 1960). [b] ΔH: The enthalpy values listed have been corrected for the change in D^{-1} with temperature as described in the text. [c] ΔH: This value for the enthalpy in CCl_4 has not been corrected for the change in D^{-1} with temperature.

solute in the sample cell. The cell temperatures were controlled to ± 0.5° by the use of water-jacketed cell holders. The temperature of the sample in the cell was obtained directly from the resistance of a thermistor press-fitted into the Teflon stopper supplied with the cell. The slit height was reduced by two-thirds to eliminate the thermistor from the light beam. The cells were allowed to come to thermal equilibrium with no light passing through them. The lamp was then moved into place, and the temperature rose to the desired value. Preventing the light beam from striking the cells until thermal equilibrium was reached served a 2-fold purpose; the final stable temperature was produced much sooner, and the effect of light on the solvents was minimized. Spectra for any one solute over the entire concentration range in a given solvent were run so that a constant slit width, between 0.17 and 0.24 mm, was obtained at the frequency of the free N-H absorption. Quartz cells with path lengths of 0.2, 1.0, 5.0, and 10.0 cm were used for the various concentration ranges in such a manner that the highest optical density in a particular series was not more than 0.7.

Dielectric Constant and Dipole Moment Measurements.—Dielectric constants were determined by the resonance method employing the apparatus described by Bender (Bender, 1946). The dipole moments were calculated in the usual manner from the distortion and

TABLE III

DIMENSIONS OF *N*-METHYLACETAMIDE AND CAPROLACTAM DIMERS NEEDED FOR CALCULATION OF ELECTROSTATIC PART OF FREE ENERGY OF ASSOCIATION[a]

Quantity	*N*-Methylacetamide	ϵ-Caprolactam
Dipole moment × 10^{18} esu-cm	4.4	3.4
r_{12} (A)	2.2	2.2
Electronic change	0.42	0.32
r_{23} (A) N—H···O	2.83	2.83
r_{14} (A) N ⟷ O	5.95	2.83
r_{13} (A) O ⟷ O	4.70	3.65
r_{24} (A) N ⟷ N	3.75	3.65

[a] The distances are taken from Figure 1.

total polarizations. Total polarizations were obtained by the method of Daniels *et al.* (1956). Distortion polarizations were calculated from refractive index and density measurements in the case of liquids, or from tables of atomic and structural refractions for ϵ-caprolactam. In order to obtain a value for the atomic refraction of nitrogen in ϵ-caprolactam, the molar refraction of liquid *N*-methylcaprolactam was determined from its refractive index. Atomic refractions of the carbon, hydrogen, and oxygen atoms were then totaled and the difference between this sum and the measured molar refraction was used as the value for the atomic refraction of nitrogen in ϵ-caprolactam.

RESULTS

The results are summarized in Tables I–III. There is a very definite effect of solvent composition on the stability and strength of the interamide hydrogen bonds of both *N*-methylacetamide and ϵ-caprolactam. The value of the equilibrium constant for ϵ-caprolactam in carbon tetrachloride, Table II, is substantially less than the value of 105 liter mole^{-1} at 26° reported by Lord and Porro (1960). The enthalpy value of −3.4 kcal mole^{-1} per hydrogen bond, however, is in better agreement with enthalpies for similar interamide hydrogen bonds as reported in the tables compiled by Pimentel and McCellan (1960). It should be noted that the study of Lord and Porro differed from that in this presentation in that they employed single-beam spectrophotometry and followed changes in the spectrum in the fundamental infrared region. Important to the conclusions of this paper, however, are the changes in the thermodynamic functions that occur with changes in the solvent composition. The magnitude of these changes were measured with a considerable degree of certainty.

Although the effect of the dipolar nature of the environment is measurable for the *cis*- and *trans*-dichloroethylene, and possibly also in chloroform and dichloroethane, it is completely masked when efficient electron donor groups are present in the solvent. The stability of the interamide complex in the three other solvents listed in Table II clearly demonstrates this masking. The extinction coefficient of the maximum free N-H absorption is greatly reduced in the ether solvents; this, accompanied by the enhanced solvent absorption, introduces a great deal of error in the value of the association constant. The relations between the stability constants, furthermore, do not reflect the basicities of the ethers: pK_a = −2.08, −3.22, and −3.27, for tetrahydrofuran, dioxane, and 1,2-dimethoxyethane, respectively (Arnett and Wu, 1962). A reasonable interpretation of the observation that dioxane and 1,2-dimethoxyethane disrupt hydrogen bonding more than tetrahydrofuran is that the number of oxygen donor atoms available per unit volume of tetrahydrofuran is only about half that in the other two solvents.

DISCUSSION

Theory.—The description of the effect of the dielectric constant of the medium on hydrogen bonding can be approached from classical electrostatic principles. The electrical free energy of the amide dipoles and the work involved in transferring these dipoles from a medium of one dielectric constant to a medium of another dielectric constant can be evaluated and related to the free energy of the hydrogen-bonding reaction. The dimerization of *N*-methylacetamide will serve as an example. *N*-Methylacetamide in dioxane has a dipole moment of 4.4 Debye units (Mizushima *et al.*, 1950). If one assumes that the charges responsible for the dipole moment reside on the oxygen and nitrogen atoms, the distance between the charges, 2.2 A, can be calculated from the known bond distances and angles of the molecule (Katz, 1957). Accordingly, the charge on the atoms would be 0.42 electronic unit. From this information, the energy of the dipole can be obtained from equation (1),

$$W = \frac{q^2}{D}\left[\frac{1}{b} - \frac{1}{r_{12}}\right] \tag{1}$$

where D is the dielectric constant, q is the charge on each pole of the molecule, and b and r_{12} are the distances indicated in Figure 1. If one makes the further assumption that the average dimensions of the dimer formed between the two *N*-methylacetamide molecules in solution are those given in Figure 1 and Table III, the energy of the polar dimer aggregate can be calculated from equation (2),

$$W = \frac{2q^2}{Db} + \sum \frac{q_i q_j}{D r_{ij}} \tag{2}$$

where $i < j$, and $j = 2, 3,$ or 4.

The energies involved in the transfer of the monomer, $\left[\frac{\Delta F}{A \to B}\right]_1$, and of the dimer, $\left[\frac{\Delta F}{A \to B}\right]_2$, from a medium of dielectric constant A to a medium of dielectric constant B are

$$\left[\frac{\Delta F}{A \to B}\right]_1 = kT \ln \frac{(\gamma_B)_1}{(\gamma_A)_1} = q^2\left[\frac{1}{b} - \frac{1}{r_{12}}\right]\left[\frac{1}{D_B} - \frac{1}{D_A}\right] \tag{3}$$

and

$$\left[\frac{\Delta F}{A \to B}\right]_2 = kT \ln \frac{(\gamma_B)_2}{(\gamma_A)_2} = q^2\left[\frac{2}{b} + \frac{1}{r_{13}} + \frac{1}{r_{24}} - \frac{1}{r_{12}} - \frac{1}{r_{14}} - \frac{1}{r_{23}} - \frac{1}{r_{34}}\right]\left[\frac{1}{D_B} - \frac{1}{D_A}\right] \tag{4}$$

where γ represents the activity coefficient of the species in question. The effect of the dielectric constant on the apparent free energy of dimerization can be expressed as

$$[\Delta F_{12}^0]_{B,\text{app}} - [\Delta F_{12}^0]_{A,\text{app}} = -RT \ln\left[\frac{\gamma_1\gamma_1}{\gamma_2}\right]_B + RT \ln\left[\frac{\gamma_1\gamma_1}{\gamma_2}\right]_A \tag{5}$$

Rearranging equation (5), and substituting equation (3) and (4) and choosing the initial solvent, A, with an infinite dielectric constant one obtains

$$[\Delta F_{12}^0]_{B,\text{app}} = [\Delta F_{12}^0]_{\infty,\text{app}} - Nq^2\left[\frac{1}{r_{14}} + \frac{1}{r_{23}} - \frac{1}{r_{13}} - \frac{1}{r_{24}}\right]\frac{1}{D_B} \tag{6}$$

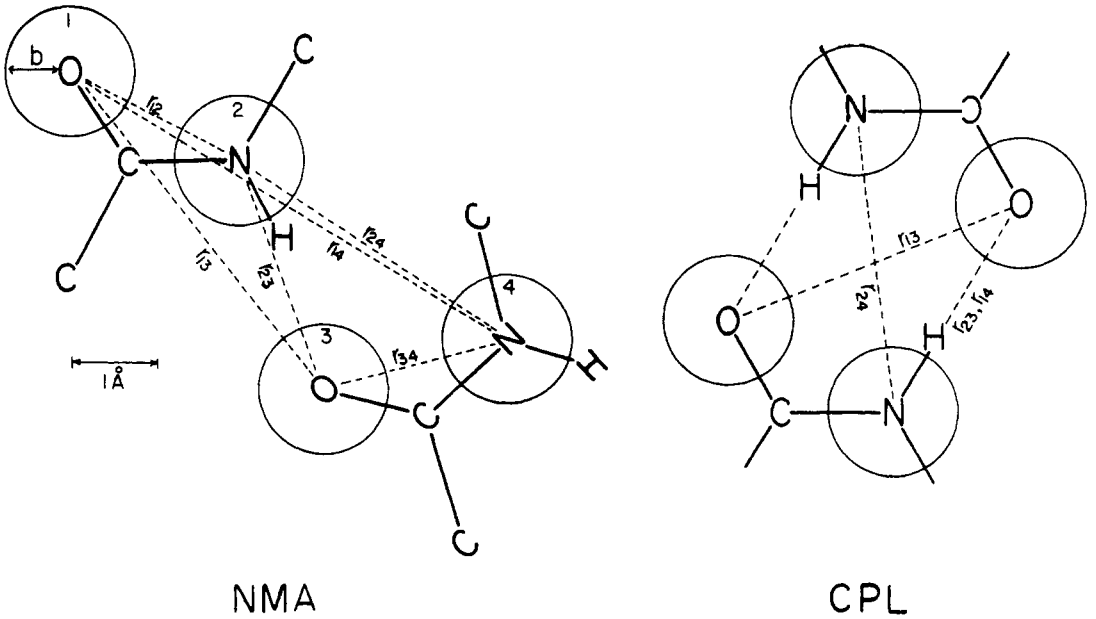

FIG. 1.—Spatial relationships in *N*-methylacetamide and caprolactam dimers. NMA: (*N*-Methylacetamide) Bond distances and angles are taken mostly from Katz, 1957. Angles around the nitrogen atom are assumed to be 120°. The 10° deviation of the hydrogen atom from the N · · · O line is based on the calculations of Schroeder and Lippincott, 1957. An N · · · O-C angle of 124° is assumed on the basis of the similarity between *N*-methylacetamide and acetyl glycine for which this angle is 124° (Carpenter and Donohue, 1950). CPL: (ε-Caprolactam). All bond angles in the amide group are assumed to be 120° and amide group is assumed to be planar. The hydrogen-bond distance of diketopiperazine, 2.85 A, has been chosen for ε-caprolactam (Corey, 1938).

For all possible orientations of the two monomers with respect to each other, including the cyclic dimer formed by CPL,

$$\frac{1}{r_{14}} + \frac{1}{r_{23}} > \frac{1}{r_{13}} + \frac{1}{r_{24}}$$

Therefore, $[\Delta F_{12}^0]_{B,\text{app}}$ decreases linearly with D_B^{-1}. The variation in the enthalpy of the reaction with the dielectric constant, on the basis of the preceding equations can be shown to be

$$[\Delta H_{12}^0]_{B,\text{app}} = [\Delta H_{12}^0]_{\infty,\text{app}} - Nq^2\left[\frac{1}{r_{14}} + \frac{1}{r_{23}} - \frac{1}{r_{13}} - \frac{1}{r_{24}}\right]\left[\frac{1}{D_B} - T\frac{dD_B^{-1}}{dT}\right] \quad (7)$$

In order to apply equation (7) it is necessary, therefore, to determine the temperature dependence of the reciprocal of the dielectric constant. The data in Table IV show that the experimental error in the evaluation of this function is too great to allow for a proper choice of the term. Another method is available, fortunately, for correcting the observed values of $\partial \ln K/\partial T$ in a particular solvent for the change in D^{-1}. This is easily done by plotting $\ln K$ versus D_{obs}^{-1} at the three temperatures of measurement and taking the difference in $\ln K$ between the resulting lines at a constant value of D^{-1}. The enthalpy of the reaction $(\Delta H_{12}^0)_{D,\text{app}}$ in a solvent which has a dielectric constant independent of temperature is then readily calculated. Figure 2 shows the result of such a treatment. It may be argued from the linear dependence of the enthalpy function on D^{-1} that dD^{-1}/dT is a constant for this solvent system; a conclusion not altogether ruled out by the imprecise data of Table IV.

TABLE IV

VARIATION OF THE RECIPROCAL OF THE DIELECTRIC CONSTANT OF *cis-trans*-DICHLOROETHYLENE SOLVENT SYSTEM WITH TEMPERATURE AT 298 K

Solvent	D	$dD^{-1}/dT \times 10^4$
trans-Dichloroethylene	2.27 ± 0.06	5.33 ± 0.43
2:1 *trans/cis*	4.06 ± 0.12	7.92 ± 0.63
1:2 *trans/cis*	6.35 ± 0.19	6.45 ± 0.52
cis-Dichloroethylene	9.07 ± 0.27	4.89 ± 0.39
		6.14 ± 0.49 avg

By combining equations (6) and (7) an expression for the entropy of association can be derived.

$$[\Delta S_{12}^0]_{B,\text{app}} = [\Delta S_{12}^0]_{\infty,\text{app}} + Nq^2\left[\frac{1}{r_{14}} + \frac{1}{r_{23}} - \frac{1}{r_{13}} - \frac{1}{r_{24}}\right]\frac{dD_B^{-1}}{dT} \quad (8)$$

Because of the nature of the solvent the second term on the right-hand side of equation (8) can be considered constant over the range of dielectric constants available, and the theory would therefore predict that the entropy should be independent of the dielectric constant. Figure 2 shows the comparisons between the experimental data and the predicted results. The rather good agreement of the enthalpy data for ε-caprolactam association with the theoretical expectations is encouraging. The deviations observed in the free energy (Figure 2) and entropy (Tables I and II) functions are readily explained.

The validity of relating a bulk parameter such as the dielectric constant to interactions at close distances such as exist in hydrogen-bonded complexes has been questioned and is debatable. Some other bulk parameter such as the vapor pressure or density might be considered as the independent variable determining the equilibrium of the hydrogen-bonding reaction. The question posed in this paper, however, is whether the interamide hydrogen bond is somewhat electrostatic in nature and whether the solvent influences the electrostatic contribution to the total hydrogen bond energy.

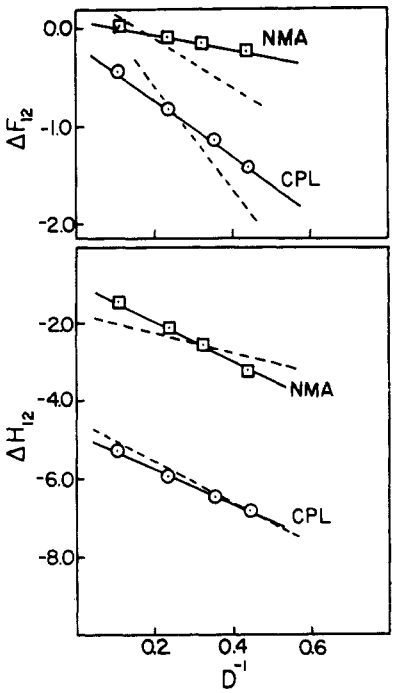

Fig. 2.—Free energy and enthalpy of hydrogen-bond formation as a function of the reciprocal of the dielectric constant. The dashed lines represent the slopes of the functions calculated from equations (6) and (7) and Table III. Enthalpy values are corrected for change in D^{-1} with temperature as explained in the text. NMA, *N*-methylacetamide; CPL, ε-caprolactam.

The properties of the solvent which are of direct bearing, therefore, are the dielectric constant and the dipole moments of the individual solvent molecules. Although there are slight differences in other bulk parameters (which reflect the fact that *cis*-dichloroethylene is weakly associated through dipole-dipole interactions), these differences are not large enough, in the opinion of the authors, to account for the differences in the equilibrium of the amide association. The heat of mixing data for mixtures of *cis*- and *trans*-dichloroethylene give some indication of the magnitude of the nonideality of the system (Amaya, 1961). Indeed, there is absorption of heat during mixing, but the size of the energy change is smaller than that for almost all the other binary mixtures of polar and nonpolar solvents investigated by Amaya. Since the heat of mixing for

TABLE V
DIPOLE MOMENTS OF AMIDES IN VARIOUS SOLVENTS AT 25°

Solute	*trans*-Dichloroethylene	*cis*-Dichloroethylene	Dioxane	1:1 *trans*/*cis*
N-Methyl acetamide	[a]	[a]	4.4[b]	—
Dimethyl acetamide	3.98	[c]	3.88	—
ε-Caprolactam	2.91[d]	2.38	3.40	—
N-Methylcaprolactam	4.25	2.84	4.11	3.27

[a] Nonlinear due to association. [b] From Mizushima *et al.* (1950). [c] Nonlinear due to conductance. [d] Not reliable due to dimer formation.

cis- and *trans*-dichloroethylene is substantially smaller than that for toluene and cyclohexane, which on the basis of molecular structure considerations would be expected to have a very small heat of mixing, it appears that the dichloroethylene system does not deviate greatly from ideality. Most of the bulk parameters would therefore probably vary in a linear manner with the mole ratio of the mixture of the isomers. The free energy and enthalpy of association do not vary directly with the ratio of the mixture, but they are expressible as linear functions of D^{-1}. This fact lends credence to the application of electrostatic principles to the system.

Implicit in the development of equations (6), (7), and (8) is the understanding that the solvent does not interact directly with the solute, or rather that there is no difference in the extent of solute-solvent interaction as the solvent composition is altered. Clearly, this is not what would be expected in consideration of the fact that *cis*-dichloroethylene has a dipole moment of 1.89 Debye units, whereas the dipole moment of *trans*-dichloroethylene is zero. Although both *cis*- and *trans*-dichloroethylene have π-electron clouds which would be presumed to complex somewhat with the N-H group of the amides, this effect should be equal for both solvents. The data offer three independent lines of evidence to support the hypothesis that *cis*-dichloroethylene interacts with *N*-methylacetamide and ε-caprolactam to a greater degree than *trans*-dichloroethylene. The dipole moment determinations on *N*-methylcaprolactam in dioxane and in the *cis*-*trans*-dichloroethylene system, Table V, show that the dipolar solvent clusters around the solute molecule thus reduc-

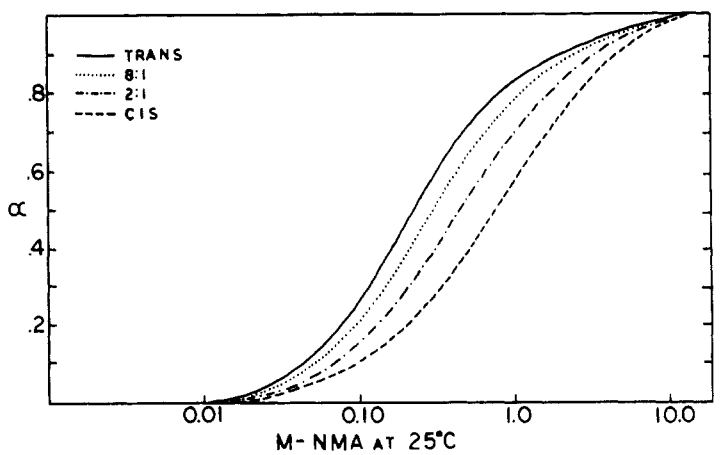

FIG. 3.—Dependence of fraction of bonded N-H groups of *N*-methylacetamide (NMA) on total *N*-methylacetamide concentration in *cis*- and *trans*-dichloroethylene. Solvent composition is indicated in terms of the mole ratios of *cis*- and *trans*-dichloroethylene.

ing its apparent dipole moment. It is not possible to measure this effect unequivocally with *N*-methylacetamide and ϵ-caprolactam because of complications arising from interamide hydrogen bonding, but it is reasonable to suppose that the effect is similar to that observed with *N*-methylcaprolactam. The graph of the fraction of hydrogen-bonded N-H groups of *N*-methylacetamide versus solute concentration in Figure 3 also shows the influence of interacting solvent dipoles. As solvent is added to pure *N*-methylacetamide it is evident that the large aggregates of *N*-methylacetamide are disrupted by *cis*-dichloroethylene more effectively than by *trans*-dichloroethylene. In this concentration range the dichloroethylene should be considered as the solute and the *N*-methylacetamide as the solvent. Certainly, the rupturing of the amide-amide hydrogen bonds is not due to dielectric constant changes since the dielectric constant of liquid *N*-methylacetamide is 178. The small amount of dichloroethylene present would not alter it significantly. Finally, the relative entropy values for the association of *N*-methylacetamide and ϵ-caprolactam in *cis*- and *trans*-dichloroethylene can easily be interpreted in terms of solute-solvent clustering. The entropy value in *trans*-dichloroethylene for the formation of the cyclic dimer of ϵ-caprolactam shows that this process entails more ordering of the system, as would be expected, than that for the formation of the linear dimer of *N*-methylacetamide. According to the application of equation (8) the entropy of the reaction in *cis*-dichloroethylene should be the same as that in *trans*-dichloroethylene. The fact that the entropy is less negative in *cis*-dichloroethylene is a result of the ordering effect of solute monomers on the solvent dipoles. Because of steric factors, as revealed by the construction of Courtauld models, it is possible to visualize the orientation of more *cis*-dichloroethylene solvent molecules around an *N*-methylacetamide monomer than around a ϵ-caprolactam monomer. The over-all change in the entropy of dimerization due to solute-solvent dipole interaction would, therefore, be expected to be greater with *N*-methylacetamide than with ϵ-caprolactam, as is observed.

Another important refinement of the above theoretical development which has been neglected is a correction for the difference between the measured solvent dielectric constant and that in the immediate neighborhood of the bonding atoms. The solvent molecules cannot completely enter the region between the charges on the amide dipoles of the dimer complex as was tacitly assumed in the derivation of equation (6). The effective dielectric constant D_{eff} is therefore less than D, and increases to a limiting value of D as the distance between the amide monomers increases. To account for this complication, equation (6) can be rewritten to give

$$[\Delta F_{12}^0]_{B,\text{app}} = [\Delta F_{12}^0]_{\infty,\text{app}} - Nq^2\left[\frac{1}{r_{14}D_{14,\text{eff}}} + \frac{1}{r_{23}D_{23,\text{eff}}} - \frac{1}{r_{13}D_{13,\text{eff}}} - \frac{1}{r_{24}D_{24,\text{eff}}}\right] \quad (9)$$

Unfortunately there is no way of arriving at the magnitude of the effective dielectric constants shown in equation (9). Depending on the magnitude of the estimated values chosen for $D_{ij,\text{eff}}$ corresponding to a given value of D, the changes in $(\Delta F_{12}^0)_{B,\text{app}}$, and consequently $(\Delta H_{12}^0)_{B,\text{app}}$, calculated from equation (9) may be somewhat greater or less than those calculated by equations (6) and (7). Therefore, any difference in the slopes of the theoretical and observed lines in Figure 2 might be a result of using wrong values of D in the calculation. This is a more serious problem for *N*-methylacetamide than for ϵ-caprolactam since with the *N*-methylacetamide dimer the electrostatic forces are transmitted through the *N*-methylacetamide molecules to a large extent. The greater coincidence of the experimentally observed and calculated slopes for ϵ-caprolactam as opposed to *N*-methylacetamide could also result from the fact that the r_{ij} values obtained from the proposed dimensions of the rather rigid cyclic ϵ-caprolactam dimer are more realistic than the corresponding r_{ij} values of the arbitrarily oriented end-to-end dimer of *N*-methylacetamide. Suffice it to say, however, the agreement between the observed enthalpy data for ϵ-caprolactam and the theoretical calculations is surprisingly good in light of the assumptions made, thereby giving credence to the theoretical approach employed. The variation in the enthalpy of association may, in summary, be expressed in terms of changes in the dielectric constant. The variation in the free energy of association, on the other hand, is determined by the entropy effect discussed above as well as by the dielectric constant.

Finally, account should be taken of the contribution of solvent-solute dipole-dipole interactions to the free energy and enthalpy of dimerization. It is not possible to rule out the proposition that the shift toward more positive values of the enthalpy with increasing dielectric constant is due to the necessity of disrupting solute-solvent complexes while concurrently forming solute-solute complexes. In fact, since we are considering a relatively short range interaction, it might well be appropriate to interpret the results entirely in terms of competition of solvent dipoles rather than in reference to a bulk parameter of the solvent such as the dielectric constant. In these terms the data readily support the conclusion that whenever there is a possibility of solute-solvent interaction, the solute-solute hydrogen bonds are weakened. The extent of hydrogen bonding, therefore, between solute molecules is strongly dependent on the molecular environment of the solvent. The linearity of the free energy and enthalpy versus reciprocal dielectric constant plots, however, strongly suggests that hydrogen bond stability is dependent to some degree on the dielectric constant of the solvent. Studies with other carefully selected solvent systems are necessary in order to make a proper choice of these alternative explanations of the data.

It has been suggested that a number of solvents with different chemical properties but with the same dielectric constant should be employed, and that only if ΔF_{ass} and ΔH_{ass} are the same in these different systems will the unique relationship between hydrogen-bond strength and dielectric constant be established. This criterion is impossible to meet since most solvents have electron donor groups which would compete to different extents for the interamide H-bonds. These complications are illustrated by the data of Table II for carbon tetrachloride, *trans*-dichloroethylene and dioxane. The only way the authors see to get around this difficulty is to vary the dielectric constant within a solvent system. Unfortunately, no matter what solvent systems are chosen, one either has to settle for different H-bonding abilities or different dipole moments of the components of the system. It is felt that the latter feature is less objectionable since the dipole-dipole interference by the solvent with the solute-solute complex is a weaker interaction than hydrogen-bond formation of the solvent with the solute.

Application to Protein Structure.– There has been a good deal of discussion, recently, on the nature and importance of intramolecular forces in proteins in aqueous solution with specific emphasis on hydrophobic interactions (Némethy and Scheraga, 1962; Tanford, 1962). The suggestion stated in the introduction that

hydrophobic bonding and hydrogen bonding operate in a concerted manner is supported by the evidence presented herein. However it appears to be essential that the intramolecular hydrogen bonds be situated in a region of the molecule which is shielded from the solvent water molecules. The studies of ϵ-caprolactam association in dioxane, tetrahydrofuran, and 1,2-dimethoxyethane, Table II, and of *N*-methylacetamide association in carbon tetrachloride, dioxane, and water (Klotz and Franzen, 1962) demonstrate that in the presence of effective hydrogen acceptors or donors interamide hydrogen bonds are readily broken. Moreover, the presence of dipoles in the immediate vicinity of the interamide hydrogen bond can lower its stability both by competitive effects and by decreasing the electrostatic energy of the hydrogen bond. Hydrogen bonds existing within hydrophobic regions, on the other hand, would have considerable stability due to the absence of competing groups and polar surroundings. This, in fact, implies that interamide hydrogen bonds exist only in regions where shielding by hydrocarbon groups occurs.

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The Conformation of Angiotensin II in Aqueous Solution*

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Some physicochemical properties of angiotensin octapeptides were studied. The molecular weight of Asp(NH_2)^1-Val^5-angiotensin II (angiotensinamide) was determined in the *p*H range 2.5–8.6 using the Archibald method of approach to sedimentation equilibrium, and was found to be 1000 ± 100 in agreement with the theoretical value for the monomer. Direct and spectrophotometric titrations showed that the *pK* values and enthalpies of ionization of the carboxyl, amino, imidazole, and phenolic groups of angiotensinamide are normal. The optical rotatory dispersions of Val^5-angiotensin II and angiotensinamide were normal, and the b_0 values obtained from Moffitt plots were 38 and 32, respectively. The optical rotatory dispersion of angiotensinamide was not significantly affected by *p*H, temperature, or the presence of urea or nonpolar solvents. The kinetics of hydrogen-deuterium exchange of angiotensinamide were also studied and the first-order rate constants obtained were of the same order of magnitude and had similar *p*H-dependence as those for poly-D,L-alanine and simple peptides. It is concluded that Val^5-angiotensin II and angiotensinamide exist in a random conformation in aqueous solutions.

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The angiotensins are peptides produced by the action of the proteolytic enzyme renin on a protein substrate contained in the α_2-globulin fraction of mammalian blood plasma (Braun-Menendez *et al.*, 1946). They are the most potent vasopressor substances known and are characterized, among other pharmacological properties, by a powerful stimulating activity on smooth muscles (Braun-Menendez, 1956). The decapeptides Ileu^5-angiotensin I (horse) and Val^5-angiotensin I (ox), and the octapeptide Ileu^5-angiotensin II (horse) have been isolated from *in vitro* renin digests of plasma