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Near Infrared Investigation of Interamide Hydrogen Bonds in Hydrogen-Bonding Solvents

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The interaction of amide groups through hydrogen bonding in the solvents methanol, ethylene glycol, chloroform, and carbon tetrachloride has been investigated by methods of near-infrared absorption spectroscopy. δ -Valerolactam was used as a model compound. The results are compared with earlier measurements in aqueous solution and in dioxane solution. All obtained data can be consistently interpreted in terms of dimerization of amide groups. Dimerization involves the formation of two $\text{NH} \cdots \text{O}=\text{C}$ hydrogen bonds. The magnitude of the overall enthalpy change in monofunctional solvents is greatly reduced as compared with inert media. The values obtained in carbon tetrachloride, chloroform and dioxane are -7 , -3 , and ± 0 kcal per mole, respectively. Dimerization of amide groups in hydroxyl-containing solvents involves changes in solute-solvent, solute-solute, and solvent-solvent interactions. Overall enthalpy values from -5 to -6 kcal per mole were obtained, in good agreement with previous work in aqueous solution.

An understanding of how and why protein molecules change their configuration requires an examination of the various kinds of bonds that might be expected to form in a native protein, and the means by which denaturing agents break these bonds; an understanding of these bonds is best gained by studying model systems (1). For investigating amide to amide hydrogen bonding by means of infrared spectroscopy, lactams are particularly suitable models. All available evidence points to the conclusion that no hydrogen-bonded chain-polymers are formed (2-6), eliminating the necessity to work at extreme dilution or to take into consideration complex multiple equilibria. Experimental measurements are facilitated by the relatively simple nature of the near infrared spectra of lactams in the 1.5μ region.

The present communication describes results obtained on $\text{C}=\text{O}$ to HN hydrogen bonding, with δ -valerolactam as a model compound, in a series of organic solvents

selected on the basis of their hydrogen-bonding properties. Measurements were made in the inert solvent carbon tetrachloride, the proton-donating solvent chloroform (which can form hydrogen bonds with $\text{C}=\text{O}$ groups (4)), and in the hydroxyl-containing solvents methanol and ethylene glycol. The data are compared with results obtained in the proton-accepting solvent dioxane (6) and with previous results obtained in aqueous solution (2). All measurements were carried out by the same experimental techniques and using the same model compound, in order to eliminate as many factors as possible except the nature of the solvent.

EXPERIMENTAL PROCEDURE

Instrumentation and samples. Absorption spectra were obtained with a Cary Model 14 recording spectrophotometer¹ in the wavelength range of

¹ Mention of commercial items is for your convenience and does not constitute an endorsement by the Department of Agriculture.

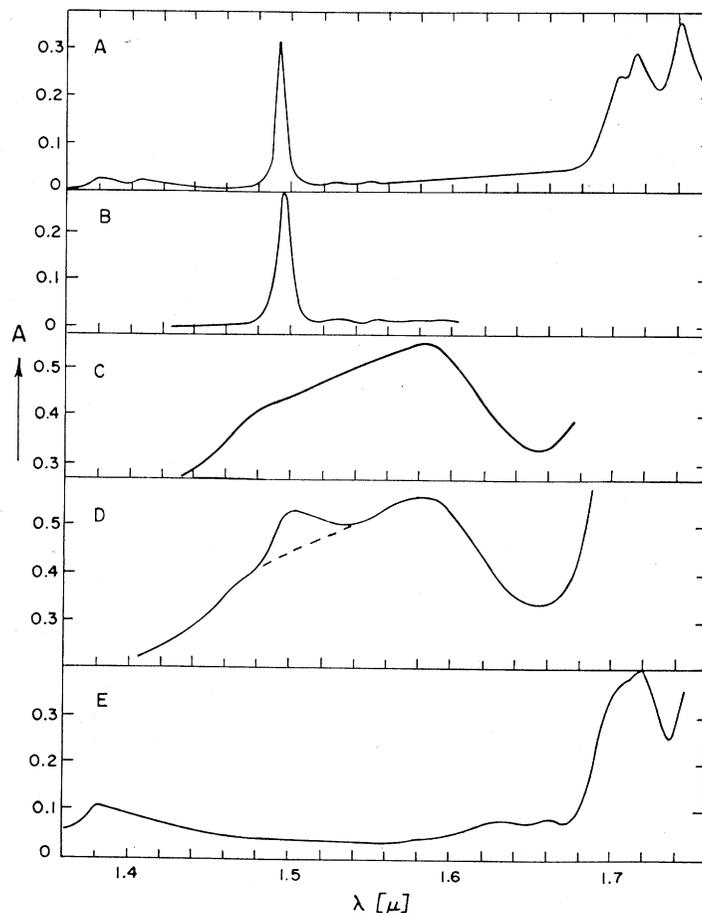


FIG. 1. Absorption spectra of δ -valerolactam at 25°. *A*, in CCl_4 ; $X = 0.006$; $d = 100$ mm. *B*, in HCCl_3 , $X = 0.004$; $d = 50$ mm. *C*, pure CH_3OH ; $d = 1$ mm (for comparison). *D*, in CH_3OH ; $X = 0.24$; $d = 2$ mm. *E*, pure lactam (all dimerized); $d = 1$ mm.

1.4–1.7 μ . Quartz cells with path lengths ranging from 2 to 100 mm were used. The 2-mm cell has been described (2); the cells with longer path length were of a conventional cylindrical double-walled design (5). Water from a constant temperature bath was circulated through the outer compartment and the temperature of the sample was measured in the absorption cell, but outside the path of the radiation beam. The sample of δ -valerolactam has been described (2). Chloroform (Spectro-Quality Reagent; Matheson, Coleman, and Bell) was separated from alcohol (added by the manufacturer as a stabilizer) by shaking with concentrated sulfuric acid, washing with water, drying with calcium chloride, distilling, and finally drying with molecular sieve. Spectral grade carbon tetrachloride was dried with a stream of dry nitrogen and with molecular sieve. Methanol and ethylene glycol (Baker Analyzed Reagent) were used without further purification.

Spectra. Figure 1 presents the observed absorption spectra. The spectra of the pure lactam and of methanol are included for comparison, and to facilitate assignment of the observed absorption bands. The spectrum obtained in ethylene glycol solution is very similar to the spectrum obtained in methanol solution and is therefore not shown. The spectra exhibit two characteristic features which are briefly discussed before proceeding with evaluation and interpretation of the data.

First, there is no evidence of an absorption band in the studied region which could be associated with NH groups involved in NH \cdots O=C hydrogen bonding. The band centering close to 1.5 μ is assigned to the first overtone of NH stretching modes of groups not involved in amide-amide bonding, for the following reasons: (i) in CCl_4 solution no dimer-band of appreciable intensity was observed in the studied wavelength region even at concentrations as high as 0.25 mole per

liter, although the corresponding fundamental in the $3\ \mu$ region is extremely intense (3-5); at such concentration the lactam is overwhelmingly dimerized in CCl_4 solution (3). (ii) The pure lactam shows no appreciable absorption around $1.5\ \mu$. Again the fundamental NH stretching mode around 3 microns is very intense (unpublished results). (iii) The concentration- and temperature-dependence of the $1.5\ \mu$ band in all studied solvents is in excellent agreement with its assignment to NH groups which are not involved in amide-amide bonding. A partial explanation for the very low intensity of the dimer band can be found in the centrosymmetric structure of dimerized lactams, which renders the first overtone of the infrared active antisymmetric stretching mode inactive. Changes in anharmonicity resulting from changes in hydrogen bonding must also be taken into consideration (7).

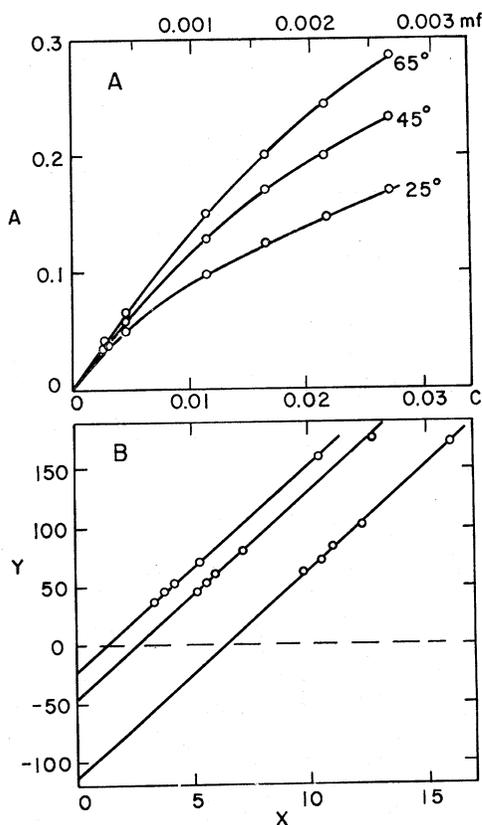


FIG. 2. A, absorbance of monomeric amide in CCl_4 solution as a function of temperature and concentration, $d = 100\ \text{mm}$; C , moles per liter; mf , mole fraction. B, graphical estimation of absorptivity and dimerization constants. $y = C_s/A$; $x = (1/A)^2 (C^2/2 + C_sC)$; slope, ed ; intercept, $-(4K_x' + 1)/2ed$.

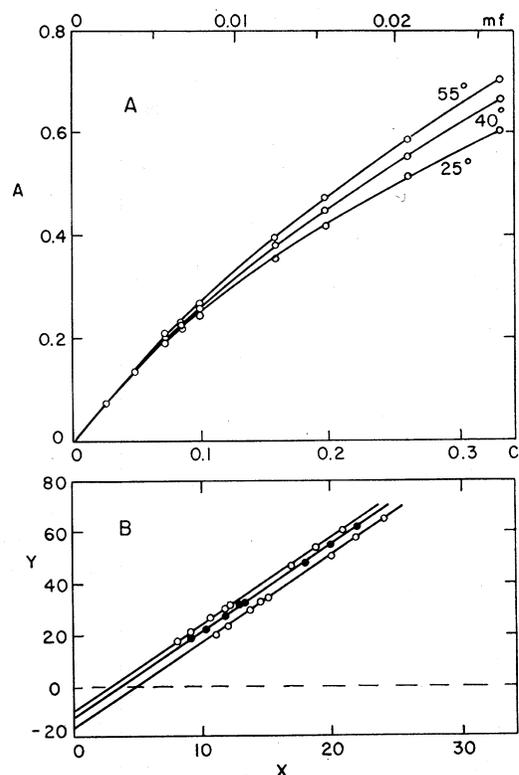


FIG. 3. A, absorbance of monomeric amide in HCCl_3 solution, $d = 50\ \text{mm}$. B, graphical estimation of absorptivity and dimerization constants (as in Fig. 2B).

A second noteworthy feature is the relatively stable frequency of the $1.5\ \mu$ band, regardless of solvent. It could be expected that the frequency is lowered in solvents where NH to solvent hydrogen bonding is expected (such as alcohols and ethers). This is indeed the case for the fundamental NH mode in dioxane solution, where a downshift of about $70\ \text{cm}^{-1}$ is observed, as compared with a carbon tetrachloride solution (unpublished results). The overtone frequency in dioxane solution is relatively unaffected as compared to CCl_4 solution (6). (In alcohol solution the fundamental NH frequency cannot be determined with any precision because of very strong interference by OH absorption.) The relative stability of the overtone frequency must be a consequence of the lower anharmonicity of NH-O modes, as compared with free NH modes (7, 8).

Thermodynamic evaluations. Dimerization constants in the various solvents were evaluated by applying the previously developed (2) equation:

$$K_x' = \frac{C - A/ed}{2(A/ed)^2} \left(\frac{C + A/ed}{2} + C_s \right), \quad (1)$$

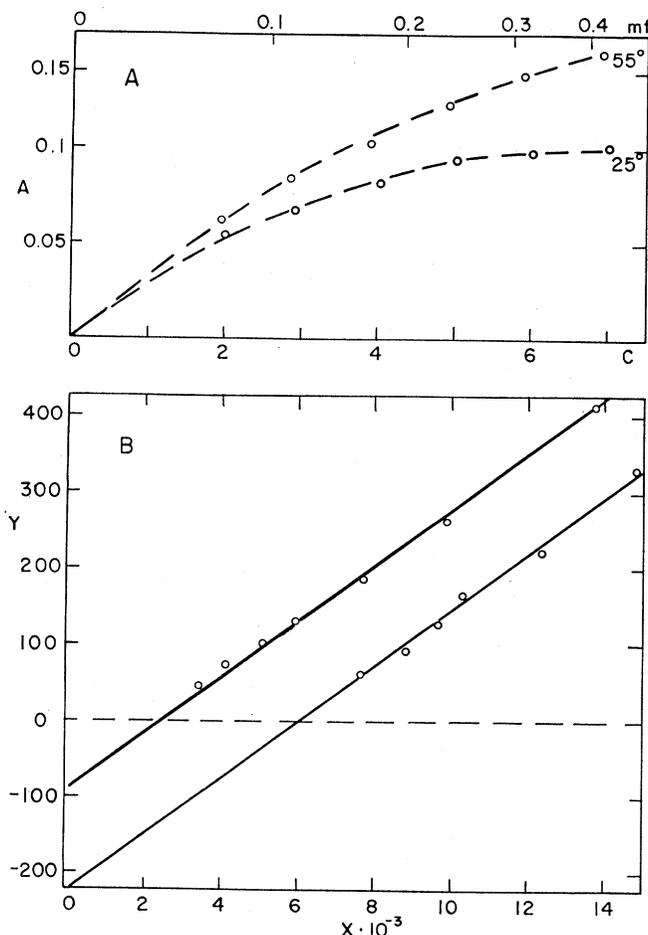


FIG. 4. A, absorbance of monomeric amide in CH_3OH solution; $d = 2$ mm. B, graphical estimation of K_x' and ϵ (as in Fig. 2B).

where K_x' is the dimerization constant in mole fraction units; C , total solute concentration in moles per liter; C_s , solvent concentration in moles per liter; A , monomer absorbance; ϵ , monomer absorptivity; and d , path length. Defining

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

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

and plotting y vs. x , a straight line is obtained with the slope ϵd and intercept $-(4K_x' + 1)/(2\epsilon d)$, provided that K_x' and ϵ are concentration independent (2). A standard state based on mole fractions was used throughout, because in some solvents high concentrations had to be employed. The thermodynamic quantities thus obtained are for the studied systems formally equivalent with

unitary quantities (1). In each solvent absorbance values were measured at several temperatures over a considerable concentration range (Figs. 2A, 3A, 4A, and 5A). The observed absorbance values were substituted into Eqs. 2 and 2a, leading to the diagrams given in Figs. 2B, 3B, 4B, and 5B. (The top and bottom lines in each lower figure are obtained from corresponding lines in the upper figures.) Values of ϵ and K_x' were calculated from the slopes and intercepts of the observed straight lines, as described. The temperature dependence of K_x' was subsequently determined in more detail by applying Eq. 1 directly to absorbance data obtained at 10° intervals for one sample in each solvent, resulting in Fig. 6. Enthalpy values were calculated in a conventional way from the slopes of the lines in Fig. 6. The estimated accuracy is ± 0.5 kcal per mole in CCl_4 and HCCl_3 solution; ± 1 kcal per mole in hydroxylated solvents.

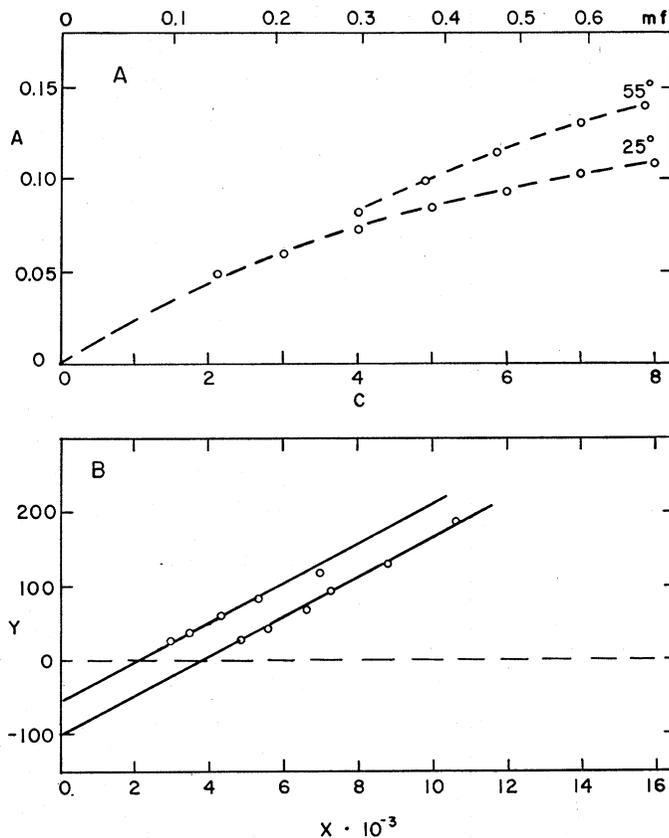


FIG. 5. A, absorbance of monomeric amide in HO(CH₂)₂OH solution; $d = 2$ mm. B, graphical estimation of K_x' and ϵ (as in Fig. 2B).

Absorbance values for the 1.5μ band were measured in a straightforward manner in carbon tetrachloride and chloroform solution. For methanol and ethylene glycol solutions a baseline procedure strictly analogous to the one used for aqueous solutions was employed (cf. Fig. 1, reference 2). The reasoning presented in connection with aqueous solution spectra (2) also applies to data obtained in methanol and ethylene glycol.

RESULTS

Carbon tetrachloride and chloroform solutions. Figure 2 presents the experimental results obtained in carbon tetrachloride solution. Corresponding information obtained in chloroform solution is given in Fig. 3. From the slopes in Figs. 2B and 3B, absorptivity values of 1.88 and 1.36 liter mole⁻¹ cm⁻¹ were obtained for the 1.5μ monomer band in CCl₄ solution and HCCl₃ solution, respectively. The obtained thermodynamic quantities are listed in Table I.

Data obtained in dioxane solution (6) are included for comparison. The enthalpy values were calculated from the temperature dependence of the equilibrium constants as evaluated by Eq. 1 and given in Fig. 6.

The enthalpy value obtained in carbon tetrachloride solution merits some attention because it can be taken, in a first approximation, as a measure of the energy of two NH -- O=C bonds under ideal conditions, where no appreciable solvent interaction is involved. Higher (5) as well as lower (3) enthalpy values have been reported by previous workers for closely related systems. These previous values were calculated from spectroscopic measurements in the 3μ region, where dimer bands are very broad and overlap with monomer bands. As seen in Fig. 1A, these difficulties are practically eliminated in the 1.5μ region.

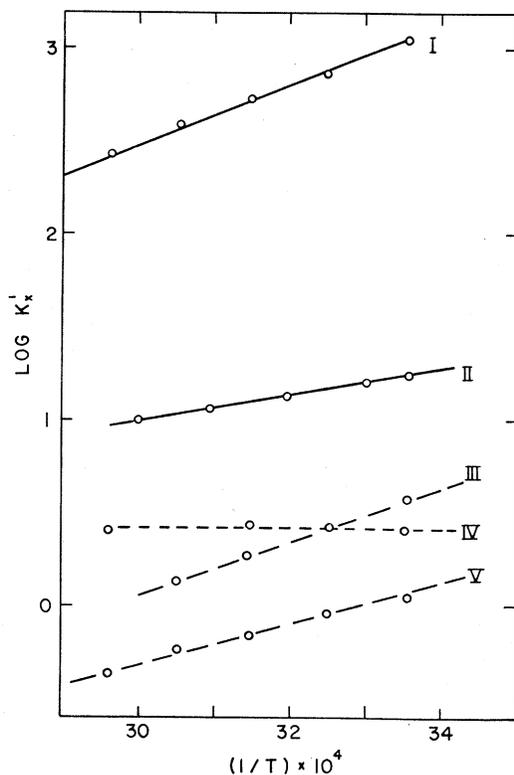


Fig. 6. Temperature dependence of $\log K_x'$. I, CCl_4 solution; II, HCCl_3 solution; III, CH_3OH solution; IV, dioxane solution (see ref. 6); V, $\text{HO}(\text{CH}_2)_2\text{OH}$ solution.

The results obtained in chloroform solution are in very good agreement with previous estimations (4). They show the substantial effect that even weak proton-donating solvents can have on the apparent bond energy of $\text{NH} \cdots \text{O}=\text{C}$ hydrogen bonds.

Methanol and ethylene glycol solutions. Figure 4 presents the results obtained in methanol solution. Because of the reduced number of hydroxyl groups per volume (as compared with aqueous solution and ethylene glycol solution), absorbance measurements of the 1.5μ NH band could be extended to lower concentrations in methanol solution than in any other investigated hydroxyl-containing solvent. As shown in Fig. 4A, meaningful data could be obtained at concentrations as low as 0.1 stoichiometric

TABLE I
THERMODYNAMIC PARAMETERS OBTAINED IN INERT AND MONOFUNCTIONAL SOLVENTS

Solvent	K_x' at 298°K	ΔH , kcal per mole
Carbon tetrachloride	1180	-7.1
Chloroform	28	-3.1
Dioxane (see ref. 6)	4	± 0

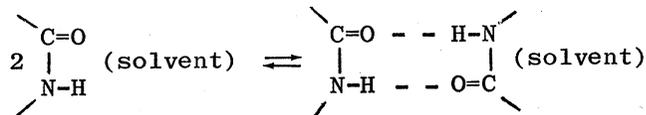
TABLE II
THERMODYNAMIC PARAMETERS OBTAINED IN HYDROXYL-CONTAINING SOLVENTS

Solvent	K_x' at 298°K	ΔH , kcal per mole
Methanol	3.7	-6.1
Ethylene Glycol	1.1	-5.1
Water (see ref. 2)	0.72	-5.5

mole fraction units.² The obtained absorbance vs. concentration curves show a remarkable similarity to corresponding data obtained in carbon tetrachloride solution (cf. Fig. 2). They lead to a temperature independent absorptivity value of $0.182 \text{ liter mole}^{-1} \text{ cm}^{-1}$, to concentration independent dimerization constants, and to a negative enthalpy change for dimerization. The obtained thermodynamic quantities are listed in Table II, along with corresponding data obtained in other hydroxylated solvents. The change of $\log K_x'$ with temperature is given in Fig. 6. The results obtained in ethylene glycol solution are given in Fig. 5. Because of the higher molecular weight of the solvent, the higher concentration of OH groups per volume, and a lower dimerization constant, meaningful measurements were not possible at as low concentrations as in methanol solution. The obtained data again lead to a constant absorptivity value and to a dimerization reaction with a negative enthalpy change.

The results obtained in methanol and ethylene glycol solution suggest that dimerization in these solvents can be consistently presented in the same way as in the previously studied aqueous solution (2):

² Stoichiometric mole fractions are defined in terms of the monomeric amide.



The thermodynamic parameters obtained in all three solvents are given in Table II. (The variation of ΔH from one hydroxylated solvent to another falls within the estimated limits of accuracy.)

DISCUSSION

The investigated solvents fall into two categories on the basis of their hydrogen-bonding capabilities: (a) monofunctional proton-donating or proton-accepting solvents which can form hydrogen bonds with NH or C=O groups but cannot form solvent to solvent hydrogen bonds; (b) hydroxylated solvents capable of forming hydrogen bonds with NH as well as C=O groups, and also capable of strong solvent to solvent hydrogen bonding. The combined evidence, obtained by applying the described experimental procedure to the same model compound in six different solvents, indicates that NH to C=O hydrogen bonding is affected in a different manner by these two types of solvents.

Even relatively weak proton donors, such as chloroform, reduce considerably the overall enthalpy change accompanying amide to amide hydrogen bond formation, because amide to solvent bonds have to be broken in the process. The numerical results obtained in chloroform solution are in full agreement with earlier estimates (4). In dioxane solution relatively strong amide-solvent bonds must be broken to permit dimerization of amide groups, with the result that the overall enthalpy change is close to zero (6). [The obtained K_x' value in dioxane solution for the studied model is primarily a consequence of entropy relations (6).]

In bifunctional solvents such as water (2) and alcohols these simple considerations are inadequate. Dimerization of the amide involves the breaking of amide-solvent bonds and the formation of amide-amide as well as additional solvent-solvent bonds. The measured enthalpy change applies to the overall process. As pointed out in connection with aqueous solutions, it is not easy to associate the obtained value of ΔH with the forma-

tion of a discrete number of hydrogen bonds (2). The distinguishing factor between solvents classified as (a) and (b) above is the ability of the bifunctional solvents to form hydrogen-bonded networks involving only solvent molecules. On the basis of the combined evidence, it appears that such solvent networks (which must be destroyed when amide-solvent bonds are present) are an important factor lending stability to amide-amide bonds in these solvents. The results obtained in methanol solution (where more precise measurements were possible than in other hydroxylated solvents) appear especially relevant.

It is difficult to say at the present stage to what extent observations based on the studied model system can be carried over to polypeptide chains, or to other model systems involving small molecules. The obtained results do indicate that for the described system the formation of NH to C=O hydrogen bonds results in a considerable negative overall enthalpy change in solvents which are capable of forming strong solvent to solvent hydrogen bonds.

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