article describes a study of the uv spectra of a number of water-soluble polypeptides and of two model alkyl amides in these solvents, together with some optical rotatory dispersion studies of the polymers. Rosenheck concluded, from a comparison of the large spectral shifts of the simple amides compared with the much smaller ones of the polypeptides in the H_2SO_4–H_2O mixtures, that the polymers were protonated to a small extent in these solutions (0–60% or 0–70% H_2SO_4). This was based on the explicit assumption that the shifts of the small amides could be taken as a measure of protonation exclusively (*i.e.*, without regard for possible medium effects). We believe that this assumption can lead to an error in the evaluation of the protonation of an amide (this manuscript and Edward and Wang[18]), and that the circular dichroic spectra are much less ambiguous in this respect. It was particularly interesting to note that Rosenheck concluded that the onset of the helix–coil transition of poly-L-glutamic acid comes in acid more dilute than 35%. We have made a preliminary measurement of the CD spectrum of this polymer and find that 10% is already in the helical form in 56.5% H_2SO_4. If his conclusion is correct, it means that the coil-to-helix transition in this case is a very long one, that is, not cooperative. We do not exclude this possibility, since the solvation requirements of the polymer may be very different with a carboxyl group in place of an ester group. We shall shortly submit a more detailed account of this transition.

Acknowledgments. J. S. thanks the National Council of Research of Italy for a Research Professorship, and thanks Professor E. Scoffone, Director of the Institute of Organic Chemistry, for his friendly and cooperative interest. We are deeply indebted to Dr. A. Fontana of this institute for the synthesis of the model diamide.

Protonation of Peptides. II. The Protonation of an Amide and of a Diamide in Dichloroacetic Acid, and the Behavior of Poly-γ-benzyl-L-glutamate in Dichloroacetic Acid and in Some Mixed Solvents

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Abstract: Ultraviolet absorption measurements were made of anisamide in dichloroacetic acid containing isopropylamine or sulfuric acid. Nmr measurements were made of the diamide N-benzoylglycine-*n*-propylamide in dichloroacetic acid–deuteriochloroform mixtures, and in dichloroacetic acid containing added sulfuric acid. It was concluded that neither amide was protonated in dichloroacetic acid solution, and that as a consequence, polypeptides like poly-γ-benzyl-L-glutamate exist in the coil conformation in dichloroacetic acid because of strong solvation rather than protonation. Optical titrations of poly-γ-benzyl-L-glutamate were carried out in mixtures of dichloroacetic acid with a number of cosolvents which brought about coil-to-helix transitions. The order of decreasing efficiency of these cosolvents in effecting the transitions was: triethylamine > acetic acid ≃ methanol > water ≃ nitrobenzene > nitroethane > formic acid > monochloroacetic acid ≃ cyanoacetic acid ≃ 1,2-dichloroethane > carbon tetrachloride. The hypothesis was made that this order represented the abilities of the various donors to form hydrogen bonds with either dichloroacetic acid monomeric molecules or with the non-hydrogen-bonded terminal carboxyl group of a chain polymer of the solvent. In either event a shift in solvent polymer–dimer–monomer proportions would take place, reducing the concentration of the chain polymer, and reducing the solvation of the peptide groups of the polymer.

This paper is concerned with the acid–base chemistry of the amide group in dichloroacetic acid as solvent, and with the helix–coil transitions of two poly-α-amino acid esters in various solvent mixtures containing dichloroacetic acid (DCA) as one component. Other liquid carboxylic acids, like formic acid (HCOOH), acetic acid (HOAc), and trifluoracetic acid (TFA), alone or in mixtures have also been used as solvents for studying helix–coil transitions. Since one implied conclusion of our present results is that each polymer and each solvent must be examined separately, some reference will be made to the basic properties of amides and polyamides in each of these solvents, as far as they are known.

Formic acid (HCOOH) is the only liquid of this group with a high dielectric constant (56.1 at 25°).[2] The constant of HOAc is 6.18 at 20°,[3] that of TFA is 8.42 at 20°,[3] and that of DCA is 7.8 at 60°.[4] Accordingly acid–base reactions in DCA and in TFA will undoubtedly be complicated by extensive ion-pair formation and possibly by further aggregation, of the kinds already known to exist in

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acetic acid solution.[5] In addition, as in acetic acid relative base strengths will depend on the strong acid which is used.

From their dissociation constants in water one would expect that the order of increasing acidity of these solvents would be: HOAc < HCOOH < DCA < TFA.[6] A quantitative comparison of the acidities of the neat liquids in terms, for example, of the H_0 function is probably inadvisable because the behavior of the aniline derivatives which make up a large part of the successful H_0 indicators may be quite different from that of amides. Certainly amides acting as bases in water–strong acid mixtures are very different from the aniline bases,[7,8] and there is some indication that they behave differently in acetic acid solution as well.[9]

Bruckenstein and Kolthoff evaluated the base strength of urea in acetic acid: $pK_A = 10.24$ (compared to 6.10 for the strong base pyridine).[10] Higuchi and Connors found that the formation constant of urea perchlorate was eight times greater than that of acetamide perchlorate (in acetic acid).[11] It is therefore safe to assume that acetamide and similar compounds (which may serve as models for peptides) are weakly protonated in this solvent.

The behavior of amides as bases in formic acid is less clear. In this medium H_2SO_4 is a strong acid, and urea is a moderately strong base.[12] Its apparent pK, from potentiometric titrations, is 1.75.[13] Acetanilide is about 13% protonated in 0.08 M solution, from indicator measurements.[14] In acetic acid acetanilide is a weaker base than acetamide, which in turn is weaker than urea with respect to $HClO_4$.[11] If the same order of base strength is found in formic acid, one would expect that acetamide or N-alkylacetamides would be at least partly protonated in formic acid. However, while Chao, *et al.*,[15] did find an increase in conductivity on adding some simple amides to formic acid they observed no formate ion bands in the ir spectra of these solutions. They concluded that there was hydrogen bonding between the amides and solvent molecules without protonation.

Very little has been reported about acid–base reactions in DCA as solvent. The potentiometric titration curves of urea and of acetanilide (*vs.* perchloric acid) are close to that of sodium dichloroacetate.[16] If the relative order of base strengths is the same in DCA as in acetic acid, this means that acetamide and other amides are strong bases in this medium with respect to perchloric acid. However, this does not necessarily mean that they are protonated by the solvent.

TFA as a solvent for amides has been examined by nmr spectroscopy[17] and by means of near-ir spectroscopy and measurements of conductivity and density.[18-20] It has been concluded that simple amides like N-methylacetamide are protonated in this medium.

Not much is known about the behavior in these four acid solvents of solutes with two or more neighboring basic groups. There is a paucity of data even about the behavior of diamines, so that it is difficult to assess the possibly opposing effects of low dielectric constants and ion-pair formation (in HOAc, DCA, and TFA) on the ratio of successive dissociation constants of a diacid base. Hall was unable to obtain satisfactory titration curves in HOAc with a number of diamines.[21] In formic acid "titration curves for diprotic bases have never been observed."[22] Nothing has been reported, to our knowledge, about the behavior of diamines in DCA or TFA. With respect to polyamides, Schaefgen and Trivisonno, using a competitive indicator in formic acid, concluded that various poly-ε-caprolactams were protonated to some extent, although to a much smaller extent than ε-caprolactam itself.[23] Although the competitive indicator method does not in itself prove that protonation took place, they also reported that the viscosity–concentration curves of the polymers resembled those of polyelectrolytes in water. Lastly, Bovey and Tiers studied the nmr spectra, among others, of diglycine, triglycine, and tetraglycine in TFA.[24] They found practically no change in the position of the protons of the terminal NH_3^+ groups and of the CH_2 groups of these compounds, and only a small change in the CONH proton position. In all probability the amino group is the only one which exists as a cation. However this does not mean that TFA is incapable of protonating dipeptides or polypeptides. The concentration of peptide was about 3 M in each case, which meant that there was a very high concentration of a strongly basic group (either the free amino or the free carboxylate or both) capable of very markedly reducing the acidity of the solvent by virtually complete reaction with it.

The present paper describes the acid–base behavior of two simple amides and a simple diamide in DCA. The simple amides are anisamide, whose uv absorption band is not obscured by solvent light absorption, and benzamide, whose protonation was studied by nmr. The diamide N-benzoylglycine-*n*-propylamide was examined by nmr because of solvent interference with uv measurements. The helix–coil transitions of poly-γ-benzyl-L-glutamate (PBLG) were then studied in a number of mixed solvents containing DCA in order to arrive at a qualitative mechanism for the effect of the solvent on the transition, in the light of the results obtained with the simple amides.

Experimental Section

Chemicals. Benzamide and anisamide were prepared from the acid or the acid chloride (Carlo Erba, R.P.) and were repeatedly recrystallized from water, mp 126.5–127 and 163–164°, respectively.

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N-Benzoylglycine-*n*-propylamide was kindly supplied by Dr. A. Fontana of this institute. It was prepared from the 4-nitrophenyl ester of N-benzoylglycine and *n*-propylamine in tetrahydrofuran. The ester was prepared from N-benzoylglycine and 4-nitrophenol in dimethylformamide.

Anal. Calcd for $C_{12}H_{16}N_2O_2$: C, 65.28; H, 7.32; N, 12.68. Found: C, 65.13; H, 7.34; N, 12.79.

TFA, DCA, and HOAc were the highest obtainable purity, and redistilled either *in vacuo* or at ambient pressure, and a middle cut was taken. HCOOH (Merck puriss.) was distilled under high vacuum at ambient temperature; a middle cut was taken and kept at −25° until used. Monochloroacetic acid (MCA) (Merck puriss.) and cyanoacetic acid (CNA) (Schuchardt Commercial Grade) were recrystallized three times from benzene. Methanesulfonic acid (Fluka puriss.), free of sulfate, was redistilled several times *in vacuo*, titer 99.0%. Sulfuric acid (99.7%) was made from concentrated H_2SO_4 (Merck puriss.) and fuming H_2SO_4 (Carlo Erba, R.P.) by the "fair and foggy" method.[25] Triethylamine (Et_3N) (Carlo Erba, R.P.) was kept over potassium metal and distilled; a middle cut (bp 89–90° (760 mm)) was taken. CCl_4 (Carlo Erba, R.P.) was redistilled. Nitroethane ($EtNO_2$) (Carlo Erba, R.P.) and nitrobenzene ($C_6H_5NO_2$) (Carlo Erba, R.P.) were redistilled. 1,2-Dichloroethane (DCE), (Carlo Erba, R.P.) was redistilled, bp 84° (760 mm). Deuteriochloroform ($CDCl_3$) (Fluka puriss.) was used as received. Poly-γ-ethyl-L-glutamate (PELG) is sample G-2 of a group of polymers which have already been described.[26] Its molecular weight in trifluoroethanol is 67,100, determined by light scattering. Poly-γ-benzyl-L-glutamate (PBLG) was prepared according to a previous article,[27] except that *n*-butylamine was used as the initiator, and the final distillation of the dioxane was made from solvent containing an excess of sodium naphthalene. The molecular weight of the present sample is 82,500, determined from viscosity. All other materials used were of high purity.

Measurements. The uv spectrum of anisamide was obtained on a Perkin-Elmer-Hitachi 124 instrument, using 1-mm quartz cells. Nmr measurements of N-benzoylglycine-*n*-propylamide were made on the Perkin-Elmer R-12 60-Mc instrument (operating temperature 37°). Tetramethylsilane (TMS) was added to each sample tube as a second reference. The primary reference for all measurements was the middle band of the proton triplet of the methyl group of the propyl radical. A preliminary spectrum of each solution was run on a 10-ppm scale. The scale was expanded to 5 ppm, and the spectrum of each half was recorded at least three times. When necessary the scale was shifted so that the CH_2 group of the glycine residue was recorded together with the reference CH_3 (in one spectrum) and the phenyl complex band (in the other spectrum). The shifts were measured from the methyl peak to the center of the glycyl CH_2 doublet and to the most prominent peak of the phenyl complex. The standard deviation of almost all groups of measurements was ±0.01 ppm. Each run was conducted on 25.0 mg of diamide in 0.5 ml of solvent, corresponding to 0.228 *M*. $CDCl_3$–DCA solutions were prepared by weight. A 1 *M* H_2SO_4 solution in DCA was prepared, and aliquots were added to known volumes of DCA, assuming volume additivity. Some measurements were made (of the phenyl bands) on a 100-Hz expanded scale without an internal reference. Optical rotatory measurements were made at 578 mμ on a Perkin-Elmer Model 141 polarimeter in a standard (10 cm) cell which was maintained at 25.0°. When DCA was the principal cosolvent, the weighed polymer samples were dissolved in DCA, and the other solvent was added. All solvent mixtures were made up by weight, while the polymer concentration was on a volume basis. The average polymer concentration was 0.3 g/100 ml. The b_0 values were calculated in the usual manner from ORD measurements made at five wavelengths between 365 and 589 mμ, assuming λ_0 212 mμ in the Moffitt–Yang equation. The b_0 values for the coil were calculated from a Drude plot.

Results and Discussion

A. Acid–Base Reactions of an Amide and a Diamide in DCA. Figure 1 shows the uv absorption spectrum of anisamide (5.0×10^{-4} *M*) in DCA, in 0.012 *M* isopropylamine, and in 0.009 *M* H_2SO_4. It was not possible to make measurements much below 265 mμ even in 0.1-mm cells because of strong solvent absorption. In water the amide has an absorption maximum at 253 mμ, with a molecular absorbancy index of 14,800. The peak of the protonated form is found at 282 mμ in 59% H_2SO_4, with an index of 16,900.[28] The figure shows that the amide in DCA has a peak at 268 mμ with an index of 11,200, which changes only slightly in isopropylamine solution, but shifts to 292 mμ with an index of 17,600 in 0.009 *M* H_2SO_4. The magnitude of this shift and the large increase in index lead to the conclusion that the sulfuric acid has protonated the amide. The question is whether it is protonated to a marked extent in DCA solution, since there is a shift in the maximum from 253 mμ in water to 268 mμ in DCA. From the values of the indexes in DCA and DCA–H_2SO_4 solution one can conclude that there is little protonation of the amide in DCA. Extensive or complete protonation by the H_2SO_4 raised the index to a value well above that in 59% H_2SO_4. The index in DCA is well below that in water. This resembles the behavior of other arylamides in HOAc, in the sense that amides (clearly unprotonated in HOAc) have lower indexes than in water, and the protonated forms have higher indexes than in water.[9] If there were already extensive protonation of anisamide in DCA, the addition of amine (*i.e.*, of strong base) should have produced a large shift in the maximum to lower wavelength, and a large decrease in the index. Since the effect of the added amine was negligible compared to that of H_2SO_4 it is concluded that there is little or no protonation of the amide in DCA. Acetamide[29] and anisamide[30] are both half-protonated in approximately 28% H_2SO_4. This suggests, but does not prove, that a simple alkylamide would exist in DCA largely or entirely as the neutral base.

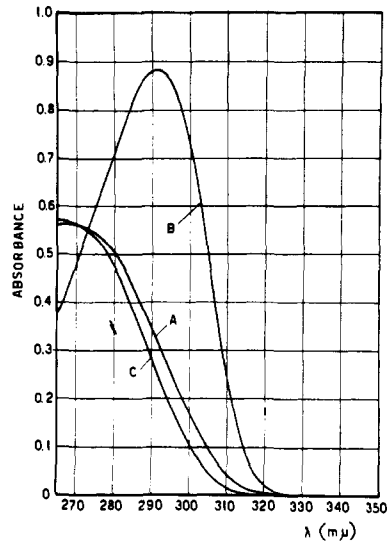

Figure 1. Uv absorption spectrum of 5.0×10^{-4} *M* anisamide in DCA (curve A), in 0.009 *M* H_2SO_4 in DCA (curve B), and in 0.012 *M* isopropylamine in DCA (curve C). Absorbance plotted against wavelength in mμ.

Figure 2 shows a typical nmr spectrum of the model diamide in $CDCl_3$, on a 10-ppm scale. The positions of

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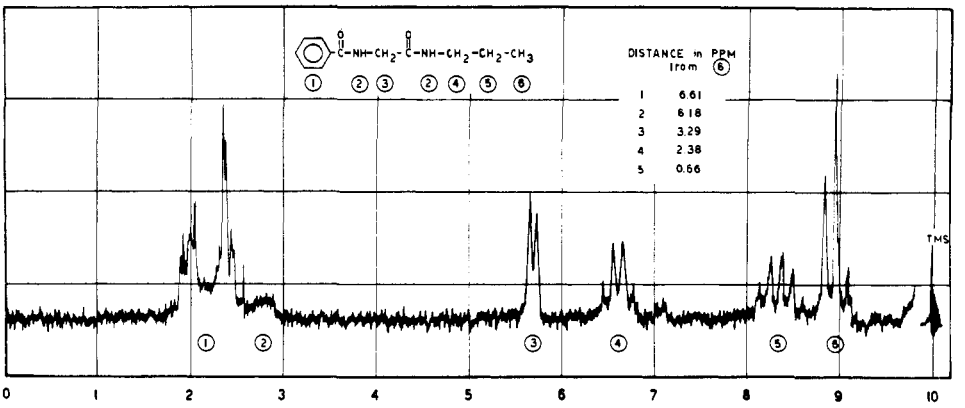

Figure 2. Nmr spectrum of N-benzoylglycine-*n*-propylamide in $CDCl_3$ (10-ppm scale).

the bands of the various groups are also shown. The difference between the position of the TMS signal and that of the central peak of the CH_3 triplet was almost constant; for example, it was 0.922 ppm in $CDCl_3$ and 0.955 ppm in DCA. All shifts were measured from the position of the highest CH_3 band. There are two NH bands. One is visible in the spectrum. The second is merged with the bands of the phenyl protons. This was determined from integration of the areas under all bands.

Table I shows the differences in chemical shift in various mixtures of $CDCl_3$ and DCA between the methyl central peak and the minimum in the glycine CH_2 doublet and between the methyl central peak and the highest peak of the phenyl protons.

Table I. Values of $(\delta_{CH_2} - \delta_{CH_3})^a$ of the Model Diamide in Mixtures of $CDCl_3$ and DCA

DCA, wt %	$\delta_{CH_2} - \delta_{CH_3}$	$\delta_{C_6H_5} - \delta_{CH_3}$
0	3.26	6.59
9	3.33	6.64
28	3.39	6.65
72	3.44	6.65
100	3.47	6.65

[a] In parts per million.

From the table it can be seen that the total relative shift of the methylene protons is 0.21 ppm over the entire range of solvent compositions, and that of the phenyl protons is 0.06 ppm. In each case the major part of the shift is found in relatively dilute acid solutions. This suggests that there is no protonation by the acid over the entire range, or else that the protonation has been completed at an early stage. In view of the effects on these spectra of added sulfuric acid in DCA, we have concluded that neither amide group of the model is protonated in pure DCA. The initial shift in the more dilute DCA solutions is very probably caused by hydrogen bonding to DCA.

The N–H protons show large shifts. In Figure 2 the broad band of one N–H group is centered at approximately 0.5 ppm upfield from the highest peak of the phenyl protons. In 9% DCA this N–H band has virtually disappeared under the phenyl bands, and the hitherto unseen second N–H band appears; it is centered approximately 0.6 ppm downfield from the same phenyl proton peak (this second band corresponds to that of a single proton, from area integration). It is probable that both N–H bands have shifted downfield on addition of DCA, although not necessarily to the same extent. From the general appearance of the curves, it is possible to say that the band which is visible in $CDCl_3$ has shifted downfield by 0.3–0.6 ppm in 9% DCA. In more concentrated acid there is a small (less than 0.1 ppm) shift upfield of the second band. Without attempting a more detailed analysis, one can say that the first large shift is probably due to hydrogen bonding of the N–H proton to DCA. The effective shift will be larger than those of the glycyl CH_2 and the phenyl groups of the diamide, since the latter are affected only indirectly by hydrogen bonding of the components of the amide group.

Figure 3 shows the chemical shifts of the methylene and phenyl protons of the diamide in DCA in the presence of added sulfuric acid. The concentration of the diamide was 0.228 *M* in each solution. The shifts are plotted against the molar ratio of H_2SO_4 to diamide. The most concentrated solution of H_2SO_4 (not shown in the figure) was 3 *M* (molar ratio 13.16). In this solution $\delta_{CH_2} - \delta_{CH_3}$ was 4.06 ppm, and $\delta_{C_6H_5} - \delta_{CH_3}$ was 6.81 ppm.

The slopes of the straight lines plotted in Figure 3 were calculated by least squares for the solutions from molar ratios 0 to 1.013 and from 1.013 to 4.39 for the CH_2 protons, and from 0 to 1.8 for the phenyl protons. The calculated intercepts in pure DCA were 3.48 and 6.68, respectively, in satisfactory agreement with the observed values. The intersection of the two straight lines in the CH_2 plot came at molar ratio 0.97. That is, the alkylamide residue is adding one proton up to this point. The continued shift of these protons in the presence of excess H_2SO_4 is probably a medium effect, similar to that reported by Edward, Leane, and Wang in an nmr study of the protonation of propionamide in H_2SO_4–H_2O mixtures.[31] The shift of the methylene protons in 3 *M* H_2SO_4 (molar ratio 13.16) is 0.08 ppm below the calculated value for the least-squares plot. Since the standard deviation of any point in the curve was ± 0.03 (largely because of the inclusion of the point in 1 *M* acid in the calculation) the value in 3 *M* acid was omitted from the analysis of the data.

The slope of the curve of the phenyl protons in Figure 3 is 0.0168, with a standard deviation of ± 0.014 for any single point. In effect there was very little change in

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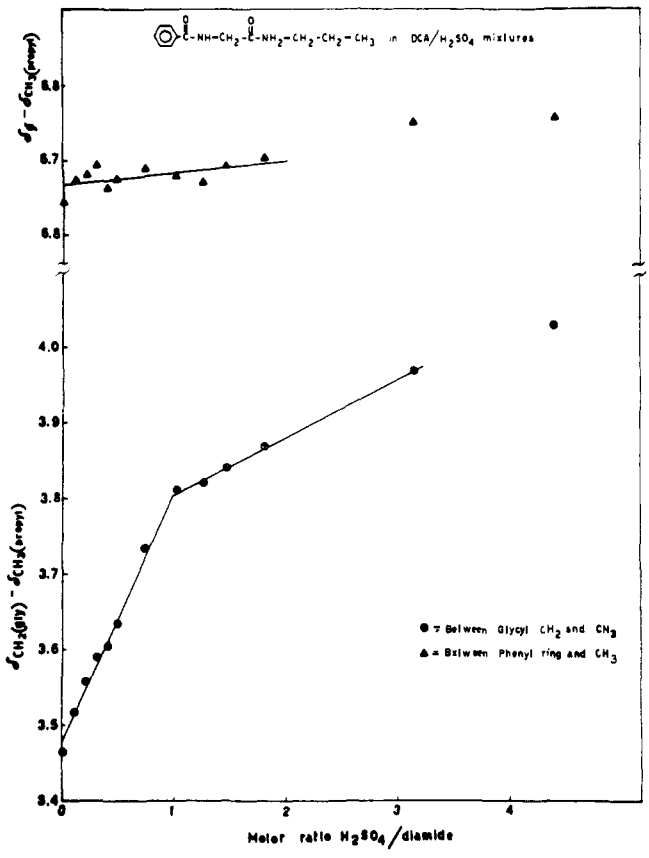

Figure 3. Chemical shifts of glycyl methylene protons and of phenyl protons of N-benzoylglycine-*n*-propylamide in DCA with added H_2SO_4, plotted against the molar ratio of H_2SO_4 to diamide. Principal peak of CH_3 triplet taken as internal reference. The straight lines represent least-squares plots.

$\delta_{C_6H_5}$ out to molar ratio 1.8. This very strongly suggests that the benzamide residue is unprotonated over the entire interval. If the small positive slope is interpreted in terms of a medium effect, the shift in 3 *M* H_2SO_4 cannot be taken as evidence of protonation, since it is 0.08 ppm lower than the extrapolated value. One would expect a relatively large downfield shift in the phenyl protons if the adjacent amide group were protonated, such as was seen for the CH_2 protons.[31] Comparable measurements made on benzamide in DCA and in DCA–H_2SO_4 solutions showed a downfield shift of about 0.18 ppm in the phenyl protons (referred to TMS) at a molar ratio of H_2SO_4 to amide of unity. In addition, the two principal bands of these protons, which are separated by 0.20 ppm in DCA, drew apart to 0.3 ppm on the addition of sulfuric acid. This latter effect is not seen in the model diamide in any of the H_2SO_4 solutions. It can be concluded that the benzamide residue of the diamide is not significantly protonated even in 3 *M* H_2SO_4.

The single N–H band which is on the low-field side of the phenyl proton peaks in DCA shifted further downfield on addition of H_2SO_4, and the second band began to emerge. At a molar ratio of unity both bands were evident. In excess sulfuric acid they shifted further downfield to unequal extents. No analysis of these changes was attempted since the bands were broad and relatively poorly defined.

The behavior of the model diamide in $CDCl_3$, in DCA, and in DCA–H_2SO_4 solutions resembles that of α-methyl

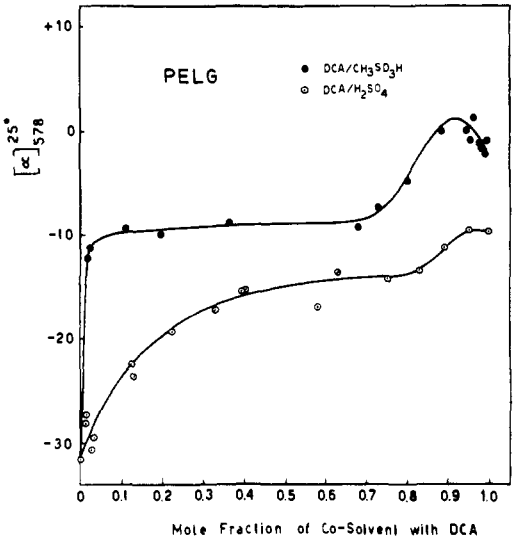

Figure 4. Specific rotation of PELG at 578 mμ plotted against mole fraction of H_2SO_4 (⊙) and of CH_3SO_3H (⊗) in DCA, temperature 25°.

aliphatic ketones in CCl_4, in TFA, and in TFA–H_2SO_4 reported by Peterson.[32] He found that there was a downfield shift of 0.30 ppm in the frequency of α-methyl protons in TFA compared to CCl_4, and a further downfield shift of 0.45 ppm on the addition of H_2SO_4 to TFA solution. He concluded that the first shift was due to solvation by hydrogen bonding to TFA, and that the second shift was due to either protonation or stronger hydrogen bonding by H_2SO_4. We draw the same conclusion for the model diamide in the solvents $CDCl_3$, DCA, and DCA–H_2SO_4. The aliphatic residue is hydrogen bonded in DCA, and is either more strongly hydrogen bonded or is protonated by H_2SO_4. In view of the stoichiometry of the reaction between the amide and H_2SO_4, it is overwhelmingly probable that the added acid is protonating the amide. This integral stoichiometry is in marked contrast to the much more gradual change produced by DCA added to $CDCl_3$ solutions of the model compound. A second conclusion is that it is exceedingly difficult to protonate an amide group (the benzamide residue) in DCA when a vicinal amide has already been protonated. No protonation was evident, even in 3 *M* H_2SO_4. Both of these conclusions are of importance in considering the condition of polyamino acid derivatives like PBLG in DCA. There is no reason to believe that the amide groups of such a polymer will differ markedly in basicity from the glycyl residue of the model diamide. We conclude, therefore, that PBLG exists in the coil conformation in DCA because it is strongly solvated by hydrogen bonding, and not because it is protonated.[33]

B. Helix–Coil Transitions in Mixtures of DCA with Various Cosolvents. Figure 4 shows the effects of added H_2SO_4 and of methanesulfonic acid on the specific rotation at 578 mμ of PELG which was originally dissolved in DCA. The PBLG could not be used for these experiments, designed to investigate possible effects of protonation of a polypeptide in a coil form, because it reacted with added H_2SO_4 to produce a pink solid, probably by splitting off benzyl groups which then polymerized, in the

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fashion of benzyl alcohol.[34] Methanesulfonic acid reacted similarly; a white solid appeared, especially in higher acid concentrations. For this reason PELG was used. The b_0 values of the H_2SO_4 solutions were zero to mole fraction 0.58; those of the methanesulfonic acid solutions were zero to mole fraction 0.11. It was assumed that the polymer was in the coil form in all the solutions studied. The effect of H_2SO_4 may be complex. A precipitate appeared between mole fractions 0.04 and 0.06, but the solutions were clear and stable on either side of this region. The general shapes of the two curves are similar at higher mole fractions, but there is a decided difference between them in more dilute cosolvent solution. The specific rotation of the polymer changes very sharply even in 0.025 mole fraction methanesulfonic acid, and is virtually constant from this point to mole fraction 0.7. In H_2SO_4 the change in specific rotation is much more gradual. From the behavior of the model diamide in DCA–H_2SO_4 mixtures, it is safe to conclude that the polymer is at least partly protonated in the H_2SO_4 solutions, from the lowest mole fractions. Methanesulfonic acid is a weaker acid than H_2SO_4 in acetic acid.[35] We assume this to be true in DCA as well. The very marked effect on the specific rotation of dilute methanesulfonic acid therefore is not due to more efficient protonation. It is reasonable to regard this as a solvation effect (with or without some protonation). Methanesulfonic acid therefore appears to solvate PELG more efficiently than H_2SO_4, and if it is assumed that it displaces DCA from the immediate neighborhood of the coil, it should produce helix-to-coil transitions more efficiently than DCA. Unfortunately, it has a very limited solubility in chlorinated solvents.

Figure 5 shows the specific rotations of PBLG solutions plotted against the mole fractions of three basic cosolvents: Et_3N, CH_3OH, and H_2O. Figure 6 shows similar plots for four diluents: CCl_4, DCE, $EtNO_2$, and $C_6H_5NO_2$. Figure 7 shows curves of the same kind for four acidic cosolvents: HOAc, HCOOH, MCA, and CNA. It was not possible to examine the polymer over the entire range of cosolvent compositions, because it did not dissolve in all of them. The curves of MCA and CNA are incomplete because these acids themselves showed a limited solubility in DCA.

For the nine cosolvents listed in Table II, it is evident that the sigmoid curves in the figures represent coil-to-helix transitions. We have assumed that similar transitions occurred with DCE and with CNA as well. Table III shows the approximate mole fractions of the various cosolvents at which the transition is half-completed, in increasing order.

The order of cosolvent efficiency in producing the coil-to-helix transition is puzzling at first glance. Et_3N is the most efficient agent of the group. If the polymer coil in DCA were actually protonated, one would say that the amine, which is undoubtedly a strong base in this solvent, simply reduced the latter's acidity and in this way brought about the transition. However, acetic acid is remarkably efficient too, much more efficient than water, which is a much stronger base than HOAc in DCA (with respect to H_2SO_4).[36]

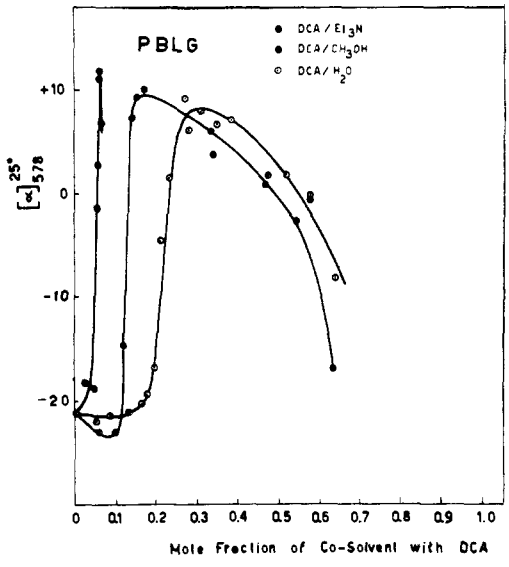

Figure 5. Specific rotation of PBLG at 578 mμ plotted against mole fraction of Et_3N (●), CH_3OH (⊗), and H_2O (⊙), in DCA, temperature 25°.

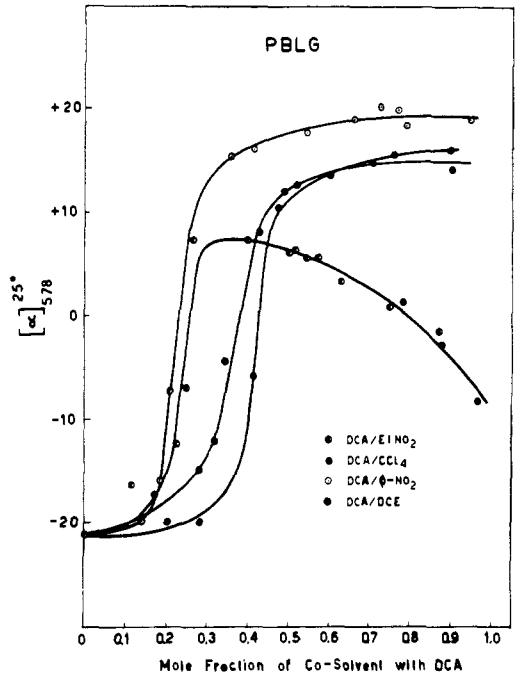

Figure 6. Specific rotation of PBLG at 578 mμ plotted against mole fraction of $EtNO_2$ (◐), CCl_4 (⊗), $C_6H_5NO_2$ (⊙), and DCE (●), temperature 25°.

The pK_A of acetic acid as a base is -6.2.[37] The H_0 is not known, but that of 85.5% DCA in H_2O is -0.19.[38] It is probable that the H_0 of the pure acid will lie between -1 and -2, since that of TFA is -3.03, measured with a nitroaniline derivative.[39] We conclude that acetic acid will not be protonated to any measurable extent in DCA solution. Water is known to be a weak base in formic

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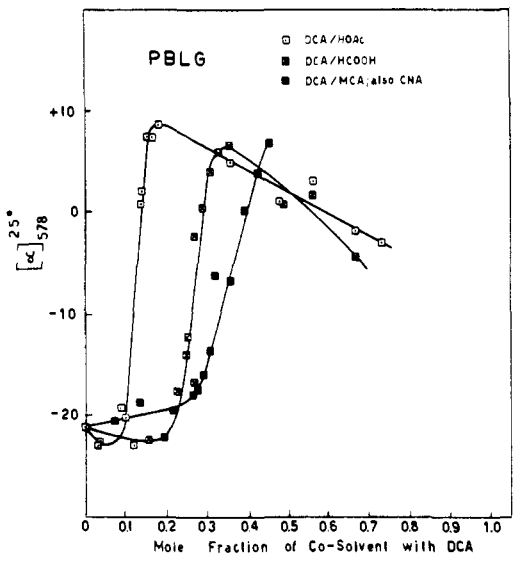

Figure 7. Specific rotation of PBLG at 578 mμ plotted against mole fraction of HOAc (⊡), HCOOH (⊠), MCA and CNA (■), in DCA, temperature 25°.

acid solution,[11] yet it is not much more efficient than the latter in producing the transition. Nitro compounds are very weak bases in sulfuric acid[40] with pK_A values of about -11[41] and are probably very weak bases in DCA, yet the two listed in Table IV are almost efficient as water. However, since the polymer in DCA is not protonated, but is solvated, we hypothesize that the order of efficiency of these helix-forming cosolvents stems from their effects on the solvent structure. We believe that Reeves[42] and Bellamy, *et al.*,[43] are correct in their evaluations of the structures of liquid carboxylic acids. These workers hypothesize that liquid HOAc, HCOOH, DCA, and TFA are not composed of cyclic dimers in the pure liquid phase, but rather consist of monomers, cyclic dimers, other cyclic forms, and open-chain polymers. It will be recalled that crystalline formic acid[44] and crystalline acetic acid[45] consist of infinite hydrogen-bonded chains, rather than closed dimers. We postulate that the effective solvation of the peptide group by DCA is due to hydrogen bonding by the terminal (nonhydrogen-bonded) COOH group in a carboxylic acid chain polymer, a group which will be capable of very strong hydrogen bonding with a suitable acceptor.[42] Dilution of the liquid acid or the addition of various acceptors (conventional bases included) will produce a shift in the distribution of the various states of organization of the liquid acid with an increased formation of dimers and monomers. There is no direct demonstration of this hypothesis for the effects of added substances on the liquid carboxylic acids themselves. However, in CCl_4 solution trichloroacetic acid has been shown to form a heterodimer with HOAc which is more stable than either homogeneous dimer.[46] Trifluoroacetic acid and

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Table II. Values of b_0 of PBLG in Solvent Mixtures

Cosolvent	Mole fraction of cosolvent	b_0
CCl_4	0.204	0
	0.900	−540
$EtNO_2$	0.063	0
	0.262	−327
	0.424	−552
CH_3OH	0.050	0
	0.166	−614
	0.620	−468
Et_3N	0.065	−542
$C_6H_5NO_2$	0.373	−610
H_2O	0.153	0
	0.254	−454
	0.384	−614
	0.633	−584
HOAc	0.025	0
	0.215	−508
	0.482	−573
HCOOH	0.101	0
	0.492	−638
	0.668	−576
MCA	0.015	0
	0.452	−362

Table III. Mole Fraction of Cosolvent at Coil–Helix Transition Midpoint

Cosolvent	Mole fraction	Cosolvent	Mole fraction
Et_3N	0.05	$EtNO_2$	0.25
HOAc	0.13	HCOOH	0.27
CH_3OH	0.13	MCA	0.36
H_2O	0.22	DCE	0.37
$C_6H_5NO_2$	0.22	CCl_4	0.43

acetic acid form mixed dimers in the gas phase which are more stable than either homogeneous dimer, and very much more stable than the heterotrimer formed between water and trifluoroacetic acid.[47] From microwave spectra the conclusion was drawn that trifluoroacetic acid and HOAc form a mixed dimer, and that it is much more stable than the heterodimer formed between trifluoroacetic acid and HCOOH. The formation of such stable heterodimers has been advanced as the reason for the supression of the catalytic effect of trifluoroacetic acid by the addition of HOAc in the chlorination of hydrocarbons in benzene[48] or in CCl_4.[49]

The order of helix-forming efficiency of the cosolvents in Table III is thus explained by the ability of the acidic or basic cosolvent to form mixed dimers or trimers with the unbonded terminal COOH group of an open-chain solvent polymer. An alternative which leads to the same result is the reaction with monomeric DCA to form heterodimers, resulting in a similar shift in the equilibrium of the various solvent species. The most efficient agent in the group would be the dichloroacetate ion (or ion pairs) since a hydrogen bond between a solvent molecule and the solvent base would be stabilized by a charge effect as well. Nitro

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compounds have not been examined in this regard, but Bellamy has cited cases where they form hydrogen bonds with various donors.[50] With respect to the inert diluents—CCl_4 and DCE—the difference between them is not very great. It is probably due to the effect of the diluent on the extent of organization of polymers of DCA in the two cosolvents. Reeves has shown from nmr studies that DCE will stabilize the monomer form of DCA more than CCl_4. This means that DCA will be more "acidic" in CCl_4 than in DCE.

Some of the results we report here are similar to earlier findings by Lotan, *et al.*[51] These workers examined the helix–coil transitions of some water-soluble polypeptides in formic acid–water mixtures and in formic acid–acetic acid mixtures, as well as in formic acid containing added formate salts. We are limiting our present conclusions to solutions of polypeptides like PBLG in DCA. It is possible, as these authors have assumed, that the polymers they studied are protonated in formic acid solution. This is a solvent which needs investigation on its own. However, it is interesting to note from the curves in their paper that acetic acid is more effective than water as a helix-forming cosolvent for formic acid in the case of at least one of their polymers. Stable heterodimers are not limited to the halogenated acetic acids as one partner; it is known that propionic and acetic acids also form stable heterodimers in the gas phase.[52]

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Oxytocin Analogs with Basic Amino Acid Residues in Positions 4 and 5. Synthesis and Pharmacological Properties of [4-Ornithine]- and [5-Ornithine]-oxytocin[1]

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Abstract: [4-Ornithine]-oxytocin, an analog of the neurohypophyseal hormone oxytocin in which the glutamine residue at position 4 is replaced by an ornithine residue, and [5-ornithine]-oxytocin, an analog in which the asparagine residue at position 5 is replaced by an ornithine residue, have been synthesized and assayed for their biological activities. The nonapeptides were synthesized stepwise by the activated ester method of peptide synthesis. The chemical properties and biological potencies of [4-ornithine]-oxytocin were identical regardless of whether the δ-amino function of ornithine had been protected during synthesis by a tosyl or a phthalyl group. Upon bioassay the [4-ornithine]-oxytocin was found to possess 163 ± 5 units/mg of avian vasodepressor activity, 58 ± 1.6 units/mg of rat oxytocic activity, 127 ± 7 units/mg of rabbit milk-ejecting activity, less than 0.1 unit/mg of rat pressor activity, and approximately 0.029 unit/mg of rat antidiuretic activity. The hydroosmotic activity of the analog was 1.60 ± 0.10% of that of crystalline deamino-oxytocin upon assay in the toad bladder system. The corresponding activity values for [5-ornithine]-oxytocin were approximately 0.07, 0.24, 0.19, 0.04, and 0.002 unit/mg and 0.046%, respectively. The striking differences in the potencies between [4-ornithine]-oxytocin and [5-ornithine]-oxytocin add further support to the contention that the biological activities of oxytocin are much less affected by structural alterations in position 4 as compared with position 5. However, dose–response analyses on the toad bladder with [5-ornithine]-oxytocin revealed that its intrinsic activity in inducing transepithelial water movement along an osmotic gradient is the same as that of the natural amphibian hormone, [8-arginine]-vasotocin. Thus it can be concluded that the asparagine residue in position 5 of oxytocin is not essential for the manifestation of intrinsic hydroosmotic activity.

In the course of the systematic study in several laboratories of the relation of chemical structure to biological activity of peptide hormones, the role of the side chains of the glutamine and asparagine residues in positions 4 and 5 of the peptide ring of oxytocin (Figure 1) has been explored. From this work[2-4] it can be concluded

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