L-α-**Aminocaprolactam (I)**, mp 66–68°, $[\alpha]^{25}$D −34.2° (in water), was kindly supplied by Allied Chemical Company (see acknowledgment).

L-**Pyrrolid-2-one-5-carboxylic acid (II)**, mp 154–157°, $[\alpha]^{25}$D −10° (in water), was purchased from Aldrich Chemical Co. and recrystallized from hot water.

1,7,7-Trimethyl-3-azabicyclo[2.2.1]heptan-2-one (III) was prepared from aminocamphonanic acid following the procedure of Noyes and Potter.[25] The aminocamphonanic acid precursor was synthesized from camphoric anhydride as described by Noyes.[24] Purification of the lactam was accomplished by sublimation at 110° (0.5 mm); mp 202–203° (lit.[25] mp 203°); $[\alpha]^{26}$D −59.2° (0.0978 g/cc, ethanol) (lit.[25] $[\alpha]^{29}$D −60.5° (0.1 g/cc, ethanol)].

The nmr spectrum ($CDCl_3$) consisted of a broad band at τ 3.05 (1 H, NH), three singlets at 8.97, 9.03, and 9.12 (9 H, CH_3), an unresolved singlet at 6.67 (1 H, CH), and a number of complex multiplets at 7.9–8.8 (4 H, CH_2).

Compound purity was determined by glpc on a 2 m × 0.25 in. column of DC 200 silicone oil at 173° and a helium pressure of 15 psi in a Perkin-Elmer Model 154D instrument. When a 15-mμ sample (13% chloroform solution) was injected, a single peak was found having a retention time of 14.5 min.

1,7,7-Trimethyl-2-azabicyclo[2.2.1]heptan-3-one (IV) was prepared from aminodihydrocamphonanic acid following the procedure of Noyes and Potter.[25] The aminodihydrocamphonanic acid precursor was synthesized from camphoric anhydride as described by Noyes.[24] Purification of the lactam was accomplished by sublimation at 105° (0.5 mm); mp 187–188° (lit.[25] mp 188–189°); $[\alpha]^{25}$D 70.1° (0.0965 g/cc, ethanol) [lit.[25] $[\alpha]^{30}$D 72.8° (0.1 g/cc, petroleum ether (bp 30–60°))].

The nmr spectrum ($CDCl_3$) consisted of a broad band at τ 3.42 (1 H, NH), three singlets at 8.83, 8.97, and 9.12 (9 H, CH_3), an unresolved singlet at 7.77 (1 H, CH), and a number of complex multiplets at 7.8–8.6 (4 H, CH_2).

An analysis by gas chromatography of an 18-mμ sample (12% chloroform solution) showed a single peak having a retention time of 15.3 min.

Acknowledgment. We want to thank Dr. D. W. Urry of the Institute for Biomedical Research Education and Research Foundation, American Medical Association for supplying us with manuscripts on his lactams prior to publication. We also desire to thank Drs. Robert Fuhrmann and Annmarie Kubanek of the Allied Chemical Company for the sample of L-α-aminocaprolactam.

Protonation of Peptides. I. The Behavior of a Model Diamide and of Poly-γ-ethyl-L-glutamate in Strong Acid–Water Mixtures

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Contribution from the Institute of Organic Chemistry, University of Padua, Padua, Italy. *Received August 5, 1968*

Abstract: Poly-γ-ethyl-L-glutamate dissolves in concentrated sulfuric acid and in methanesulfonic acid. Circular dichroism measurements showed that it was in the coil form in both solvents. The addition of water produced the α-helical form of the polymer. At room temperature the transition took place in 57–62% sulfuric acid by weight, and in 75.5–78% methanesulfonic acid. The diamide N-benzoylglycyl-*n*-propylamide was examined spectrophotometrically in aqueous mixtures of the two acids in order to determine the region in which the second protonation occurred. Assuming that the glycylamide group was protonated first, the benzamide portion was half-protonated in 74% sulfuric acid. Protonation was not complete in 99.0% methanesulfonic acid, and it was tentatively concluded that the second protonation was much less than half-completed in 75% acid. From these results it was hypothesized that in sulfuric acid–water mixtures no more than 50% of the amide groups of the polymer helical residues were protonated early in the transition, and that the percentage might be much less in methanesulfonic acid–water solutions in which the transition started. The cooperative character of the helix–coil transition was evident from the relatively small change in acidity which was required to produce it, in contrast to the range of acidity which is required for extensive amide protonation.

The connection between the helix–coil transition of polyamino acid derivatives and the protonation of the amide groups of these compounds is not clear at the present time. Klotz and his coworkers have maintained that in trifluoroacetic acid–chlorinated hydrocarbon mixtures there is an initial protonation of amide groups at low acid concentration without disruption of the helix, followed (at much higher acid concentration) by an additional reaction between the trifluoroacetate–amide ion pairs and trifluoroacetic acid. The latter reaction, which forms homoconjugate ion pairs, is presumably responsible for the disruption of the helix. From the examination of simple models and of polymers they have shown some evidence for this thesis by near-infrared spectroscopic studies, and density, nmr, and conductivity measurements.[2-4] Stewart, *et al.*, compared the nmr spectra of poly-L-alanine and poly-DL-alanine[5] with those of the simple models N-methylacetamide and N,N-dimethylacetamide[6] in trifluoroacetic acid–chloroform mixtures. They concluded that whereas there is

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[1] On leave of absence from the Polytechnic Institute of Brooklyn, Brooklyn, N. Y.; author to whom inquiries should be addressed.

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[6] W. E. Stewart, L. Mandelkern, and R. E. Glick, *ibid.*, **6**, 150 (1967).

protonation of the simple amides this does not occur with the polymers: the latter are probably solvated by the acid by hydrogen bonding, rupturing the intramolecular bonds in the helix. Quadrifoglio and Urry found that in trifluoroacetic acid–chloroform mixtures (to 10 wt % acid) and in dichloroacetic acid–chloroform mixtures (to 30 wt % acid) there was no change in the amplitude of the CD minimum at 222 mμ of the α-helix of poly-γ-benzyl-L-glutamate, ruling out protonation in these regions.[7] Veis and his coworkers found conductivity increases in formic acid solutions of various simple amides, but no indication of the presence of formate ion in the ir spectra and no indication of amide group protonation in the near ir spectra of these solutions.[8] They concluded that formic acid solvated the amides by hydrogen bonding, forming aggregates which gave conducting solutions, but did not directly protonate the amide bond. They subsequently applied these findings to the mutarotation of solutions of poly-L-proline and other polymers in formic acid.[9] On the other hand, Lotan, *et al.*, in a study of the behavior of poly-N^5-(3-hydroxypropyl)-L-glutamine and similar polymers dissolved in mixtures of formic acid with added formates, water, or acetic acid, attributed the helix-breaking ability of pure formic acid to its ability to protonate amide groups.[10]

The question of polyamide protonation is clearly a complicated one, and may well have different answers for different compounds in different media. The following areas are involved, at least in part: the acid–base chemistry of the amide group in water-strong acid mixtures, its chemistry in solvents like formic acid and dichloro- and trifluoroacetic acids (with varying proportions of different cosolvents), the difficulty in protonating an amide group when its nearest neighbor is already protonated, (and this in a variety of solvents), and the increased resistance to protonation caused by the intramolecular hydrogen bonding in the helix. In some of these areas very little is known. The present paper is concerned with amide and polyamide acid–base chemistry in water–strong acid mixtures.

The protonation of simple amides has been extensively studied in sulfuric acid–water mixtures.[11] Two difficulties have been apparent for some time. First, there are spectroscopic complications in the evaluation of the ratio of the concentrations of the protonated and unprotonated forms, because either spectrum may show strong medium effects. The uv spectrum of the protonated form and, on occasion, that of the free base may shift with increasing acid concentration. In general, no isosbestic point is observed.[12] A number of empirical methods have been developed to correct for these shifts.[13,14] Secondly, amides, like a number of other types of neutral bases, do not show the same behavior as the substituted primary anilines which were originally used by Hammett and Deyrup to establish the H_0 acidity scale.[15] A necessary (but not sufficient[16]) requirement for the evaluation of the pK_a of a weak base is that the logarithm of the ratio of conjugate acid concentration to base concentration must be a linear function of the $-H_0$ values of the various strong acid–water mixtures, with a slope of unity (eq 1). Amides

$$pK_A = H_0 + \log([BH^+]/[B]) \qquad (1)$$

show striking differences from the primary anilines in this regard, with slopes which range from 0.4 to 0.8 for different compounds.[17,18] As a consequence, the pK_A values of amides which are based on the H_0 scale, are not true equilibrium constants, but are numbers which represent the H_0 values of acid solutions in which half-protonation of the bases has taken place.[19] There is now in existence a separate acidity scale for amides—the H_A scale—based on primary aromatic amides.[20] It covers the range from 16 to 82% H_2SO_4. The indicators and a large group of other primary amides show the required unit slope on the H_A scale.[21] This scale changes much more slowly with acid concentration than does the H_0 scale in more acidic solutions. Thus, 80% H_2SO_4 is at least two logarithmic units less acid toward these amides than to primary anilines.

The effect of an already protonated group on the basicities of vicinal unprotonated amides is not known at the present time. In a poly-α-amino acid chain one would expect that the protonation of a single amide group would make that of either neighboring amide more difficult, if only because of the effect of the positive charge, and the small number of atoms between the groups. There may be a polyelectrolyte effect as well (the effect of the charge density of the partly protonated chain). No suitable simple models have been successfully examined in this regard. Goldfarb, *et al.*, reported pK_A values of a number of simple alkyl amides and of dipeptides, tripeptides, and higher peptides in sulfuric acid–water mixtures, basing their calculations on uv absorption spectra.[22,23] Plots of the extinction coefficients at 205 mμ against the H_0 values of the solutions were found to yield sigmoid curves. It was necessary to assume two equilibria and to calculate two pK values for each base in order to fit the curves. However, since this was the case for acetamide and other monoamides, and since from freezing-point measurements it was known that monobasic amides add only one proton in absolute sulfuric acid,[24] the second equilibrium was attributed to solvation without protonation. No account was taken of shifts in the spectra with changing acid concentration. In addition, Edward and Wang pointed out the implicit assumption which was made in calculating the pK_A

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values: that the amide group behaved like a Hammett base.[18] These latter workers attempted to correct the calculations by placing the true value of the pK between the two values reported for each compound. On this basis they concluded that the amide group of glycinamide had pK_A = −4.91 (the amino group being protonated first). This corresponds to a sulfuric acid content of about 63% when half the amide groups are protonated. However, the van't Hoff *i* factor of the same compound in 99.9% H_2SO_4 is 2.7.[25] This means that 70% of the compound exists in the form BH_2^{2+}. It is not reasonable for a base to show 50% protonation in 63% H_2SO_4 and 70% protonation in 99.9% H_2SO_4. The H_0 values of the two solutions are −4.9 and −10.6, respectively.[26] If one extrapolates the H_A scale to 100% H_2SO_4, there is a difference of at least two units between the acidities of the two solutions. For this reason we believe that a more detailed examination is necessary of the spectra of the various compounds for which pK_A values have been reported before conclusions can be drawn about the protonation of vicinal amide groups. In view of the complexities in the spectra of aliphatic amides,[18] it may be preferable to find another method of analysis.

In their paper O'Brien and Niemann also reported that the *i* factor of the diamide benzoylglycinamide was 2.9 in 99.9% H_2SO_4.[25] Since acetamide is half-protonated in 28% H_2SO_4,[27] and benzamide is half-protonated in 35% H_2SO_4,[28] it is reasonable to conclude that the glycinamide portion of the diamide is completely protonated, and that 90% of the benzamide groups are protonated. However, the uncertainty in the *i* factor is of the order ±0.1. Hence from this datum nothing can be concluded about the difficulty of protonation of neighboring amide groups, since the compound in question may be virtually completely protonated in H_2SO_4. Accordingly in the present research, a similar compound, N-benzoylglycyl-*n*-propylamide, was prepared, and its absorption spectrum was studied in sulfuric acid–water mixtures and in mixtures of methanesulfonic acid and water. At the same time the polymer poly-γ-ethyl-L-glutamate (PELG), which dissolves in both concentrated sulfuric acid and in methanesulfonic acid, was examined by circular dichroism in mixtures of either acid with water in order to locate the region of the helix–coil transition in these strongly acid media.

Experimental Section

1. **Materials. A. Chemicals.** Methanesulfonic acid (Fluka puriss.) was distilled several times *in vacuo* (<0.01 mm), a middle cut being retained. The final product contained no free sulfate. Titration showed an acid content of 99.0% by weight. Concentrated sulfuric acid (Merck puriss.) was used directly. Its titer was 95.7% by weight. Sulfuric acid (99.7%) was prepared by the "fair and foggy" method[29] from the Merck product and fuming sulfuric acid (Carlo Erba, R.P.). All acid mixtures were analyzed by potentiometric titration. Dimethylformamide was dried over P_2O_5 and fractionally distilled under reduced pressure, retaining a middle cut. N-Benzoylglycyl-*n*-propylamide was kindly supplied by Dr. A. Fontana of this institute. *Anal.* Calcd for $C_{12}H_{16}N_2O_2$: C, 65.28; H, 7.32; N, 12.69. Found: C, 65.13; H, 7.34; N, 12.79. Poly-γ-ethyl-L-glutamate (PELG) is sample G-2 of a group of polymers whose preparation and properties have already been described.[30] Its molecular weight, in trifluoroethanol, determined by light scattering, is 67,100, and is 71,100 from viscosity measurements in dichloroacetic acid. Other materials were of the highest obtainable purity.

B. Preparation of PELG Solutions in Acid Mixtures. A known quantity of polymer was introduced into a 25-ml volumetric flask. A weighed quantity of pure or concentrated acid was added. After the polymer had dissolved the desired amount of water was added to the cooled solution. After it came to room temperature, dilution was made to the mark by adding a separately prepared solvent mixture of the same composition as that in the flask. An alternative procedure consisted of adding weighed quantities of water to cooled solutions of the polymer in concentrated or pure acid to the extent needed to bring the final volume of the mixture to 25 ml at room temperature. The total interval of time between addition of pure acid to the polymer and the completion of the CD spectrum was less than 1 hr in all cases. In a control experiment, 250 mg of polymer was dissolved in 50 ml of concentrated H_2SO_4, and stood at room temperature for 16 hr. The polymer was then precipitated in ice and water, filtered, washed well with water, dried *in vacuo* at 50°, dissolved in methylene chloride, reprecipitated with ether, washed, and dried. It was then analyzed for free carboxyl groups by the method of Sela and Berger.[31] The analysis showed that there was approximately one free carboxyl group in each four amino acid residues. Another control was dissolved in DMF and precipitated in a sulfuric acid–water mixture to be certain that the acidity was not due to entrapped H_2SO_4 in the polymers. The intrinsic viscosity of the sample recovered from the concentrated H_4SO_4 was measured in dichloroacetic acid. It was virtually the same as that of the starting material, from which it was concluded that even the prolonged treatment with H_2SO_4 did not break amide bonds in the backbone, but rather hydrolyzed some of the ester groups. In the light of these results, because of the much shorter period of time required for sample preparation and CD measurements, and in view of the known stability of ethyl acetate in pure sulfuric acid (for short periods of time),[32] it appears safe to conclude that the polymer solutions whose spectra are reported were not seriously decomposed.

2. **Measurements.** CD spectra were recorded on a Roussel-Jouan 185 Model II Dichrograph, using 1-mm fused quartz cells. Uv absorption measurements were made on a Perkin-Elmer-Hitachi EPS-3 spectrophotometer, and on a Perkin-Elmer-Hitachi 124 instrument, using 1-mm quartz cells. The results of CD spectra are generally reported in terms of Δε, the so-called molecular extinction coefficient, rather than the molecular absorbancy index. For consistency's sake, all spectrophotometric results in this paper are reported in terms of ε. Potentiometric titrations were performed with a Metrohm Model E 388 potentiometer with a Metrohm UX combined electrode.

Results and Discussion

A. The Protonation of the Diamide. Table I shows the spectrophotometric behavior of the model diamide in the two acid systems. The concentration of the amide was of the order of 3.6×10^{-4} *M*. The table lists the locations of the two maxima in each curve, their molecular extinction coefficients, and the coefficients at 228 and 258 mμ wavelengths which were selected for certain calculations.

In both groups of solutions the first maximum, near 195 mμ, is undoubtedly associated with the glycylamide part of the molecule. It shows relatively little change in position with increasing acid concentration, but there are marked changes in the extinction coefficient. A plot of ε_1 at 195 mμ against per cent H_2SO_4, $-H_0$, or $-H_A$ shows a decrease to 40% H_2SO_4 (or its equivalent), zero

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Table I. Spectrophotometric Behavior of the Model Diamide in Acid–Water Mixtures

Acid, wt %	λ_1 max, mμ	ε_1 max	λ_2 max, mμ	ε_2 max	ε_{228}	ε_{258}
A. Sulfuric Acid–Water Mixtures						
0	193.5	37,500	228	11,400	11,400	1,303
23.8	196	34,450	229.5	11,060	10,870	1,414
40.2	195	31,620	232	11,070	10,650	1,887
56.5	195	31,885	236.5	11,180	9,237	5,286
67.9	194.5	26,790	243	11,080	6,560	7,159
70.5	194.5	26,637	245	11,605	6,051	8,345
73.8	195	24,700	248	11,840	5,368	9,823
74.8	195	24,453	249	12,610	4,532	10,640
77.0	195	24,840	252	13,440	4,408	12,060
83.0	195	22,840	255	14,040	3,135	13,450
95.7	195	21,520	258	15,250	2,206	15,250
99.7	195	19,700	258	15,390	1,850	15,390
B. Methanesulfonic Acid Solutions						
53.4	195	30,180	232	11,267	10,980	1,594
74.9	195	28,828	236	11,016	9,520	3,060
84.8	195.5	25,496	242	11,428	7,565	6,010
89.5	195.5	23,961	245	12,159	6,330	8,250
93.1	196	22,460	248	13,037	5,340	9,840
95.0	197	21,243	249	13,094	4,620	10,600
96.6	197	20,944	250	13,414	4,345	11,310
98.8 (99.0)	197	19,788	252	14,270	3,650	12,730

slope from 40 to 56% H_2SO_4, and a continuing decrease to 99.7% acid. If the changes are ascribed to protonation alone, this would require two equilibria over the acid range for the alkylamide residue alone.

This is the type of curve found by Goldfarb and his coworkers[22,23] and their interpretation—which, among other things, ignored spectral changes due to solvation—was correctly criticized by Edward and Wang.[18] We conclude that part of the decrease in the extinction coefficient is due to a solvent effect; this is evident in the more acid solutions, since, unlike the benzamide residue, the coefficient of the glycinamide portion does not approach a limit in 95.7% acid. Since benzamide[21] is a weaker base than acetamide,[27] one would expect the aliphatic residue of the model to be completely protonated before the complete protonation of the aromatic portion.

The second band, which starts at 228 mμ, undergoes progressive shifts with increasing acid concentration. Its final position is at 258 mμ, representing a total change of 30 mμ. At the same time the extinction coefficient at the maximum increases, particularly beyond 70% H_2SO_4 and beyond 85% methanesulfonic acid. Figure 1 shows a set of five spectra in sulfuric acid solutions. Plots of the molecular extinction coefficients against wavelength for all the solutions of Table I did not pass through an isosbestic point. It can be concluded that the shifts in the second band (and perhaps changes in the coefficients) are due to both protonation and medium effects. The diamide shows similar behavior in the methanesulfonic acid solutions.

The spectra in both sets of solutions were analyzed by the method of Davis and Geissman,[14] at 228 mμ (the band maximum wavelength in water) and at 258 mμ (the corresponding wavelength in H_2SO_4). The molecular extinction coefficients are shown in Table I. The differences ($\varepsilon_{258} - \varepsilon_{228}$) were plotted against the $-H_0$ values of the sulfuric acid solutions[26] and of the methanesulfonic acid mixtures.[33] The required acidity values were obtained by linear interpolation. The curves are shown in Figure 2.

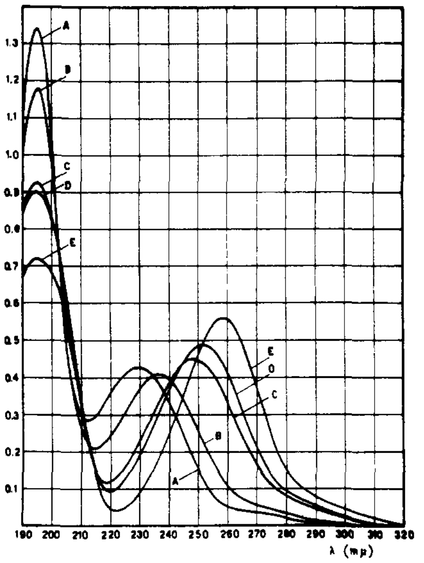

Figure 1. Uv spectra of the diamide model compound N-benzoylglycine-*n*-propylamide in H_2SO_4–H_2O mixtures. The spectra have been recorded in 1-mm cells: A, 3.890×10^{-4} *M* in 23.8% H_2SO_4; B, 3.672×10^{-4} *M* in 56.5% H_2SO_4; C, 3.726×10^{-4} *M* in 73.8% H_2SO_4; D, 3.636×10^{-4} *M* in 77.0% H_2SO_4; E, 3.672×10^{-4} *M* in 99.7% H_2SO_4.

In the sulfuric acid system the inversion point of the sigmoid curve (or at least the region of maximum slope) falls between $-H_0 = 6.14$ and $-H_0 = 6.275$. This corresponds to a solution whose composition is 74.3% H_2SO_4 by weight. The benzamide residue is assumed to be half-protonated in this solution. In turn, the H_A of this solution is -4.1, and if this acidity scale applies to the second protonation step of diamides, this also represents the pK_A of the aromatic portion. Calculations of the diprotonated fractions of the model compound from the extinction coefficients of the various species in water, in H_2SO_4, and in the intermediate mix-

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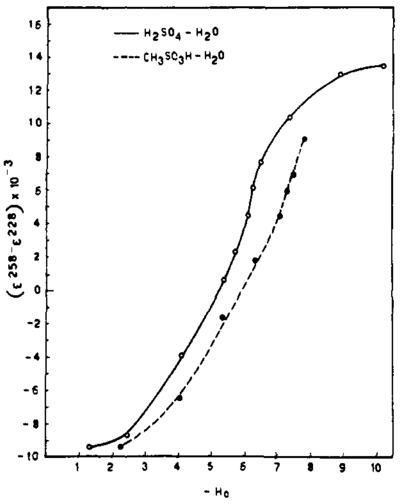

Figure 2. Analysis of the uv spectra by the method of Davis and Geissman.[14] $\varepsilon_{258} - \varepsilon_{228}$ values plotted *vs.* $-H_0$ values of the sulfuric acid solutions and of the methanesulfonic acid mixtures.

tures produced contradictory results. This probably occurred because some of the coefficients changed to some extent in the different solutions. The Davis and Geissman method rests on the validity of Beer's law, and as they pointed out it is much more reasonable to make calculations over a limited acid composition range. Accordingly, the assumption was made that in 74.3% H_2SO_4 the diprotonated fraction was 0.5. This is a reasonable assumption because benzamide and N-methylbenzamide are half-protonated in 35% H_2SO_4, and the protonation is essentially complete in 59% H_2SO_4.[28] If the benzamide residue of the diamide is half-protonated in 74.3% acid, this higher acid concentration is understandable only on the basis that the glycinamide portion was virtually completely protonated in a less acid solution. The extinction coefficient of the diprotonated species at 258 mμ was taken as 15,390, and its coefficient at 228 mμ was taken as 1850 (Table I, values in 99.7% H_2SO_4). Equation 2 was then used[14]

$$\begin{aligned}\varepsilon_{258} - \varepsilon_{228} &= [(\varepsilon u)_{258} - (\varepsilon u)_{228}] \\ &+ [(\varepsilon i)_{258} - (\varepsilon u)_{258} + (\varepsilon u)_{228} - (\varepsilon i)_{258}]I \quad (2)\end{aligned}$$

in which $(\varepsilon u)_{258}$ is the coefficient of the un-ionized form at 258 mμ, ε_{228} is that at 228 mμ, $(\varepsilon i)_{258}$ and $(\varepsilon i)_{228}$ are the corresponding coefficients of the diprotonated form, ε_{258} and ε_{228} are the experimental values from Table I, and I is the fraction which is diprotonated. With these various assumptions a value of −3040 was found for the term $[(\varepsilon u)_{258} - (\varepsilon u)_{228}]$. This value is uncomfortably small in the light of the data in Table I, but it is probable that there are compensating effects in the values of the other coefficients.

The calculated values of the logarithm of the ratio of concentrations of the diprotonated to monoprotonated forms were plotted against $-H_A$ from 57 to 83% H_2SO_4, and gave a straight line. A least-squares analysis showed the slope to be 1.01 ± 0.13, and the pK_A was −4.12 ± 0.05. These values are surprisingly good; the point at 83% was well off the curve, and contributed heavily to the large standard deviation. The $-H_A$ value of that solution may well be in error, since it was obtained by extrapolation. It is not known at present whether the H_A scale can be applied to diamides. These results suggest that it does describe their behavior. The situation may resemble that of the H_+ function, which is parallel to the H_0 function in concentrated H_2SO_4, and is only slightly displaced from it.[34] In the absence of an appropriate scale, we will assume that the pK_A of the diprotonated diamide is −4.1. Since the pK_A of N-methylbenzamide is −1.7,[21] this means that the protonation of a benzamide residue in an already protonated diamide is made more difficult by 2.4 pK units.

The application of this ΔpK to aliphatic diamides and to aliphatic polypeptides is questionable. Propionamide is the only aliphatic amide for which an analysis has been carried out in relation to the H_A scale.[21] The slope of the straight line in the plot of the log of the indicator ratio against $-H_A$ is only 0.7. If this is true of all aliphatic amides, a different acidity scale—one which changes with acid concentration even more slowly than H_A—will be needed for them. For the moment we will assume that the H_A scale applies to most aliphatic amides and diamides. The "pK" of acetamide is taken as −1.4,[27] that of propionamide is 0.9[18] or 1.1,[35] and an average value for a single amide group of this kind would be −1.2. If there is a neighboring amide group of the same kind, it will be further assumed that the second "pK" will be lowered by 2.4 units; this would mean that the diamide would be half-protonated in 67% H_2SO_4. This analysis will be discussed further in part B.

In 99% methanesulfonic acid the diamide appears to be incompletely diprotonated, since the curve in Figure 2 does not approach a limit. This reflects the much lower acidity of methanesulfonic acid compared to sulfuric acid.[33,36] It is possible to say from the data of Table I that the benzamide residue of the diamide would be diprotonated to a small extent in 75% methanesulfonic acid, and that in this region of acid concentration an aliphatic diamide would be diprotonated to a somewhat greater extent. A more quantitative estimate is not possible.

B. Helix–Coil Transitions of PELG in Strongly Acid Aqueous Solutions. Figures 3, 4, and 5 show the CD spectra of PELG in various sulfuric acid–water mixtures. In 95.7% H_2SO_4 the spectrum is that of a polypeptide in the random coil configurations.[37] The positive dichroic band at 220 mμ did not change on addition of water before the transition. A sharp conformational change is evident between 38 and 43% water (57–62% H_2SO_4). In 57% acid the spectrum is that of a polypeptide which is beginning to assume the conformation of a right-handed α-helix. The values of the two negative bands are roughly half of those expected for a pure α-helix. It was not possible to go to still lower acidities because the polymer precipitated.

Figures 6 and 7 show the CD spectra of the polymer in two different methanesulfonic acid–water solutions. In 99% acid the spectrum is that of a polypeptide in the random coil conformation. As water is added (to about 20%) there is a marked increase in the weak positive dichroic band at 220 mμ. The nature of this band is

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(34) J. C. D. Brand, W. C. Horning, and M. B. Thornley, *J. Chem. Soc.*, 1374 (1952).

(35) J. T. Edward, J. B. Leane, and I. C. Wang, *Can. J. Chem.*, **40**, 1521 (1962).

(36) R. A. Craig, A. B. Garrett, and M. S. Newman, *J. Am. Chem. Soc.*, **72**, 163 (1950).

(37) G. Holzwarth and P. Doty, *ibid.*, **87**, 218 (1965).

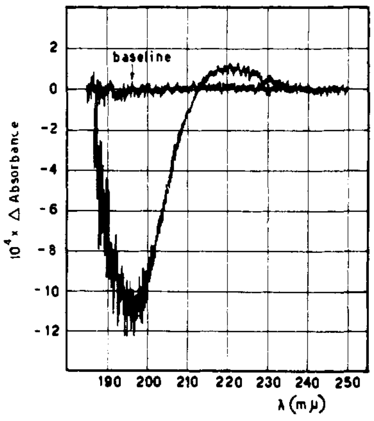

Figure 3. Original CD spectrum of PELG in 96% H_2SO_4. The polymer concentration was 0.2728 g/l. in a 1-mm cell.

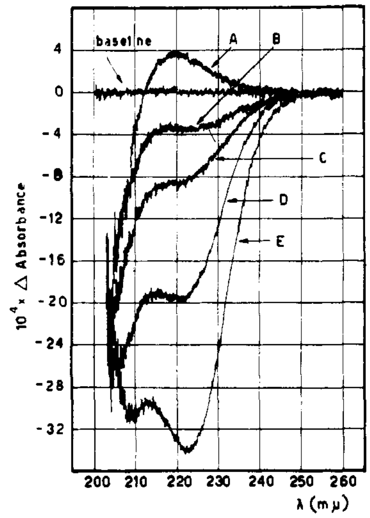

Figure 4. Original CD spectra of PELG in H_2SO_4-H_2O mixtures recorded in a 1-mm cell: A, 0.93 g/l. in 95.7% H_2SO_4; B, 1.22 g/l. in 66.2% H_2SO_4; C, 0.97 g/l. in 60.7% H_2SO_4; D, 1.07 g/l. in 59.4% H_2SO_4; E, 1.08 g/l. in 56.3% H_2SO_4.

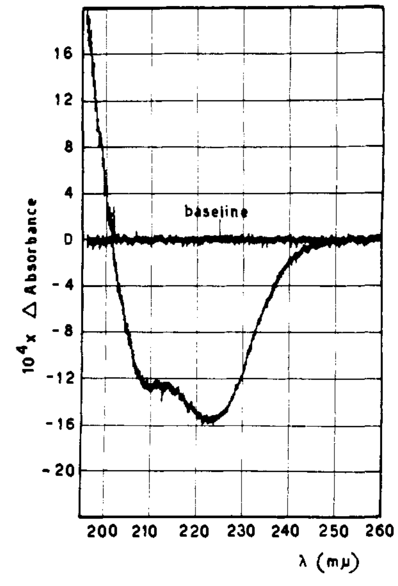

Figure 5. Original spectrum of PELG in 56% H_2SO_4. The polymer concentration was 0.39 g/l. in a 1-mm cell.

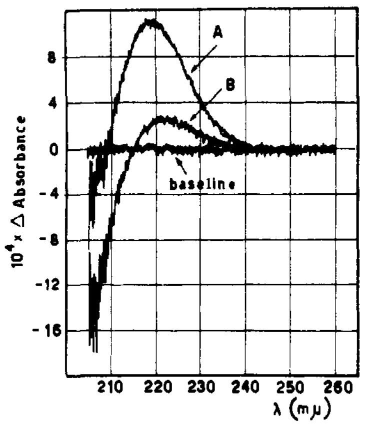

Figure 6. Original CD spectra of PELG in methanesulfonic-water mixtures: A, 1.049 g/l. in 99.0% methanesulfonic acid; B, 1.043 g/l. in 83.6% methanesulfonic acid.

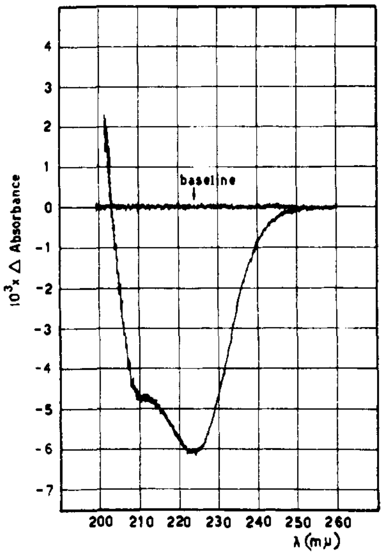

Figure 7. Original CD spectrum of PELG in 66.5% methanesulfonic acid. The polymer concentration was 1.133 g/l. in a 1-mm cell.

not well understood at the present time,[38] but since it is increasing as the acidity of the solution is decreasing and since the polymer is still in the coil form, it is probably associated with the solvation of the coil by the added water (water is only 60% protonated in methanesulfonic acid solution.[36] A further increase in the water concentration produces an extremely marked change in the spectrum. In 75% acid solution two negative bands appear at 222 and near 210 mμ. With still more water the intensities of the two bands increase. Figure 7 shows the CD spectra in 67% acid; in this solution the bands attain the numerical Δε values which are characteristic of a right-handed α-helix. The addition of water beyond this point causes the polymer to precipitate.

Figure 8 shows values of Δε at 222 mμ plotted against solvent composition for the H_2SO_4–H_2O system, and for the methanesulfonic acid–water mixtures. The sharp

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(38) S. Beychock in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, Inc., New York, N. Y., 1967, p 306.

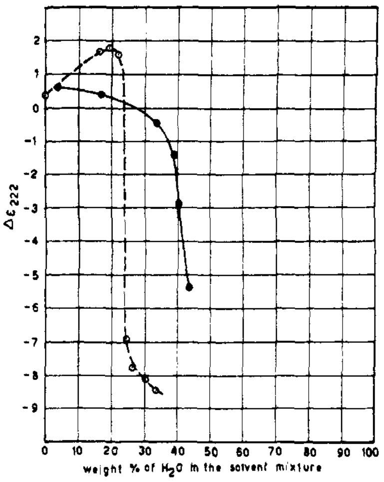

Figure 8. Δε ellipticity values at 222 mμ as a function of the solvent mixture composition: ------, methanesulfonic acid solutions; ——, sulfuric acid solutions.

transition in each medium is evident. In sulfuric acid solutions it takes place between 57 and 62% H_2SO_4. In methanesulfonic acid solutions it is even sharper: between 75.5 and 78% acid.

In part A it was concluded that the model diamide is diprotonated to the extent of 50% in 77.4% H_2SO_4. It can be assumed that an alkyl diamide would be diprotonated to the same extent in 67% H_2SO_4 and less so in 75% methanesulfonic acid. The acid solutions in which the helix–coil transitions are taking place would thus produce some diprotonation of an alkyldiamide, but probably less than 50%. A monoamide would be virtually completely protonated in these latter solutions, since acetamide is half-protonated in 28% H_2SO_4.[27] This will also be the case for isolated amide groups in the polymer coil sections. If the average coil section has more than three residues probably every alternate amide group will be protonated in somewhat less acid solutions. The work required for such an arrangement might be a little more than that required for protonating single amide groups which are much further apart in the coil, but it will certainly be much less than that needed for protonating two adjacent groups. In other words, in the solutions in which the helix–coil transitions are just beginning (about 75% for methanesulfonic acid, and not known for H_2SO_4), every other amide group in the coil sections will have been protonated. It is assumed in this discussion that the amide groups in the helical sections are not protonated, since the n–π* transition at 222 mμ which is evident in the CD spectra would disappear on protonation.[7] Additional protonation in the coil can take two forms: direct addition of a proton to a group which is located between two already protonated neighbors, or a rearrangement of both the neutral and conjugate acid amides. The first process is equivalent to the triprotonation of a triamide. We have not yet examined a triamide model in this respect, but in view of the incomplete diprotonation of the model diamide in solutions which are more acid than those in which the transitions occur, it is almost certain that triprotonation would not be found to any significant extent except in systems which are far more acid than any encountered here. The second possibility is a rearrangement of the neutral and conjugate acid amide groups in the coil so that there will be two neighboring protonated amide groups, followed by a neutral group, a conjugate acid group, and the previous alternation of uncharged and charged amides. This process will require more work than the protonation of alternate groups, and probably somewhat more work than the diprotonation of an isolated pair of neighboring groups, but it will take place much more readily than the triprotonation of a triamide sequence. It is this step, or a succession of such steps, which could initiate the disruption of the helix in these solutions. This part of the discussion will be limited to the methanesulfonic acid system, since the transition is incomplete in the sulfuric acid solutions. In 75% acid the polymer is largely in the helical conformation, and in 78% acid it is mostly in the coil form. This is a small range of acidity. It represents a change of only 0.3 H_0 unit, and less than this (although how much less is not known) in H_A units. If the helix remained stable over this acid interval, the increase in coil amide protonation would be small over the same interval. There would be a corresponding small increase in charge density in the coil sections, but any possible electrical effect would be minimized because of the high concentration of the surrounding solvent ions. It appears improbable therefore that the disruption of the helix can be attributed to coulombic effects. However, the introduction of additional diprotonated diamide segments into the coil (additional, since there may already be some present before the transition starts) would have another consequence. The slow rate of change of the H_A scale with acid concentration compared with that of the H_0 scale has been attributed to the greater hydration requirement of an amide conjugate acid compared to that of an anilinium ion.[21] It has also been concluded that an amide conjugate acid requires more solvation than the free amide.[39] Additional protonation of the coil amide groups will therefore bring more water of solvation into the region of the polymer. If the postulated diprotonated segment is near or next to the edge of a helical segment, its associated solvent molecules could hydrogen bond to the helical end. If this step takes place, a further rearrangement in the coil could occur, the latter would be lengthened, an additional amide group could be protonated, more water would be brought into the vicinity of the coil, and the process could become extensive over a small range of acidity.

An estimate of the extent of protonation of the polymer at the beginning of the transition requires a knowledge of the fraction of polymer which is in the helical form. We shall assume from the Δε values that in the most dilute sulfuric acid solution in which the transition is visible (57%) half of the polymer is in the helical form. This means that somewhat more than 25% but less than 50% of the polymer amide groups are protonated at this point. In 75% methanesulfonic acid, from the Δε values in Figure 8 about 75% of the polymer appears to be in the helical conformation. On this basis somewhat more than 12% of the polymer amide groups but less than 25% are protonated near the beginning of the transition.

Just prior to the submission of this manuscript we read with interest the article by Rosenheck.[40] The

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[39] L. M. Sweeting and K. Yates, *Can. J. Chem.*, **44**, 2395 (1966).

[40] K. Rosenheck in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968, p 517.

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.

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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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Contribution from the Institute of Organic Chemistry, University of Padua, Padua, Italy. *Received August 5, 1968*

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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(1) On leave of absence from the Polytechnic Institute of Brooklyn, Brooklyn, N. Y.; author to whom inquiries should be addressed.

(2) J. E. Johnson and R. H. Cole, *J. Am. Chem. Soc.*, **73**, 4538 (1951).

(3) W. Dannhauser and R. H. Cole, *ibid.*, **74**, 6105 (1952).

(4) P. Walden, *Z. Physik. Chem.* **70**, 569 (1910).

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