than is ester oxygen. Values of $k(OH + S)$ in units of $10^7\ M^{-1}\ sec^{-1}$ are

CH_3CONH_2	$CH_3CON(CH_3)_2$	CH_3CO_2H	$CH_3CO_2CH_3$
19	350	1.4	8

Implications for Radiolysis of Peptides

The determination of the sites of attack by OH radicals on simple amides and the observed activation of N-methyl groups by the amide nitrogen have been found to be of considerable importance in the study of more complex peptides.[21] From a comparison with the transient absorption spectra and the extinction coefficients of radicals derived from α-CH_3 and N-CH_3 certain generalities are apparent. For instance, the radicals $\dot{C}H_2CONHR$ (where R = H, $CH_2CO_2^-$, or $CH_2CONHCH_2CO_2^-$) and $\dot{C}H_2CON(CH_3)_2$ have absorption maxima above 400 nm with $\epsilon \sim 1000\ M^{-1}\ cm^{-1}$. On the other hand, the radicals $CH_3CONH\dot{C}HR$ (where R = H or CO_2^-) and $CH_3CON(CH_3)\dot{C}H_2$ have main absorption maxima in the region 235–270 nm, and much higher extinction coefficients, $\epsilon \sim 6000$–$14{,}000\ M^{-1}\ cm^{-1}$. These differences are being exploited further to establish sites of free radical attack on peptides.

Acknowledgment. The assistance of Dr. P. Neta in preliminary experiments is gratefully acknowledged.

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Interaction of Anhydrous Formic Acid with Model Amides[1,2]

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Received November 4, 1969

Abstract: The interaction of anhydrous formic acid with model amides has been studied using infrared, nmr, viscosity, conductance, and density measurements. Several equilibria are needed to describe this interaction over the entire mixing range of acid and amide: hydrogen bonding is prevalent over the entire mixing range, protonation of the amides occurs at high acid concentrations, and ion pair formation is present at intermediate acid concentrations. The data are consistent with protonation and hydrogen bonds involving the acidic proton of formic acid and the amide carbonyl oxygen. The degree of protonation, as measured directly by the specific conductance, reflects the basicities of the different amide carbonyls.

The use of nonaqueous solvent systems containing organic acids in the study of polypeptide conformational stability is well documented.[4] Solvent systems containing halogenated acetic acids, such as trifluoroacetic and dichloroacetic acids, have been widely used in the study of the helix $\rightleftharpoons$ random chain interconversion.[5,6] The interaction of these acids with model amides[5,7] and polypeptides[8,9] has been investigated by a variety of physicochemical techniques. Although conflicting results have been presented for polypeptide systems[7,9,10] there is general agreement that trifluoroacetic and dichloroacetic acids act as strong acids toward amides and protonate extensively.

In contrast, less attention has been given to the weaker, unsubstituted fatty acids, such as formic and acetic. Conformational transitions have been reported in formic[11] and acetic[12] acids but no detailed study of the action of these acids on amides or polypeptides has been carried out. From previous investigations in this laboratory involving gelatin[13] and derivatives of poly-L-proline[11] in formic acid, the usefulness of anhydrous formic acid in the study of polypeptide conformational stability became apparent. Formic acid appeared to interact in some specific manner with polypeptides, the degree of interaction being easily regulated by the addition of basic solvent components, such as amides and dimethylsulfoxide. In this respect, the formic acid–polypeptide systems seemed ideal models for the study of solvent–backbone interactions. In addition, Chao, Veis, and Jacobs[14] have shown that formic acid is a good solvent for differentiating the basicity of various model amides. The high dielectric constant of formic acid, *viz.* ϵ 58,[15] and the resistance to solvolytic degradation shown by polypeptides in it further recommended its use as a general

(1) A report of work done under Contract No. 12-14-100-8933(73) with the U. S. Dept. of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.

(2) Presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.

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polypeptide solvent in preference to the halogenated acids.

Thus, investigations were undertaken to (1) elucidate the equilibria governing formic acid interaction with the -CONH- group and (2) examine the ability of formic acid to differentiate among the basicities of peptide bonds formed from various amino acids. A variety of physicochemical techniques has been used to characterize the interactions present in the anhydrous acid. The results of these experiments are reported here. Details concerning the second question can be found in the companion paper[16] which follows.

Experimental Section

Materials. Formic acid, obtained in a practical grade of 97+% purity from Eastman Organic Chemicals, was purified after the method used by Schlesinger and Martin[17] and Chao, *et al.*[14] To remove the last traces of water, formic acid was stirred with boric anhydride for at least 48 hr. The acid was then distilled from a fresh portion of anhydride under reduced pressure at 25°. Formic acid produced in this way, and having a specific conductivity, κ_{25}, at 25° in the range 6.20–6.60 × 10^{-5} ohm^{-1} cm^{-1}, was considered pure enough for physical studies. This conductivity range was also used by Schlesinger and Martin[17] and Chao, *et al.*[14] Although formic acid slowly decomposes at room temperature,[18] it was found to be stable for several weeks, if stored in a dessicator over Drierite. The specific conductance was checked before use, and if it differed from the value obtained after distillation, fresh solvent was prepared.

Trifluoroacetic acid, obtained from Eastman Organic Chemicals, was distilled under reduced pressure at room temperature. The specific conductance at 25° was 2.3 × 10^{-6} ohm^{-1} cm^{-1}. This acid was used without further purification and stored over anhydrous $CaSO_4$.

Dimethyl sulfoxide, a Crown-Zellerbach spectrograde product, contained appreciable amounts of water, as indicated by the refractive index at 589 mμ. The method of Kolthoff and Reddy[19] was used to prepare the anhydrous liquid. Removal of water was accomplished by shaking the dimethyl sulfoxide for 24 hr with Woelm, neutral, chromatographic alumina (activity I), which had been ignited to 600° in a muffle furnace to drive off the water present, and allowed to cool *in vacuo*. Dimethyl sulfoxide was then distilled from the alumina under reduced pressure at 55°. Dimethyl sulfoxide produced in this way gave κ_{25} = 3.41 × 10^{-7} ohm^{-1} cm^{-1}, and was used for all further experiments. It was stored over Drierite and its conductivity was checked before each use.

N,N-Dimethylacetamide (Eastman Organic Chemicals) was purified by vacuum distillation at 45°. This material gave n^{25}D 1.4358 (lit.[20] 1.4358) and κ_{25} = 6.22 × 10^{-7} ohm^{-1} cm^{-1}. No change in n^{25}D or κ_{25} was observed over many months, if the material was stored over Drierite.

N-Methylacetamide, obtained from Eastman Organic Chemicals, was purified using the fractional freezing procedure of Lagowski.[21] Liquified N-methylacetamide was allowed to crystallize slowly at room temperature. After 3 days, 90% of the liquid had frozen. The remaining liquid was discarded and the freezing was repeated. The N-methylacetamide thus produced had κ_{35} = 2.3 × 10^{-6} ohm^{-1} cm^{-1} and was used without further purification.[21]

N$^{\alpha}$-Acetylglycinemethylamide was synthesized according to the method of Applewhite and Niemann[22] from glycine ethyl ester hydrochloride. The product was recrystallized twice from methanol–ethyl ether and dried *in vacuo* at 60°. Purity was checked by thin layer chromatography using 60/40 (v/v) $CHCl_3$–methanol as a developing solvent. Only one spot appeared after two recrystallizations, mp (uncorrected) 159° (lit.[22] 157.5–158.0°).

Anal. Calcd for $C_5H_{10}N_2O_2$: C, 46.14; H, 7.75; N, 21.53. Found (Midwest Microlab, Inc.): C, 46.10; H, 7.75; N, 22.10.

N$^{\alpha}$-Acetylglycinamide was purchased from Cyclo Chemical Corp. (Lot No. K-5733) and was of Grade I purity.

Anal. Calcd for $C_4H_8N_2O_2$: C, 41.37; H, 6.89; N, 24.14. Found (Midwest Microlab, Inc.): C, 41.21; H, 7.06; N, 24.02.

2,5-Piperazinedione (diketopiperazine) was obtained in reagent grade from Eastman Organic Chemicals.

Preparation of Solutions. Solutions involving liquid components were prepared by volume at room temperature, 23–25°. Mixing of strongly interacting components was accompanied by the evolution of a considerable amount of heat and thus had to be carried out slowly. Nevertheless, preparation of these solutions was rapid enough so that the use of a drybox was found unnecessary. Dissolution of solid peptides in formic acid, on the other hand, had to be carried out in a drybox under dry nitrogen.

Since we were interested in computing results for all amides in the same terms, the relative concentration of amide groups, concentrations were calculated in terms of the mole fraction of formic acid, X_{FA}, using the equation

$$X_{FA} = \frac{N_{FA}}{N_{FA} + N_{-CONH-}}$$

where N_{FA} = the number of moles of formic acid and N_{-CONH-} = the number of moles of peptide or amide units.

Infrared Studies. Experiments in the fundamental region, 900–4000 cm^{-1}, were carried out using a Beckman IR-12 spectrophotometer. This instrument, with its grating monochromator, had a wave number of accuracy and reproducibility of ±0.6 cm^{-1} and ±0.3 cm^{-1}, respectively, in the region 900–1800 cm^{-1}. In this region, resolution was maintained at better than 3 cm^{-1} by suitable adjustment of the gain and slit programs. Scanning speeds were selected to eliminate tracking error.

A double-beam internal reflectance attachment, Model 12, obtained from Wilks Scientific Corp., was used to obtain reflectance spectra; 45° germanium reflector plates, with dimensions 50 × 20 × 2 mm, were used in the standard liquid and flow-through sample holders. When temperature control was desired, water coolant was circulated on one side of the reflector plate. The water spectrum could be easily cancelled by using a similar arrangement in the reference beam. All infrared spectra reported are double-beam scans, using air, solvent, or unmixed components in the reference beam.

Conductivity Measurements. Resistances in the range 0.2–2.5 × 10^6 ohms were measured using an Industrial Instruments, Inc. Model RC-16B-1 conductivity bridge with an accuracy of ±1%. A 1000-cps current was generally used, although for liquids with very high resistances, such as pure solvents, the 60-cps frequency had to be used. The sensitivity of the bridge is 1/3% of the scale reading.

For liquids with low conductances, a cell with constant 0.0784 cm^{-1} at 25°, obtained from LKB Instruments, Inc., was used. The cell had platinized platinum electrodes and was calibrated by the manufacturer.

Specific conductivities in the range 1–2000 × 10^{-5} ohm^{-1} cm^{-1} were measured with a cell of constant ~0.8 at 25° (LKB Instruments, Inc.). Because of the continual contact of the platinized electrodes with strong acid, the cell constant changed slightly from time to time. Frequent calibrations were performed with standard 0.01 *N* KCl, obtained from Industrial Instruments, Inc. The specific conductance of this standard is κ_{25} = 1.4114 × 10^{-3} ohm^{-1} cm^{-1}, from the data of Jones and Prendergast.[23] Cleaning of the cell was accomplished by thoroughly flushing with distilled water, absolute ethanol, and reagent grade acetone. The cell was then dried with a stream of dry air. No change in cell constant was observed using this cleaning procedure. Before filling, the cell was flushed with a small amount of the test solution.

A Wilkens-Anderson constant temperature bath provided temperature control to 25.00 ± 0.01°. Temperature equilibration was accomplished in either of two ways. In one procedure, the conductance cell was placed in the water bath for about 5 min, then removed, and dried quickly, prior to resistance measurements. Readings could not be obtained in the water bath due to the large capacitance effect created by water.[24] In the second method, the cell was equilibrated for 7–10 min in a bath containing light paraffin oil, which was submerged in the water bath and stirred vigorously

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(24) T. Shedlovsky in "Technique of Organic Chemistry," 3rd ed, Vol. I, A. Weissberger, Ed., Interscience Publishers, New York, N. Y., 1959, Part IV, p 3011.

to maintain thermal equilibrium. Measurements could then be made without removing the cell from the oil bath. Temperatures were monitored using a calibrated mercury thermometer from Fisher Scientific Co., and a Yellow Springs Model 42-SC telethermometer, equipped with microprobes. Equilibration times given above were found to be adequate.

Specific conductivities were calculated using the equation

$$\kappa_{25}' = \frac{\theta}{R}$$

where θ = the cell constant in cm^{-1}, and R = the resistance in ohms. Since all solutions had conductivities much higher than pure formic acid, it was unnecessary to apply solvent corrections to the values calculated. All conductances were corrected for the viscosities of the solutions by multiplying by the relative viscosity

$$\kappa_{25} = \eta_{rel}\kappa_{25}'$$

where κ_{25} = the corrected conductance, κ_{25}' = the observed conductance, and η_{rel} = the viscosity of the solution relative to formic acid.

Viscosities. Flow times for solutions and solvents were measured with Cannon-Ubbelohde No. 75 suspended level dilution viscometers having flow times of approximately 2 min for distilled water. The viscometers were rigidly suspended in the water bath and isolated from the vibrations produced by the heating, cooling, and stirring mechanisms. Flow times obtained with electrical timers were reproducible to ±0.2%.

Solution viscosities, η_{rel}', were calculated relative to formic acid

$$\eta_{rel}' = \frac{t_{25}^{soln}}{t_{25}^{FA}}$$

where t_{25}^{soln} and t_{25}^{FA} are the flow times for solution and solvent, respectively, at 25° and in the same viscometer. The relative viscosity obtained in this manner was corrected for the difference in pressure head between solution and solvent resulting from the density difference

$$\eta_{rel} = \frac{d_{25}^{soln}}{d_{25}^{FA}}\eta_{rel}'$$

where η_{rel} is the corrected relative viscosity, and the densities of the solution and solvent at 25° are given by d_{25}^{soln} and d_{25}^{F}, respectively.

Densities. Density measurements were made using the type of pycnometer designed by Lipkin, *et al.*,[25] for extremely volatile liquids. The U-shaped pycnometer consisted of capillary side arms graduated 10 divisions/cm with a bulb of 1-ml capacity at the lower end of one side arm. The procedure in obtaining the density was to (1) weigh the pycnometer empty, (2) fill and weigh the pycnometer again, (3) place it in the thermostated water bath, and (4) read the liquid levels in the side arms no later than 15 min after filling. The actual volume was obtained from a calibration curve, which indicated the volume as a function of the sum of the side arm readings. Calibration of each pycnometer was performed with formic acid. The precision of this method was 0.005 g/ml, and from a comparison of determinations using pure N,N-dimethylacetamide with literature values, this is also believed to be the accuracy. Calculations, including air buoyancy corrections, were carried out as suggested by Bauer and Lewin.[26] Apparent specific volumes, ϕ_{v_2}, were calculated using the equation

$$\phi_{v_2} = \frac{1}{\rho_0}\left[1 + \frac{\rho_0 - \rho_1}{C_2}\right]$$

where ρ_0 = density of pure solvent (g/ml), ρ_1 = solution density, and C_2 = concentration of solute (g/ml).

Nmr. Proton magnetic resonance spectra were obtained on a Varian Associates Model A-60 analytical spectrometer, operating at 60 MHz and a field strength of about 14,092 G. Resolution, *i.e.*, the full line width at half the maximum amplitude, was 0.3 Hz. Spectral reproducibility with an environmental temperature variation less than ±3° was 0.6 Hz. Spectra were run at room temperature, and 1 or 2 drops of tetramethylsilane (TMS) was added to each sample to provide an internal reference absorption. The frequency shift, $\Delta\nu$, in cycles per second from TMS was obtained from the spectra. Chemical shifts, δ, were calculated using the equation

$$\delta = \frac{\Delta\nu \times 10^6}{60 \times 10^6} = \text{ppm}$$

Results

The interaction of anhydrous formic acid with N,N-dimethylacetamide, N-methylacetamide, and dimethyl sulfoxide was investigated using infrared spectroscopy, nmr, conductivity, viscosity, and density.[27] Properties of the binary liquid mixtures were studied over the entire mixing range and compared with those of solutions containing trifluoroacetic acid.

Infrared spectra of the binary mixtures are extremely complex owing to high molar extinction coefficients of the pure compounds and the extensive hydrogen bonding present in the mixtures. However, changes of acid and amide carbonyl frequencies with increasing concentration of acid are marked. The amide I band carbonyl stretch of DMA is shifted 55 cm^{-1} to lower frequency from 1650 cm^{-1} in pure DMA to 1595 cm^{-1} in dilute solution in FA. Spectral changes for the two acids are similar, although larger effects are seen with the stronger acid TFA, where the carbonyl absorption frequency drops from 1785 cm^{-1} in pure TFA to 1775 cm^{-1} at $X_{TFA} = 0.10$ in DMA.

Evidence for an additional interaction is provided by difference spectra, *i.e.*, spectra taken using unmixed components in the reference beam. Such spectra were obtained only for the equivolume mixing ratios. Figure 1 is a difference spectrum of a mixture of FA and DMA with a mole fraction of acid of 0.71. Clearly an excess of unbonded DMA is present in the reference beam. A shoulder at 1550 cm^{-1} and a band at 1375 cm^{-1} appear which result from the interaction of FA with DMA. From an examination of the spectrum of potassium formate in FA these bands are assigned to the asymmetric and symmetric stretching frequencies of the carboxylate ion.[28-30] It is apparent that a significant concentration of formate ion is present in the FA–DMA mixture of Figure 1.

An absorbance–concentration curve was constructed for potassium formate in FA using the optical density at 1550 cm^{-1} relative to a base line absorption at 1480 cm^{-1}. This curve was linear in the range 0–11 *M* potassium formate and was used to estimate the formate ion concentrations in FA–DMA solutions containing 0.71 and 0.80 mole fraction of acid. These results along with the calculated molarity of DMA are shown in Table I. Unfortunately, reliable data on formate ion

Table I. The Formate Ion Concentration in FA–DMA Mixtures as a Function of DMA Concentration

Mole fraction of formic acid	Molarity of DMA (calcd)	Molarity of formate ion (found)
0.71	6.25	6–7
0.80	4.67	~3

(25) M. R. Lipkin, J. A. Davison, W. T. Harvey, and S. S. Kurtz, Jr., *Ind. Eng. Chem., Anal. Ed.*, **16**, 55 (1944).

(26) N. Bauer and S. Z. Lewin in ref 24, Part I, p 131.

(27) Abbreviations adopted in this paper: FA = formic acid, TFA = trifluoroacetic acid, NMA = N-methylacetamide, DMA = N,N-dimethylacetamide, DMSO = dimethyl sulfoxide, AGA = N-acetylglycinamide, AGMA = N-acetylglycinemethylamide, DIKETO = 2,5-piperazinedione.

(28) H. Junge and H. Musso, *J. Chem. Soc., B*, 389 (1968).

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(30) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958.

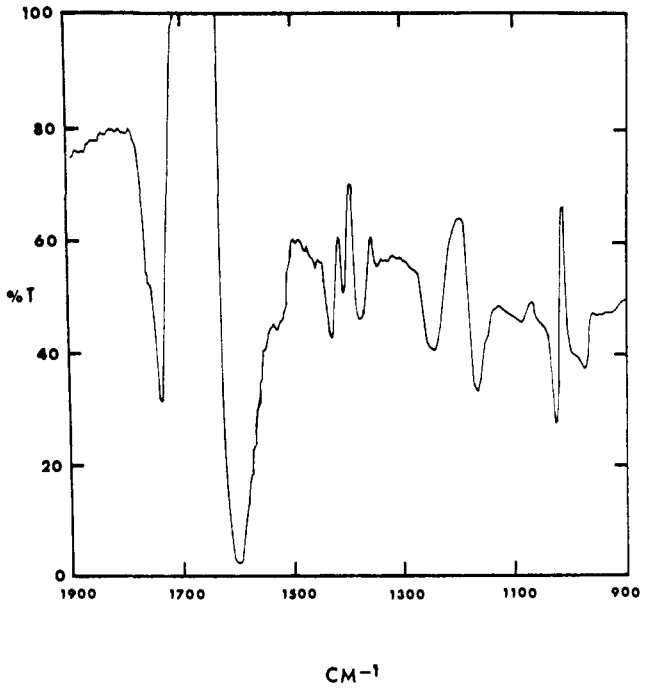

Figure 1. Infrared spectrum of a mixture of formic acid and N,N-dimethylacetamide (mole fraction of acid = 0.71) taken with pure, unmixed components in the reference beam.

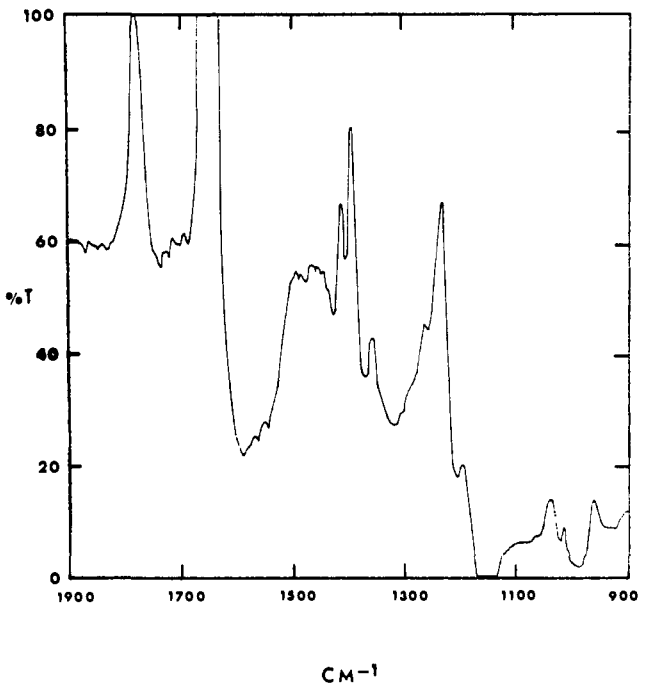

Figure 2. Infrared spectrum of a mixture of trifluoroacetic acid and N,N-dimethylacetamide taken with unmixed components in the reference beam (mole fraction of acid = 0.55, 50 vol % acid).

concentrations could not be obtained for other mixing ratios due to the opacity of the 1500-cm^{-1} region upon further addition of either FA or DMA. It should be noted that a higher formate ion concentration is found at the higher DMA concentration. This will be discussed below in conjunction with conductivity results.

Frequency shifts for the system FA–NMA parallel those for the disubstituted amide, although the presence of the amide II band precluded direct observation of the carboxylate ion.

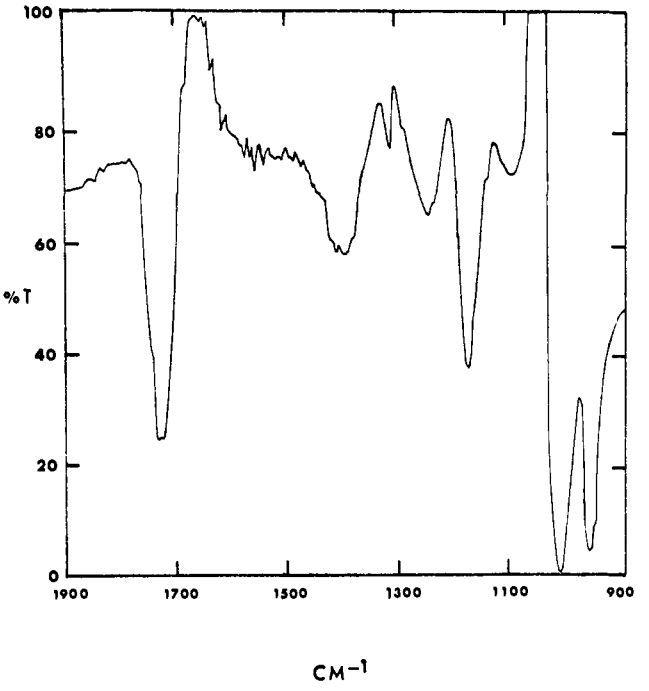

Figure 3. Infrared spectrum of a mixture of formic acid and dimethyl sulfoxide taken with unmixed components in the reference beam (mole fraction of acid = 0.65, 50 vol % acid).

The absorption of the trifluoroacetate ion at 1500 cm^{-1} is seen in Figure 2, a difference spectrum for the TFA–DMA system. TFA has been shown to protonate model amides[5,7] and thus, similar infrared spectral changes are observed in the 1550-cm^{-1} region for both FA and TFA.

The interactions of FA and TFA with DMSO are shown in Figures 3 and 4. Chao, *et al.*,[14] found that DMSO interacted less strongly with FA than model amides. In agreement with their observation, no evidence could be obtained for the presence of ions in the FA–DMSO system. This may be due to a lower ionic concentration and the small molar extinction coefficient for the formate ion band. Nevertheless, strong hydrogen bonding is present since the bonded sulfoxyl exhibits a strong band at 1005 cm^{-1} and only a small shoulder at 1050 cm^{-1} appears corresponding to free S=O.

The presence of the carboxylate ion is apparent in the difference spectrum of Figure 4 for TFA–DMSO. Asymmetric and symmetric stretching frequencies are located at 1720 and 1330 cm^{-1}. Since DMSO has only one electronegative site, protonation by TFA must occur at the sulfoxyl oxygen.

The results of nmr experiments are in essential agreement with the infrared evidence for hydrogen bonding and protonation.[31] The chemical shifts observed for the acidic proton of FA in mixtures containing model amides, DMSO, and acetonitrile are shown in Figure 5 as a function of the mole fraction of acid. In all cases except acetonitrile a downfield displacement is observed relative to anhydrous formic acid resulting, in part, from strong hydrogen bonding of acid to amide.[32,33]

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(32) J. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1965.
(33) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959.

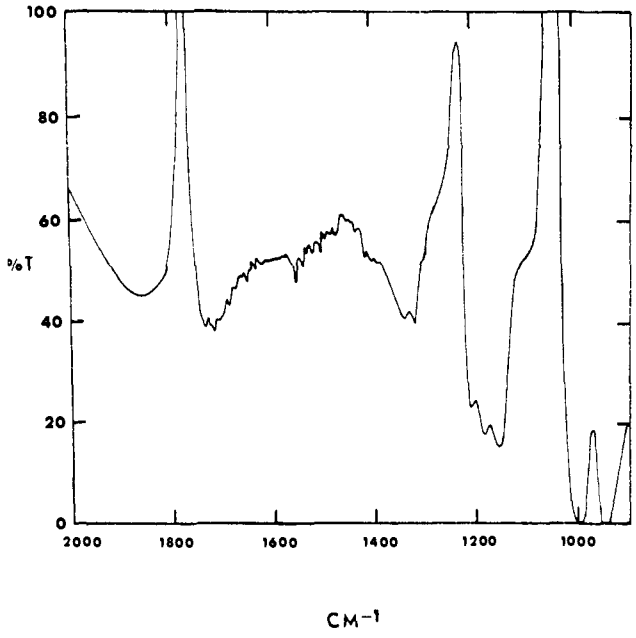

Figure 4. Infrared spectrum of a mixture of trifluoroacetic acid and dimethyl sulfoxide taken with unmixed components in the reference beam (mole fraction of acid = 0.48, 50 vol % acid).

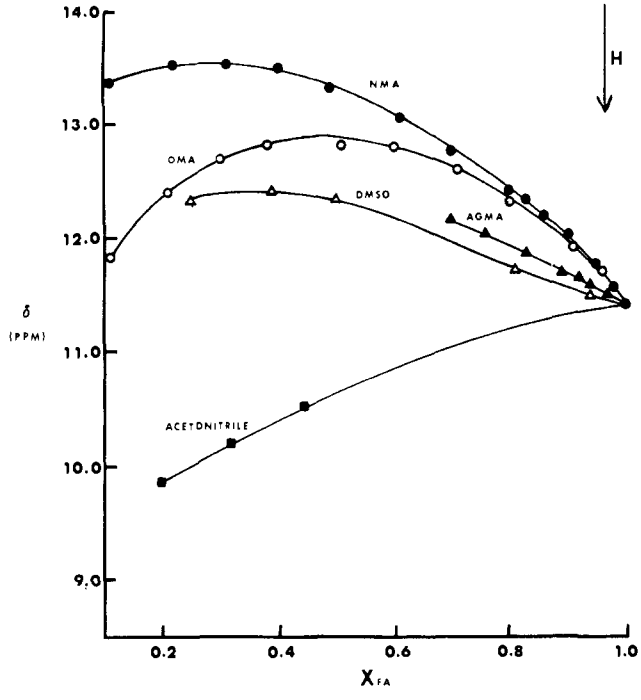

Figure 5. Chemical shift of the acidic proton (parts per million from TMS) of formic acid as a function of concentration in binary mixtures with amides, dimethyl sulfoxide, and acetonitrile. See text for abbreviations.

The maximum displacement, however, is clearly not at the equimolar mixing ratio. The presence of the protonation reaction should be reflected in these concentration curves.

The upfield displacement observed in FA–acetonitrile mixtures is consistent with the chain hydrogen bonding proposed for pure formic acid[34,35] and the depolymerizing effect of acetonitrile, an inert diluent.[14]

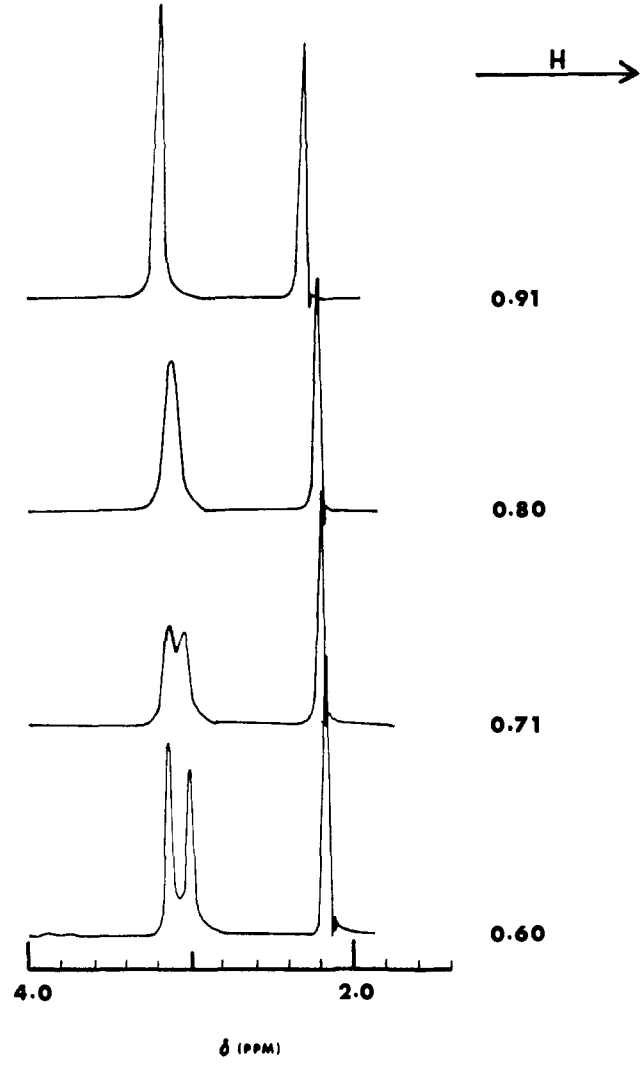

Figure 6. Nmr spectra of the methyl protons of N,N-dimethylacetamide in formic acid, parts per million from TMS, concentration indicated at the right of each spectrum as mole fraction of acid.

Strong protonating solvents bring about the collapse of the N-methyl doublet of dimethylamides.[32,33] Upon protonation the barrier to rotation is reduced and the two methyl groups have a similar time-averaged chemical environment. Protonation occurs primarily at the carbonyl groups, but a small amount of N-protonation is believed to be responsible for the unrestricted rotation.[36,37] Collapse of the N-methyl doublet is clearly diagnostic of proton transfer to the amides[38] and consequently the chemical shifts of the N-methyl protons of DMA were examined in FA and TFA. Figures 6 and 7 show the concentration dependence of δ for DMA in the two solvents and for the doublet collapse. In TFA, protonation is sufficient at $X_{TFA} \approx 0.3$ to bring about complete loss of the doublet, whereas a mole fraction of FA of about 0.75 is required to accomplish the same degree of protonation.

Relative viscosities of solutions of NMA and DMA in FA at 25° are shown in Figure 8. A maximum is seen for the FA–DMA system at 0.70 mole fraction of acid. In contrast, the viscosity of FA–NMA mixtures

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(37) G. Fraenkel and C. Franconi, *ibid.*, 82, 4478 (1960).
(38) Proton transfer is used synonymously with protonation.

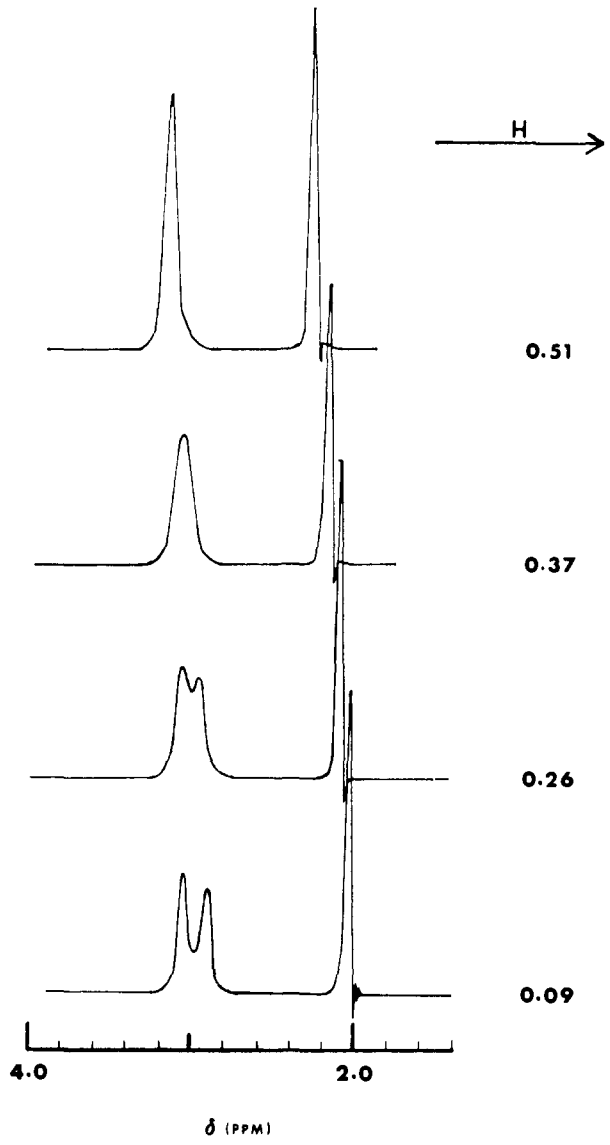

Figure 7. Nmr spectra of the methyl protons of N,N-dimethylacetamide in trifluoroacetic acid, parts per million from TMS, concentration indicated at the right of each spectrum as mole fraction of trifluoroacetic acid.

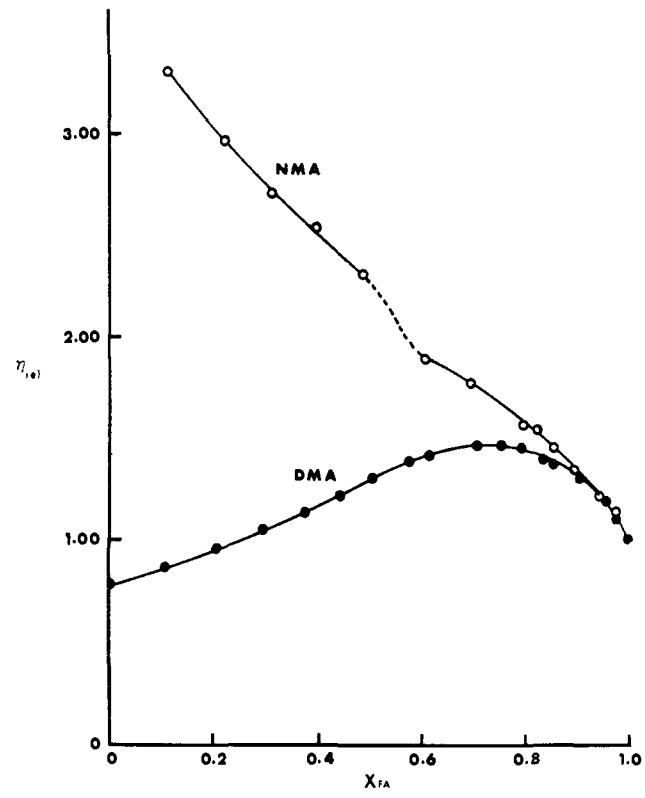

Figure 8. Relative viscosities at 25° of solutions of N-methylacetamide and N,N-dimethylacetamide in formic acid. X_{FA} = mole fraction of formic acid.

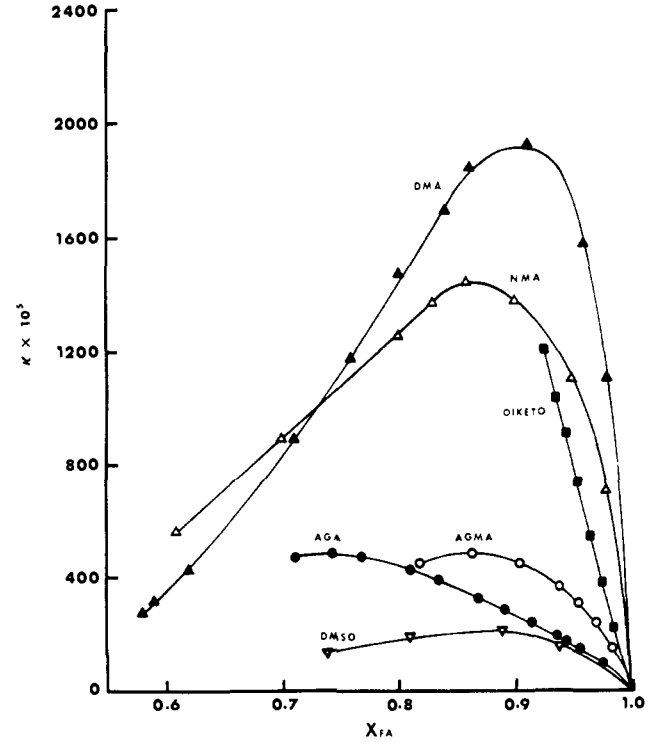

Figure 9. Specific conductivities (ohm^{-1} cm^{-1}) at 25° of model compounds in formic acid plotted against the mole fraction of acid. For abbreviations, see text. Data for DMSO are those of Chao, *et al.*,[14] corrected for viscosity effects.

increases continuously as the proportion of NMA increases. This latter observation agrees with the extensive chain association proposed for pure NMA by LaPlanche, *et al.*[39]

Since pure DMA has a lower viscosity than pure FA, the maximum seen in the mixture viscosity clearly reflects an associative DMA–FA interaction. There is no adequate theory for computing the viscosity of a mixture of two strongly interacting liquids in terms of the interaction intensity. It seems likely, however, that the viscosity maximum should correspond to the concentration at which deviations from solution ideality are greatest. In the present case, the observed viscosity maximum is markedly shifted from the 1:1 molar mixing ratio (X_{FA} = 0.5).

Specific conductivities were determined for the various amides and model compounds in FA at 25°. The relative viscosity of each of the mixtures was determined and the viscosity–conductance products were compared.

Figure 9 was constructed from the viscosity-corrected conductance data. The curves for DMSO, NMA, and DMA are similar to those reported by Chao, *et*

(39) L. A. LaPlanche, H. B. Thompson, and M. T. Rogers, *J. Phys. Chem.*, **69**, 1482 (1965).

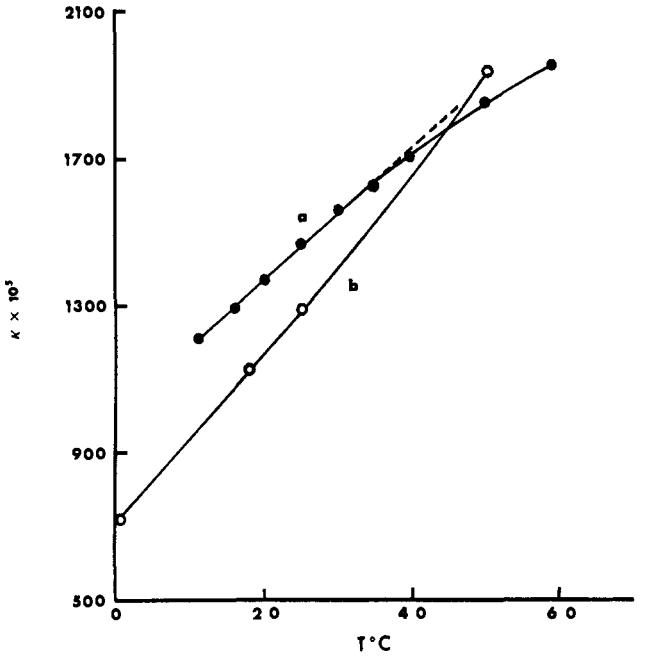

Figure 10. Temperature dependence of the specific conductance (ohm^{-1} cm^{-1}) of DMA in formic acid (at $X_{FA} = 0.90$), curve a, compared to 0.1 *N* aqueous KCl, curve b; data on aqueous KCl taken from "International Critical Tables," Vol. VI, McGraw-Hill Book Co., New York, N. Y., 1929, p 234.

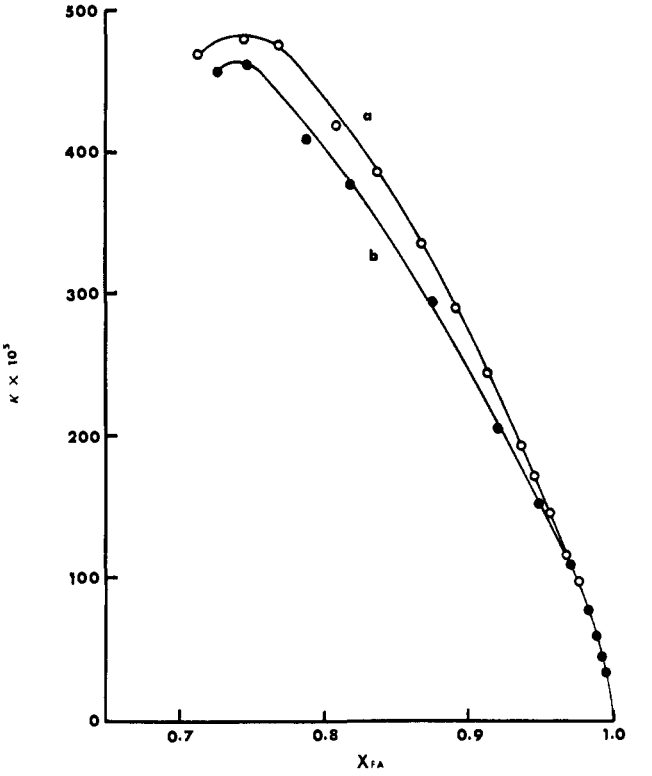

Figure 11. Specific conductivities (ohm^{-1} cm^{-1}) of solutions of N-acetylglycinamide in formic acid at two temperatures: curve a, 25°; curve b, 20°; X_{FA} = mole fraction of formic acid.

al.,[14] but the apparent conductance values are higher due to the viscosity correction. The important point is that the relative order of the conductance maxima is maintained and the maxima appear at values greater than $X_{FA} = 0.5$.

The temperature dependence of the conductance of the FA–DMA system (at 0.90 mole fraction of acid) is given in Figure 10. For comparison the aqueous KCl system is included. It is clear that any major contribution to the conductance from a Grotthuss type of conductivity, as originally proposed by Chao, *et al.*,[14] must be discounted. If the unusually high conductance of these systems were due to a mechanism of proton transfer involving migration down a hydrogen bonded chain, an increase of temperature should have the effect of decreasing the observed conductance, since hydrogen bonding should be less extensive at the elevated temperatures. Figure 11 illustrates the conductance–concentration curves for N^{α}-acetylglycinamide in FA at two temperatures. Again a higher conductance is observed at the higher temperature. However, near $X_{FA} = 1.0$ the two curves are identical.

Table II. Apparent Partial Specific Volumes of NMA and AGMA in FA as a Function of Solute Concentration

FA-NMA		FA-AGMA	
$(M)^{1/2}$	ϕ_{v2}	$(M)^{1/2}$	ϕ_{v2}
0.71	0.98	0.28	0.87
1.11	1.00	0.44	0.83
1.56	1.01	0.60	0.89
1.79	1.02	0.85	0.84
1.96	1.02	1.03	0.84
2.10	1.02	1.19	0.84
2.47	1.03	1.46	0.84
2.72	1.03	1.68	0.86
		1.88	0.85

Systems in which ionization equilibria are present often exhibit electrostriction, and, consequently, solution densities greater than that of either pure component, with a maximum at some specific mixing ratio. Densities were determined for solutions of NMA and AGMA in FA. Apparent specific volumes are given in Table II. Although no electrostriction is apparent in the FA–NMA system, a small amount is indicated in the case of AGMA.

Discussion. It is clear from a variety of techniques that several equilibria are important in FA–model amide systems. Hydrogen bonding of FA to the amide carbonyls is strong and dominant over much of the mixing range ($0 < X_{FA} < 1.0$).

$$HCOOH + O{=}C \rightleftharpoons HCOOH{\cdots}O{=}C \qquad (1)$$

In addition, at high acid concentrations ($0.9 < X_{FA} < 1.0$) FA is found to protonate the amides.

$$HCOOH + O{=}C \rightleftharpoons HCOO^- + H^+O{=}C \qquad (2)$$

From the parallel behavior or DMSO and DMA in FA, it is concluded that both hydrogen bonding and protonation equilibria involve only the peptide carbonyl function.

Equilibrium 2 implies protonation and dissociation into ions. The conductance data presented above indicate this to be the case at $X_{FA} > 0.9$. This is apparent from the sharp rise in the specific conductance with addition of small amounts of solute and the small temperature dependence exhibited in Figure 11 at $X_{FA} > 0.9$. However, as the concentration of solute is increased *i.e.*, below $X_{FA} = 0.9$, the degree of pro-

tonation and/or the amount of dissociation into free ions appear to decrease since the solution conductance rapidly reaches a maximum and then decreases. Infrared measurements of the concentration of formate ion in solutions beyond the conductance maximum were presented in Table I. As can be seen, the formate ion concentration appears to increase as the concentration of DMA increases. This observation, coupled with the fact that the conductance is decreasing in this concentration range, indicates that dissociation into ions is incomplete. Thus, the spectral concentration of $HCOO^-$ will be higher than the concentration of free $HCOO^-$ as determined conductometrically. A reasonable explanation appears to be the formation of associated ions, or ion pairs. Such ionic association is difficult to reconcile with the generally accepted theory for the behavior of 1:1 electrolytes in solvents of high dielectric constant.[40] Nevertheless, this type of association is not without precedent. Recently, D'Aprano and Fuoss[41] showed that nonconducting solutions of picric acid in acetonitrile became highly conducting upon addition of solvents, such as water, methanol, and ethanol, which are basic relative to picric acid. The conductance increased sharply on first addition of the basic solvent and continued to increase with the content of basic solvent, regardless of whether the dielectric constant increased or decreased. Specific solvent interaction of the basic solvent with the picrate ion was cited as the reason for the apparent inapplicability of simple electrostatic effects.

Specific solvent–solute interaction has been demonstrated in the FA–amide systems. The competing hydrogen bonding equilibrium is thought to be responsible for the decrease in the dissociation constant for equilibrium 2.

From the conductance data it is clear that dissociation into ions is complete at $X_{FA} > 0.9$. Thus, the initial rise in specific conductivity with the addition of solute, as well as the value of the conductance maximum, generally at $X_{FA} = 0.9$, directly reflects the degree of protonation by FA, and, consequently, the basicity of the solutes. In particular, FA appears to be probing the electron density at the oxygen carbonyl, providing a convenient method for the comparison of amide and peptide bonds.

Acknowledgments. The authors are grateful to Dr. Philip J. Chenier of the Department of Chemistry, Loyola University, Chicago, Ill., for taking the nmr spectra and assisting in their interpretation.

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(41) A. D'Aprano and R. M. Fuoss, *J. Phys. Chem.*, 73, 400 (1969).

Basicity Differences among Peptide Bonds[1,2]

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Received November 4, 1969

Abstract: The basicities of peptide bonds formed from various amino acids were investigated using anhydrous formic acid as a solvent probe. Conductivity measurements of the degree of protonation by formic acid gave the order of decreasing carbonyl basicity as: proline peptide bonds > serine > glycine ~ alanine. The peculiar position occupied by proline was established and discussed in terms of recent conformational analysis and solvent–polymer interaction.

Recent theoretical treatments of polypeptide conformation have achieved some success in predicting the stability of structures[4-6] of various polypeptides. Calculations of the dimensions of stable, isolated polypeptide helices[7] as well as the mean square properties of random chain polymers[8] give results in reasonable agreement with experimental data. However, some of the subtle effects observed in the study of polypeptide conformation and structural interconversions in solution[9] seem beyond the scope of existing treatments. Examples can be easily found among investigations involving conformational titrations wherein differences in the conformational stability of various polypeptides are explained on the basis of "side chain effects." Fasman's review[9] effectively illustrates the disparity between current theory and experiment. A compilation is given of a series of homopolypeptides with regard to the stability of their α-helical form in various nonaqueous solutions. Hydrophobic interactions are absent in these systems. However, optical rotatory dispersion measurements on these polymers, generally

(1) A report of work done under Contract No. 12-14-100-8933(73) with the U. S. Dept. of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.
(2) Presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.
(3) Submitted by C. F. N. in partial fulfillment of the requirements for the Ph.D. degree at Northwestern University, Chicago, Ill., Aug 1969.
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(6) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, 23, 283 (1968).
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