

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY, EVANSTON, ILLINOIS]

Protonation of Amides in a Helix-Breaking Solvent¹BY I. M. KLOTZ, S. F. RUSSO,² SUE HANLON,³ AND M. A. STAKE⁴

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Helix-coil transformations produced by trifluoroacetic acid have generally been ascribed to the ability of the acid to form competing hydrogen bonds. A variety of physical measurements (specific volumes, conductance, infrared, nuclear magnetic resonance) with a model amide, N-methylacetamide, in trifluoroacetic acid all show that the amide is protonated by strong acids. In polypeptides such protonation in itself should produce configurational changes due to electrostatic effects.

Helix-coil transformations of polypeptides have been produced, among other methods, by varying the proportion of polar and apolar components in appropriate mixed solvents. Particularly effective polar components have been acids such as dichloroacetic and trifluoroacetic acid.

The effectiveness of these acids in producing transitions in optical rotatory and viscosity parameters of solutions of polypeptides⁵⁻⁷ has been attributed to the ability of the acids to break peptide bonds primarily by forming their own competing hydrogen bonds with the peptide C=O and N-H groups.⁷⁻⁹ On the other hand, dichloroacetic and trifluoroacetic acids are strong acids and it has long been recognized that even a moderately strong acid such as formic is capable of protonating an amide group to some extent.¹⁰⁻¹² If we are to understand the molecular basis of the action of strong acids on polypeptides, it seems in order, therefore, to examine the possibility of protonation of peptides by dichloroacetic or trifluoroacetic acid. For this purpose we have used N-methylacetamide as a model amide¹³ and have investigated a number of physicochemical properties that might be expected to reflect the presence of ions produced on protonation of the amide by a strong acid.

Experimental

Partial Specific Volumes.—Densities of solutions of N-methylacetamide and of N,N-dimethylacetamide were measured in each of several solvents using standard pycnometric procedures. The pycnometers had volumes of 2-6 ml. The precision in apparent specific volumes is about 0.002 ml./g.

Conductances.—Electrical conductances were obtained from measurements of the resistances of solutions by a Model RC-1B conductivity bridge made by Industrial Instruments, Inc. The conductance cell, containing nonplatinized platinum electrodes, had a cell constant of 0.348 cm.⁻¹. Resistances were measured

with a precision of at least 2% in the range of 50 to 250,000 ohms. All measurements were carried out at 25°.

The resistance of trifluoroacetic acid was found to be considerably larger than 250,000 ohms and was negligible, therefore, in comparison to the amide and salt solutions. Most measurements were performed in a drybox to avoid possible artifacts due to water. Purposeful addition of up to 0.5% water to trifluoroacetic acid did not produce a significant increase in conductance.

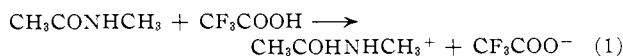
Infrared Spectra.—Absorption spectra in the 1.4-1.6 μ region were recorded with a Cary Model 14R spectrophotometer. The instrument was operated in the mode which sent the near-infrared radiation first through the monochromator and then through the absorption cells. Both sample and reference cells were mounted in thermostated cell holders. Temperatures were measured by a thermistor sealed into the stopper of the absorption cell. Quartz cells with optical paths of 0.5 to 5 cm. were used. Spectra in the 6 μ region were recorded with a Baird Associates, Inc., Infrared Recording Spectrophotometer Model 4-55.

Nuclear Magnetic Resonance.—All measurements were made with a Varian Associates A-60 Analytical n.m.r. spectrometer equipped with a V-6040 variable temperature controller. Pyrex n.m.r. tubes of 5.00 mm. (o.d.), purchased from Varian Associates, were filled with about 1 ml. of solution and closed with a plastic cap. The temperature at the sample was maintained within $\pm 2^\circ$. For most occasions the CH₃-C peak of N-methylacetamide served as an internal standard; in some cases tetramethylsilane or the sodium salt of 3-(trimethylsilyl)-1-propanesulfonic acid provided the reference resonance. Spectra were recorded in sections, usually the N-H region, the solvent region, and the CH₃ peaks, the amplitude and radiofrequency field being adjusted in each region to give the maximum signal to noise ratio.

Materials.—N-Methylacetamide, purchased from Eastman Organic Chemicals, was distilled at atmospheric pressure through a Vigreux column, and the fraction boiling at 205-206° was collected. N,N-Dimethylacetamide from the same supplier was distilled in a similar fashion and the fraction boiling at 164-165° was collected. Trifluoroacetic acid, from the same supplier, was also distilled and the fraction boiling at 70.5-71.0° was collected and stored in a drybox. For use in the conductance measurements this acid was distilled from a pot containing P₂O₅. Potassium trifluoroacetate was purchased from Peninsular Chem-Research, Inc., and was stored and measured out in a drybox.

Results

Apparent Specific Volumes.—One of the distinctive characteristics of ionic aqueous solutions is the large electrostriction produced in the solvent by the charged solute species. If N-methylacetamide becomes protonated in trifluoroacetic acid



one might reasonably hope to see an electrostrictive effect in this solvent too.

Measurements of the densities of solutions of this amide in trifluoroacetic acid were converted into apparent specific volumes, ϕ_{v2} , with the use of the equation

$$\phi_{v2} = (1/\rho_0) \left[1 + \frac{\rho_0 - \rho_1}{C_2} \right] \quad (2)$$

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(2) Part of this paper constituted the Ph.D. dissertation (Northwestern University, 1964) of S. F. Russo.

(3) National Science Foundation Fellow 1961-1962; Public Health Service Postdoctoral Fellow 1962-1963.

(4) Public Health Service Postdoctoral Fellow 1963-1964.

(5) J. T. Yang and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

(6) G. E. Perlmann and E. Katchalski, *ibid.*, **84**, 452 (1962).

(7) G. D. Fasman in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962.

(8) C. H. Bamford, W. E. Hanby, and F. Happy, *Proc. Roy. Soc. (London)*, **A206**, 407 (1951).

(9) S. J. Singer, *Advan. Protein Chem.*, **17**, 1 (1962).

(10) J. R. Schaefgen and C. F. Trivisonno, *J. Am. Chem. Soc.*, **73**, 4580 (1951).

(11) P. R. Saunders, *J. Polymer Sci.*, **43**, 273 (1960).

(12) B. Becker and A. W. Davidson, *J. Am. Chem. Soc.*, **85**, 159 (1963).

(13) I. M. Klotz and J. S. Franzen, *ibid.*, **84**, 3461 (1962).

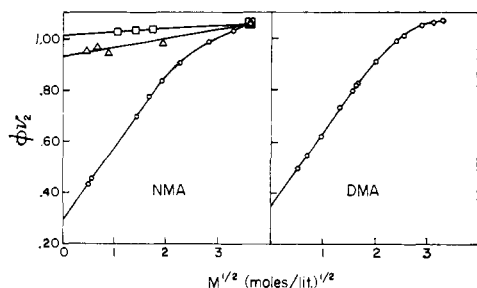


Fig. 1.—Apparent specific volumes, ϕv_2 , as a function of the square root of the molar concentration of amide, N-methylacetamide (NMA), or N,N-dimethylacetamide (DMA). Solvents are: trifluoroacetic acid, O; acetic acid, Δ ; carbon tetrachloride, \square .

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

The results for N-methylacetamide are shown in Fig. 1 for each of three separate solvents, trifluoroacetic acid, acetic acid, and carbon tetrachloride. In the last solvent, ϕv_2 for the amide varies only slightly with concentration, and all values are near that for pure liquid N-methylacetamide, 1.06 ml./g. Such behavior is in accord with the fact that no protonation of amide can occur from carbon tetrachloride. The observations in trifluoroacetic acid (Fig. 1) stand in marked contrast, however, ϕv_2 dropping very sharply with decreasing concentration of amide. Extrapolation to zero concentration of solute leads to a ϕv_2^0 of 0.30 ml./g., a value markedly smaller than 1.06 for pure liquid solute. Similar behavior is observed with N,N-dimethylacetamide in trifluoroacetic acid (Fig. 1). For both solutes, furthermore, ϕv_2 varies linearly with $C^{0.5}$ (but not with C) over a wide concentration range at the dilute end, as has been found to be true for ionic solutions in aqueous solvents.¹⁴⁻¹⁶ Volume measurements thus point strongly to the presence of large concentrations of ionic species in solutions of N-methylacetamide in trifluoroacetic acid.

It is of interest to note in passing that ϕv_2 values for N-methylacetamide dissolved in acetic acid are close to those in CCl_4 (Fig. 1), but, nevertheless, significantly lower. Apparent volumes thus indicate the possible presence of a small fraction of ions when the amide is dissolved in this weak acid. With this evidence alone, however, one cannot make a strong argument, for the small drop in ϕv_2 could be attributed to hydrogen bonds between the CH_3COOH solvent and $\text{CH}_3\text{CONHCH}_3$ solute.

Conductance.—Ionic solutions, in general, should be good conductors of electricity, but some caution must be observed in interpreting observations with solvents of low dielectric constant since ion pairs, which would be nonconducting, may be formed. Trifluoroacetic acid has a dielectric constant^{17,18} of 8.3 and thus would be expected to be a solvent conducive to ion-pair formation. That such indeed is the situation was shown in preliminary measurements of the conductance of the salt $\text{K}^+\text{CF}_3\text{COO}^-$ in trifluoroacetic acid (Fig. 2). Equivalent conductances, Λ , for this salt are about 1–4 $\text{ohm}^{-1}\text{equiv.}^{-1}\text{cm.}^2$, about 50-fold smaller than for aque-

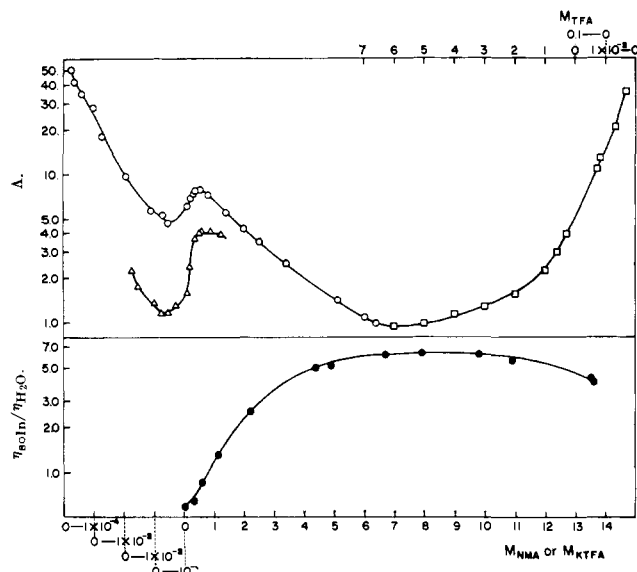
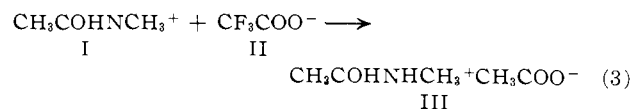


Fig. 2.—Top: equivalent conductances at 25° as a function of molar concentration of solute; N-methylacetamide in trifluoroacetic acid, O; potassium trifluoroacetate in trifluoroacetic acid, Δ ; trifluoroacetic acid in N-methylacetamide, \square . Note that the scale for the abscissa has been expanded in increasing steps at low concentrations, so that the rise in conductance with dilution can be represented adequately. Bottom: relative viscosity ($\eta_{sp01n}/\eta_{\text{H}_2\text{O}}$) of solutions of N-methylacetamide in trifluoroacetic acid, as a function of the molar concentration of amide.

ous solutions. Since the viscosities of the two solvents are similar in magnitude, the solute, $\text{K}^+\text{CF}_3\text{COO}^-$, must exist predominantly as ion pairs in trifluoroacetic acid. As one expects for such a system,¹⁹ there is a sharp upward turn in conductance as one goes toward very dilute solutions in which ion pairs must dissociate due to mass-law effects.

With this background we may turn to solutions of N-methylacetamide. The conductance of this solute in solutions of trifluoroacetic acid (Fig. 2) is remarkably high compared to that of $\text{K}^+\text{CF}_3\text{COO}^-$, a striking indication of the presence of ions. Their origin must be the protonation reaction of eq. 1.

The general shape of the curve in Fig. 2 is typical of an ion-pair interacting system in nonaqueous solvents.¹⁹ At very low concentrations the conductance of N-methylacetamidinium trifluoroacetate is very high and drops steeply with increasing concentration. Such behavior is indicative of the association reaction to ion pairs (III). From conductance data alone it is



not possible to tell whether the associated form (III) is an ionophore¹⁹ or ionogen. On the other hand, the very small value of the apparent specific volume, ϕv_2 , of N-methylacetamide at concentrations of 0–0.1 M points strongly to the presence of ionic species rather than molecular complexes in the associated state (III). The strong electrostrictive effect at these concentrations also favors structure IV rather than V for the ion pair.

As one proceeds to concentrations above about 0.05 M , conductances rise again and reach a maximum near 0.5 M N-methylacetamide. Similar trends have

(19) R. M. Fuoss and F. Accascina, "Electrolytic Conductance," Interscience Publishers, Inc., New York, N. Y., 1959, pp. 5, 9, 249–271.

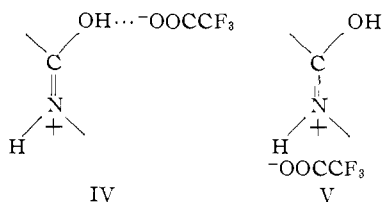
(14) D. O. Masson, *Phil. Mag.*, [7] 8, 218 (1929).

(15) W. Geffcken, *Z. physik. Chem.*, **A155**, 1 (1931).

(16) O. Redlich, *J. Phys. Chem.*, **44**, 619 (1940).

(17) W. Dannhauser and R. H. Cole, *J. Am. Chem. Soc.*, **74**, 6105 (1952).

(18) F. E. Harris and C. T. O'Konski, *ibid.*, **76**, 4317 (1954).



been observed with other electrolytes in solvents of low dielectric constant and have attributed to the appearance of clusters of three ions.¹⁹ Such configurations would be expected to arise from the interaction of an ion-pair dipole with another anion or cation, and the cluster formed would convert an ion pair into a conducting triplet. It is the increase in concentration of triple-ion cluster that produces the minimum and subsequent upswing in conductance in the range between 0.05 and 0.5 *M*. In the present system, the triple ion may be either of the following two species, (II⁻)(I⁺)(II⁻) or (I⁺)(II⁻)(I⁺), where I and II represent the species shown in eq. 3.

Above 0.5 *M* concentration the conductance of *N*-methylacetamide solutions drops once again. Above this concentration, in a complementary manner, the viscosity of the solution (Fig. 2) begins to rise rapidly and reaches a maximum at about 6 *M* (60 mole %) amide. It seems, therefore, that the conductance is responding largely to the viscosity of the solution.

Above a concentration of amide which exceeds that of acid, equivalent conductances were computed in terms of concentration of trifluoroacetic acid since the acid would be the limiting component required for the protonation reaction (eq. 1). Equivalent conductances so computed show a step rise (Fig. 2) in solutions above 7 *M* in amide. This rise reflects in part the turn-around in viscosity (Fig. 2). However, it is obvious that the drop in viscosity is small compared to the increase in Λ . Recalling that pure *N*-methylacetamide has an unusually high dielectric constant²⁰ of 185 we are inclined to attribute the increase in Λ at high concentrations of amide to the increasing dissociation of ion pairs with their increasing dilution in solvent mixtures of increasing dielectric constant. In accord with this interpretation are some measurements of the conductances of trifluoroacetic acid-dimethylacetamide solutions which are invariably lower than those of corresponding solutions with methylacetamide. Dimethylacetamide has a dielectric constant²¹ of only 36.

In summary, then, solutions of *N*-methylacetamide-trifluoroacetic acid show conductance properties which accord fully with the behavior expected for ion pairs and higher ionic clusters in nonaqueous solvents. Again it seems evident that the protonation reaction of eq. 1 has proceeded very extensively to the right-hand side.

Infrared Spectra.—It has been shown previously¹³ that when $\text{CH}_3\text{CONHCH}_3$ is in the unassociated form in carbon tetrachloride, dioxane, or water, the overtone of the N-H stretching vibration of the amide falls at 1.47–1.48 μ . Upon formation of an interamide hydrogen bond, $\text{N}-\text{H}\cdots\text{O}=\text{C}$, two characteristic bands appear in all solvents, a major peak at 1.525

μ and a minor one at around 1.575 μ . When *N*-methylacetamide is dissolved in trifluoroacetic acid, however, none of these characteristic features of the spectrum is evident. Instead, the spectrum at the lowest concentration of *N*-methylacetamide, shown in Fig. 3, exhibits a double-humped band between 1.510 and 1.525 μ as well as a slight shoulder between 1.48 and 1.49 μ . Thus, the amide must be in a different state in this solvent than in those previously examined.

One might be inclined to attribute all of the observed differences to hydrogen-bonded associations between the amide and the carboxyl groups of the acid. This possibility, however, was excluded by the information provided by spectra of *N*-methylacetamide in acetic acid. As Fig. 4 shows, the major peak appears at 1.485 μ in the spectrum of this amide dissolved in CH_3COOH despite the provision of the carboxyl group to which the amide is presumably hydrogen bonded.²²

It thus seemed likely that the shoulder at 1.48 μ observed in the spectra of *N*-methylacetamide in trifluoroacetic acid is due to amide N-H hydrogen bonded to the carboxyl groups of the acid. The 1.51 and 1.525 μ bands, however, are inexplicable on this basis. Hence, on spectroscopic grounds alone, one would suspect that these peaks are due to the strong acidity of trifluoroacetic acid and the consequent protonation of the amide by this solvent (eq. 1). This conjecture was supported by the conductance and volume experiments described above, as well as by further spectroscopic observations on *N*-methylacetamide in a variety of strong mineral acids.

Protonation of *N*-methylacetamide in a solvent mixture consisting of perchloric acid, dioxane, and water has been followed by the technique of nuclear magnetic resonance.²³ Under conditions where these data indicate protonation of the amide (predominantly on the oxygen), the near-infrared spectra of *N*-methylacetamide, shown in Fig. 4, reveal a peak at 1.51 μ together with a shoulder at about 1.525 μ . Solutions of *N*-methylacetamide in other strong acids, such as concentrated sulfuric and concentrated hydrochloric, also exhibit maxima at 1.51 μ . In the case of the spectrum in HCl (Fig. 4), the peak is symmetrical about 1.51 μ without any evidence of a shoulder in the 1.53– μ region. Thus it seems clear that a 1.51- μ peak in the spectra of *N*-methylacetamide in trifluoroacetic acid can be identified as that due to the overtone of the NH group of the amide in the protonated state.

The 1.525- μ peak of *N*-methylacetamide in trifluoroacetic acid as well as the shoulder observed in other oxy acids are probably due to additional hydrogen bonding between the NH of the protonated series and either undissociated acid or the acid anion. The lowest concentration of amide in trifluoroacetic acid which can be examined spectroscopically is still quite concentrated from the standpoint of the conductometric behavior (see Fig. 2). The spectrum at the lowest concentration of amide shown thus represents the spectrum of several protonated amide species—simple ion pairs as well as triple and perhaps higher polyions. The NH-stretching overtone in these higher ionic

(22) The additional small peak at 1.538 μ in this spectrum seems to be a characteristic of the solvent-associated amide as it is a persistent feature at lower amide concentrations.

(23) A. Berger, A. Loewenstein, and S. Meiboom, *J. Am. Chem. Soc.*, **81**, 62 (1959).

(20) B. J. Bass, W. I. Nathan, R. M. Meigham, and R. H. Cole, *J. Phys. Chem.*, **68**, 509 (1964).

(21) G. R. Leader and J. F. Gormley, *J. Am. Chem. Soc.*, **73**, 5731 (1951).

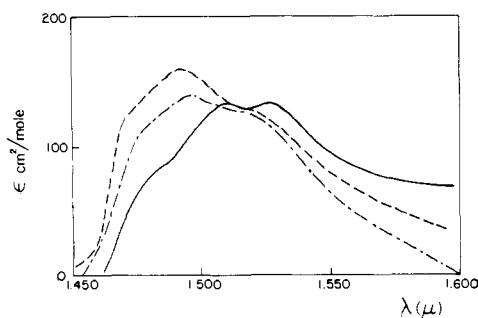


Fig. 3.—Spectra of N-methylacetamide (NMA) in trifluoroacetic acid, with dimethylacetamide (DMA) in reference cell: —, 0.466 *M* NMA vs. 0.35 *M* DMA; - - - -, 3.00 *M* NMA vs. 2.40 *M* DMA; - · - ·, 4.00 *M* NMA vs. 3.33 *M* DMA.

species would be expected to fall at a wave length higher than that of the unassociated protonated species.

As demonstrated in Fig. 3, the spectrum of N-methylacetamide in trifluoroacetic acid changes markedly with concentration. As the ratio of acid to amide decreases, the absorption at 1.48 and 1.47 μ continues to rise, indicating that the percentage of total amide which is unprotonated increases with increasing concentration of amide. The 1.48- μ band has been identified as that of a specific hydrogen-bonded complex between the acid solvent and the amide. The 1.47- μ shoulder is very likely attributable to amide which is unassociated with solvent. This is indicated by the spectra of N-methylacetamide in solutions of trifluoroacetic acid containing variable amounts of the salt, potassium trifluoroacetate. As the concentration of the potassium trifluoroacetate is increased, a 1.47- μ peak appears in the near-infrared spectra; this is what one would expect from a reversal of the protonation reaction eq. (1), as well as from competition of the acid anion for any undissociated CF_3COOH .

A very brief qualitative survey of spectra in the 6- μ region was also made. Quantitative measurements would be difficult in this range because the absorptions are so strong that exceedingly thin optical paths must be used. For our measurements, a thin film of liquid was placed between two Irtran plates and mounted in the spectrophotometer.

Liquid N-methylacetamide shows two absorption peaks, as expected, at 6.05 and 6.40 μ , ascribed to the $\text{C}=\text{O}$ stretching and $\text{N}-\text{H}$ bending vibrations. Liquid trifluoroacetic acid was found to have a band at 5.6- μ , attributable to the COOH group. Potassium trifluoroacetate dissolved in trifluoroacetic acid showed a new band at 6.15 μ which is clearly due to COO^- groups.

A solution of 5.1 *M* N-methylacetamide in trifluoroacetic acid showed two peaks, at 5.6 and 6.2 μ , and no clear absorption peaks at 6.0 or 6.4 μ . The 6.2- μ band indicates the presence of COO^- ions which should form on protonation (eq. 1) of the amide. The band at 5.6 μ , which is definitely smaller than in pure trifluoroacetic acid, indicates some residual acid. In general, then, the changes in spectra are in accord with the changes in structure to be expected if the acid solvent protonates the amide.

Nuclear Magnetic Resonance.—A typical n.m.r. spectrum of a solution of N-methylacetamide in trifluoroacetic acid is shown in Fig. 5. The hydrogens

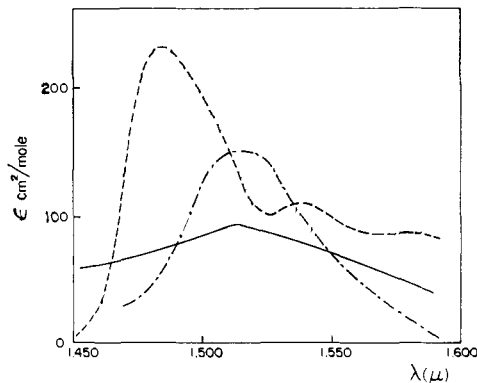
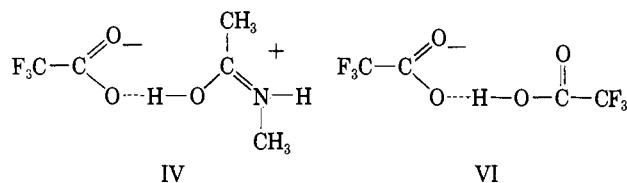


Fig. 4.—Spectra of N-methylacetamide (NMA) in strong and weak acids, with dimethylacetamide (DMA) in reference cell: - - - -, 0.419 *M* NMA vs. 0.329 *M* DMA in acetic acid; - · - ·, 1.10 *M* NMA vs. 0.89 *M* DMA in 8.4 *M* HClO_4 , 2.5 *M* dioxane, 19.2 *M* H_2O ; —, 1.478 *M* NMA vs. 1.209 *M* DMA in 10.7 *M* HCl , 36.5 *M* H_2O .

of the CH_3-C of the amide have been used as the zero point. Reading downfield the next band at 40 c.p.s. is known to be attributable to the $\text{N}-\text{CH}_3$ and that at 370 c.p.s. to amide $\text{N}-\text{H}$, so that the peak at 800 c.p.s. can be assigned to CF_3COOH .

There is a marked shift in the position of this OH peak of trifluoroacetic acid with concentration of amide added to the solvent. This shift to low fields is illustrated in Fig. 6. Our results with N-methylacetamide-trifluoroacetic acid are fully analogous to those published by Reeves²⁴ for corresponding solutions with N-methylformamide. In both cases the chemical shift to low field implies the formation of a very strong hydrogen bond. In both cases such a hydrogen bond would exist if the acid protonated the amide (eq. 1) and products such as the following formed



In view of the following experiments, it seems likely that the hydrogen bond of structure VI²⁵ is the primary reason for the downfield shift in resonance of CF_3COOH . If $\text{CF}_3\text{COO}^-\text{K}^+$ is added to pure CF_3COOH , downfield shifts are obtained (Fig. 6) which are essentially identical with those for solutions of amide in trifluoroacetic acid. When $\text{CF}_3\text{COO}^-\text{K}^+$ is added, only structure VI, and not IV, can be formed. Similar shifts are obtained when $\text{CF}_3\text{COO}^-\text{K}^+$ is added to a solution of 0.5 *M* amide in trifluoroacetic acid. The solubility of $\text{CF}_3\text{COO}^-\text{K}^+$ in trifluoroacetic acid is only about 2 *M*, and hence this salt could not be added to concentrations high enough to parallel the full curve (Fig. 6) for amide.

Discussion

The sum of the four or five different experimental approaches used seems to point inescapably to the conclusion that N-methylacetamide is protonated in trifluoroacetic acid solutions. In a sense this conclusion

(24) L. W. Reeves, *Can. J. Chem.*, **39**, 1711 (1961).

(25) The existence of structures such as VI has also been shown by infrared studies; see W. Klemperer and G. C. Pimentel, *J. Chem. Phys.*, **22**, 1399 (1954).

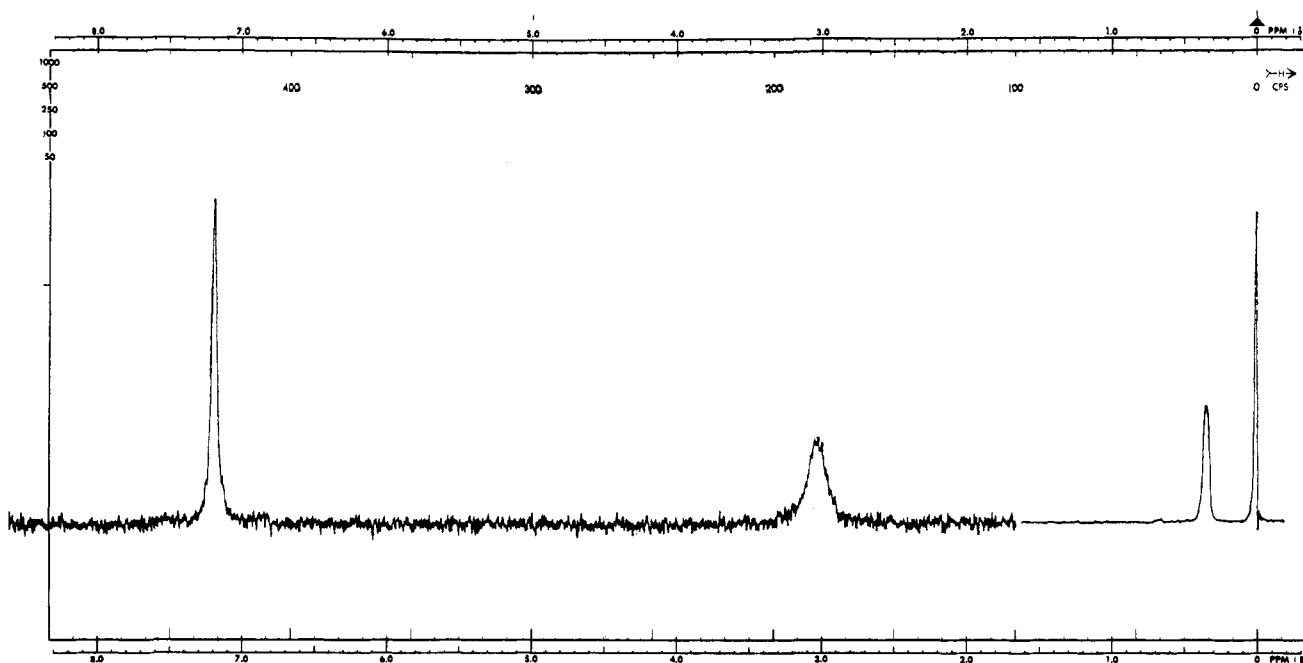


Fig. 5.—Nuclear magnetic resonance spectrum of solution of 6.0 *M* *N*-methylacetamide in trifluoroacetic acid, at 25°. Abscissa scale actually in use is 0–1000 c.p.s.

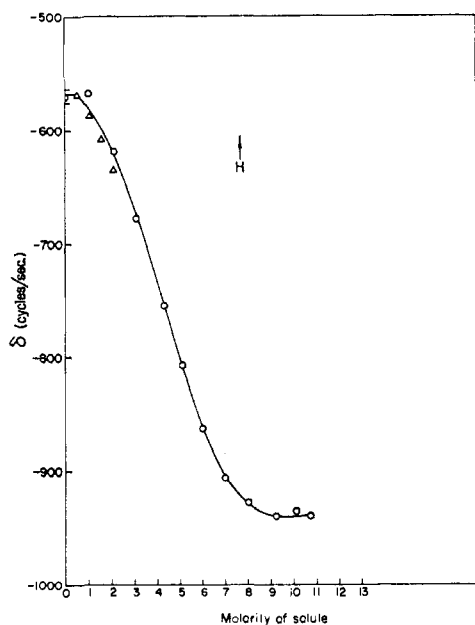


Fig. 6.—Chemical shifts δ (relative to $\text{CH}_3\text{-C}$) of resonance of CF_3COOH upon addition of solutes: O, *N*-methylacetamide; Δ , potassium trifluoroacetate.

could have been anticipated from various uncoordinated separate reports in the literature. Almost

40 years ago Conant and Hall²⁶ showed that “superacids” such as perchloric and sulfuric acid would neutralize amides in suitable solvents. (In the present studies trifluoroacetic acid provides both the solvent and the “superacid”). Also other acids have been found to protonate amide groups to varying extents as judged from separate viscosity,¹⁰ light scattering,¹¹ phase diagram,¹² conductance,²⁷ or nuclear magnetic resonance²⁴ experiments. In essence, then, the present investigation has merely taken one amide-acid system and examined it thoroughly by a variety of modern and classical techniques. Thus, overwhelming evidence has been provided that the model amide, $\text{CH}_3\text{-CONHCH}_3$, becomes protonated when dissolved in a strong acid solvent.

One would expect the amide groups of polypeptides to show similar basic properties and to become protonated in strongly acidic solutions. The electrostatic effects produced thereby should cause configurational changes in the polypeptide. Some experiments in this connection have been reported in a preliminary fashion^{28,29} and will be discussed in detail presently.

(26) J. B. Conant and N. F. Hall, *J. Am. Chem. Soc.*, **49**, 3062 (1927).

(27) Y. I. Bokhovkina and I. M. Bokhovkin, *J. Struct. Chem. USSR*, **26**, 1872 (1956).

(28) S. Hanlon, S. F. Russo, and I. M. Klotz, *J. Am. Chem. Soc.*, **85**, 2024 (1963).

(29) S. Hanlon and I. M. Klotz, *Federation Proc.*, **23**, 215 (1964).