to take part in the chain transfer reaction, either because it is evaporated, as has been suggested in the case of polymethyl methacrylate,[13] or because it is resonance-stabilized by an unsaturated chain end. In either case the effect is to decrease the number of unzipping chains produced by each initiation step, thereby reducing the quantum yield of monomer, and to introduce a first-order chain termination step, thereby increasing the intensity exponent. The verification of such a mechanism would be very difficult, particularly when one considers such complications as the effect of melt viscosity, which depends on temperature and molecular weight, and on diffusion-controlled termination reactions.

SUMMIT, NEW JERSEY

[CONTRIBUTION FROM THE WEIZMANN INSTITUTE OF SCIENCE]

Nuclear Magnetic Resonance Study of the Protolysis and Ionization of N-Methylacetamide[1]

BY A. BERGER, A. LOEWENSTEIN AND S. MEIBOOM

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The rate and mechanism of protolysis in N-methylacetamide (I) in aqueous solution has been investigated by the nuclear magnetic resonance technique. The protolysis was found to be both acid and base catalyzed, the reaction rate being proportional to the amide concentration and to the hydrogen ion or hydroxyl ion concentration. It was found that in N,N-dimethylacetamide (II) free rotation around the C–N bond occurs on acidification. This phenomenon is closely related to the protolysis in I, both being due to protonation at the nitrogen atom. It could be shown, however, from a study of the n.m.r. spectrum in very acidic solution, that the protonation occurs predominantly at the oxygen, both ionic species being in equilibrium. A detailed mechanism for the exchange reactions is proposed.

Introduction

In this paper we report measurements of the protolysis kinetics in N-methylacetamide by the nuclear magnetic resonance (n.m.r.) technique.[2] This compound is of special interest, as it provides the simplest model of the RCONHR′ grouping characteristic of peptides. Its chemical and physical properties have been studied extensively.[3–7]

Exchange rates of the amide hydrogens in proteins[8] and polypeptides[9] have been measured by isotope labelling and the results obtained were interpreted in terms of the extent and stability of the hydrogen bonds formed by the amide groups. However, in the absence of stable hydrogen bonds, *e.g.*, in low molecular weight peptides, the exchange is too fast to be measured by isotope labelling. Direct measurements of the reaction constants in the absence of hydrogen bonding, as well as an understanding of the reaction mechanism can be obtained from n.m.r. spectra. This technique is a powerful tool in the study of fast exchange reactions, being applicable to reactions with half-life times between about 1 and 0.001 of a second.

The n.m.r. spectrum of N-methylacetamide in aqueous solution at *p*H 5 is shown in Fig. 1. From left to right (in order of increasing frequency) this spectrum shows a single line due to the C-methyl hydrogens, a doublet due to the N-methyl hydrogens, the water line and a broad line due to the amide hydrogen. The splitting of the N-methyl resonance is a result of spin–spin interaction with the amide hydrogen. The sharpness of the components of this doublet shows that the rate of exchange of the amide hydrogen is relatively slow, the half-life time being more than one second.

The resonance of the amide hydrogen would be expected to show a triplet structure because of spin–spin interaction with the N^{14} nucleus (spin 1), with a superposed quadruplet structure from interaction with the N-methyl group. However, because of the fast quadrupole relaxation of the N^{14} nucleus only one broadened line is actually observed.[10]

Figure 2 shows the changes in the N-methylacetamide n.m.r. spectrum when the *p*H of the solution is varied. The observed changes are the result of the increased rate of exchange of the amide hydrogen. Note the broadening of the water line, which is the result of hydrogen exchange between the water and the NH group.

An additional and unexpected feature of the spectrum, which is not resolved in Figs. 1 and 2, should be mentioned. It was found that a spin–spin interaction of about 0.45 c./s. exists between the C-methyl and the N-methyl hydrogens. These interactions are observed at the highest resolution obtainable and with very slow passage. In acidic and basic solutions, where the amide hydrogen exchanges rapidly, and its spin–spin interactions are averaged out, both methyl resonances are quadruplets (see Fig. 3). At a *p*H of about 5, where the ex-

(1) This research has been sponsored in part by the Air Force Office of Scientific Research of the Air Research and Development Command, USAF, through its European Office, under Contract No. AF 61 (052)-03.

(2) While this work was in progress, M. Takeda privately communicated to us that he had measured the protolysis of N-methylacetamide in acid solution by the n.m.r. technique.

(3) S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, Inc., New York, N. Y., 1954, pp. 117–152; "Advances in Protein Chem.," **9**, 299 (1954).

(4) L. R. Dawson, P. G. Sears and R. H. Graves, THIS JOURNAL, **77**, 1986 (1955).

(5) A. R. Goldfarb, A. Mele and N. Gutstein, *ibid.*, **77**, 6194 (1955).

(6) J. Bello, *J. Phys. Chem.*, **60**, 1341 (1956).

(7) M. Davies, J. C. Evans and R. L. Jones, *Trans. Faraday Soc.*, **51**, 761 (1955).

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(10) H. S. Gutowsky, D. W. McCall and C. P. Slichter, *J. Chem. Phys.*, **21**, 279 (1953).

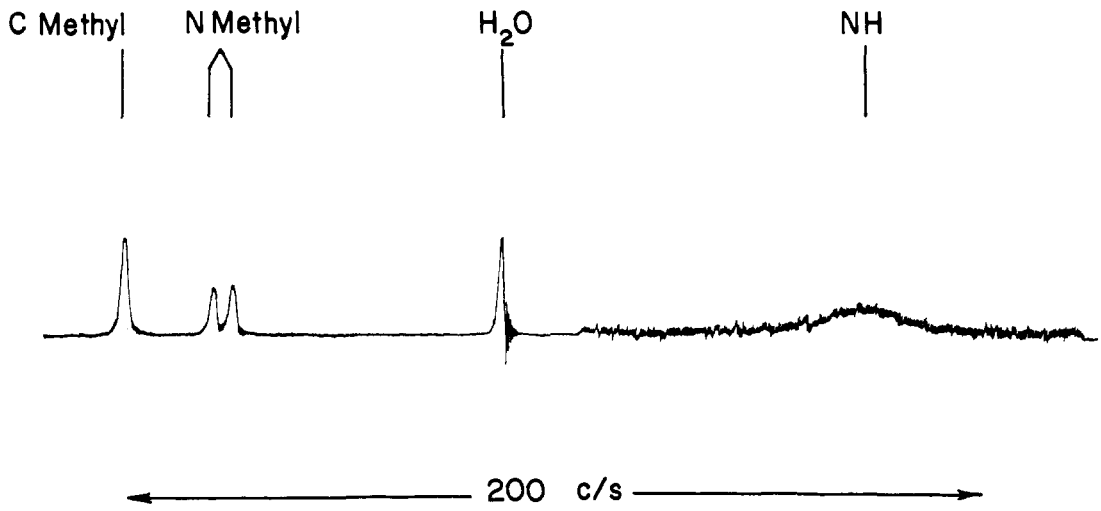


Fig. 1.—N.m.r. spectrum of N-methylacetamide in aqueous solution at *p*H 5. Frequency increases from left to right. Proton frequency 31.65 Mc./s. The H_2O resonance was recorded at a lower gain, and the NH resonance at a higher gain than the methyl lines.

change is slow, both N-methyl components are quadruplets (see Fig. 4). In this case the C-methyl resonance is not well defined and probably consists of two overlapping quadruplets, indicating the presence of a small spin-spin interaction with the amide hydrogen. The existence of these spin–spin interactions has been taken into account in the quantitative interpretation of the exchange broadening of the N-methyl group.

In elucidating the mechanism of hydrogen exchange, two other problems were found to be closely related to it. The first one is the question whether the protonation in acid solution occurs at the oxygen or at the nitrogen atom. Observations reported in literature[3,5,6] seem to be inconclusive. We found that under extremely acid conditions, the N-methyl resonance again becomes a doublet, indicating that the protonation occurs almost exclusively at the oxygen.

The second problem is that of free rotation around the C–N bond. In the closely related compound N,N-dimethylacetamide, the absence of free rotation in the pure compound has been demonstrated[11] by the observation of two N-methyl lines in the n.m.r. spectrum. This doublet is the result of the non-equivalence (*cis* and *trans*) of the two N-methyl groups. We found that in aqueous solution this doublet collapses into one line on acidification, indicating the onset of free rotation. The implications of these observations will be discussed below.

Experimental

Some details of the n.m.r. spectrometer used in this investigation have been given previously.[12]

N-Methylacetamide was prepared as follows. A solution of methylamine in excess ethyl acetate was kept for 2 weeks at room temperature in a tightly stoppered bottle. By this time only about 5% of the original amount of methylamine remained, as found by anhydrous titration. The mixture was then fractionated *in vacuo*. The product, collected between 110 and 114° at 30 mm., melted at 29–30°.

Solutions of known hydrogen ion concentration were prepared as follows: In the acidic range (*p*H 0.8–2.0) measured volumes of standard hydrochloric acid were added to the N-methylacetamide solutions. The hydrogen ion concentration was calculated, applying a correction for bound hydrogen ions. The correction values were taken from potentiometric titration curves (Fig. 5) obtained with methylacetamide solutions at the required concentrations.

In the basic range (*p*H 8.0 to 9.5) the solutions were prepared by adding small quantities of NaOH (0.1 or 0.01 *N*) to the N-methylacetamide solutions. The base was added and the sample-holder was filled in an atmosphere of nitrogen. The hydrogen ion concentration was measured by means of a *p*H meter (Metrohm type E 148c with type U glass electrode). The *p*H readings were corrected for the effect of amide concentration on the glass electrode potential, the correction values being taken from the potentiometric titration curves given in Fig. 6. This figure shows that the titration curves are shifted to higher *p*H readings with increased amide concentration, the shift being roughly proportional to the amide concentration. This effect, together with the difficulty of working with weakly buffered solutions in this *p*H range, reduced the accuracy of the *p*H determinations. The accuracy in hydrogen ion concentration in this range is estimated at ± 0.1 *p*H unit.

Interpretation of the N.m.r. Spectra

The exchange rates were determined from the broadening of the N-methyl doublet and of the water line, the procedure being similar to the one described in a previous paper.[13] The resonance of the amide hydrogen is too broad to give useful information.

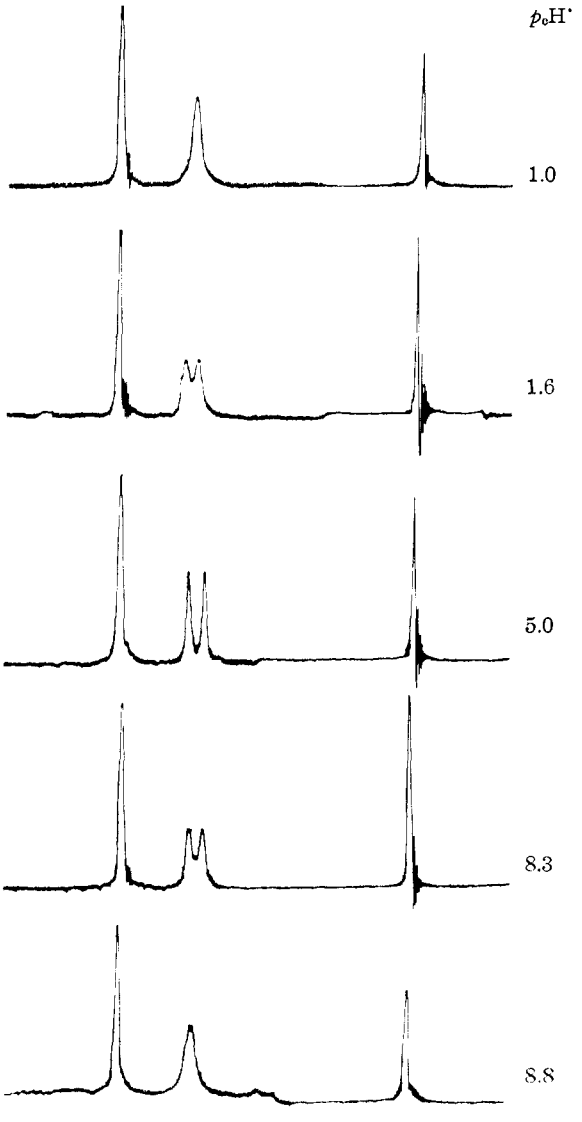
The quantitative interpretation of the exchange broadening of the N-methyl doublet is complicated by the fact that, as described in the Introduction, each of its components is actually a closely spaced quadruplet. The derivation of equations for the exchange broadening in this case presents in principle no difficulties. However, the final equations become very cumbersome, and the necessary numerical calculations too laborious to be worthwhile. Instead, equations for the exchange broadening of a simple doublet were used and the quadruplet structure taken into account by the introduction of an effective line width (see Appendix A).

At relatively low rates of exchange, where the N-methyl resonance shows doublet structure, the ratio

(11) W. D. Phillips, *J. Chem. Phys.*, **23**, 1363 (1955); H. S. Gutowsky and C. H. Holm, *ibid.*, **25**, 1228 (1956).

(12) E. Grunwald, A. Loewenstein and S. Meiboom, *ibid.*, **27**, 630 (1957).

(13) A. Loewenstein and S. Meiboom, *ibid.*, **27**, 1067 (1957).



N-methylacetamide, 2.6M.

Fig. 2.—Spectra of N-methylacetamide in aqueous solution (2.6 M) at different p_cH values.

of maximum to central minimum of the resonance line served as a measure of the exchange rate. The curves used are those of Fig. 3 of reference 13. At higher rates of exchange a single line is observed and its width at half amplitude measures the exchange rate. The relevant curves are given in Fig. 4 of reference 13.

The width of the water line was measured from the decay of the "wiggles" on fast passage. Equation 6 of reference 13 was used to evaluate the mean residence time of a hydrogen atom in water, τ_W. It was found that the viscosity of the N-methylacetamide solutions increased appreciably with concentration, and a correction for the increased line width due to this effect was applied. This correction was computed on the assumption that the natural line width of the water line in the absence of exchange is proportional to the viscosity of the solution. Observed values for the ratio of the viscosity of the solution to the viscosity of water are given in Table I. Viscosities were measured with an Ostwald viscosimeter.

TABLE I

COMPARISON OF THE RATES OBTAINED FROM THE N-METHYL DOUBLET WITH THOSE OBTAINED FROM THE WATER LINE

[Amide] (M)	[H$^+$] (M)	pO^{-s}/[H$^+$] (M)	η_{rel}[a]	τ_W (sec.)	R[b] (sec.$^{-1}$)	R'[c] (sec.$^{-1}$)	$p = R'/R$
			Acidic range				
6.2	0.11		3.52	0.17	43	47	1.08
4.3	.12		2.44	.49	34	26	0.78
4.1	.14		2.35	.25	62	60	0.97
2.7	.17		1.75	.38	66	75	1.14
2.1	.45		1.52	1.52	25	19	0.76
2.1	.15		1.52	0.62	56	59	1.05
						Av.	0.96
			Basic range				
3.1		4.8	1.89	0.61	25	37	1.48
3.1		7.2	1.89	0.40	42	60	1.43
1.6		12.1	1.36	1.07	35	49	1.40
0.8		18.5	1.18	1.41	72	76	1.07
5.5		7.1[d]	3.10	0.58	10	10	1.00
5.5		15.8[d]	3.10	.29	34	30	0.88
3.3		10.0[d]	1.97	.69	27	25	0.92
2.0		14.4[d]	1.49	.74	46	52	1.13
1.1		13.2[d]	1.30	1.73	47	40	0.85
						Av.	1.12

[a] Ratio of viscosity of solution to viscosity of water. [b] Specific rate as determined from the N-methyl resonance (eq. 1). [c] Specific rate as obtained from the water line (eq. 2). [d] These measurements were made at a later date than the main body of observations, with the special purpose to investigate whether the apparently systematic deviation of p from unity of the earlier measurements was significant. A special effort was made to observe the rather small broadening of the water line as accurately as possible.

Results

In the n.m.r. technique the quantity measured is essentially the mean lifetime, τ_A, of a molecule between successive proton exchanges. We call the reciprocal of this quantity the "specific rate," R which is related to the reaction rate, d[amide]/dt, by the equation

$$R \equiv \frac{1}{\tau_A} = \frac{1}{[\text{amide}]} \frac{d[\text{amide}]}{dt} \qquad (1)$$

Results for the specific rate, as evaluated from the broadening of the N-methyl doublet, are given in Fig. 7 for the acidic range (pH < 2). This figure gives R as function of hydrogen ion concentration for a number of amide concentrations. Figure 8 gives similar results for the basic range (pH > 8). From these figures it can be seen that the specific rate is essentially independent of amide concentration in both ranges. The specific rate is proportional to hydrogen ion concentration in the acidic range and to the reciprocal of the hydrogen ion concentration in the basic range. The scatter of the points is within the limits of the experimental precision. An independent determination of the specific rate R of the amide can be obtained from the broadening of the water line. If it is assumed that the exchange reaction always results in the transfer of a proton between amide and water (*i.e.*, no direct transfers between amide molecules occur), the mean residence time of a proton in the amide (τ_A) and that of a proton in water (τ_W) are propor-

Fig. 3.—C-Methyl (left) and N-methyl (right) resonances of N-methylacetamide at very high resolution; pH about 13. The spin–spin interaction between the two methyl groups is about 0.45 c./s.

Fig. 4.—C-Methyl (left) and N-methyl (doublet at right) resonances of N-methylacetamide at very high resolution; pH about 5.

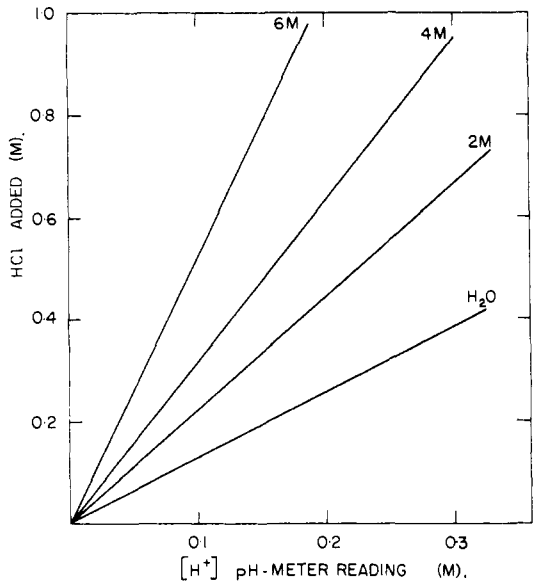


Fig. 5.—Potentiometric titration curves of aqueous N-methylacetamide solutions, acidic range. The concentration of HCl is plotted against hydrogen ion activity, as measured with a glass electrode.

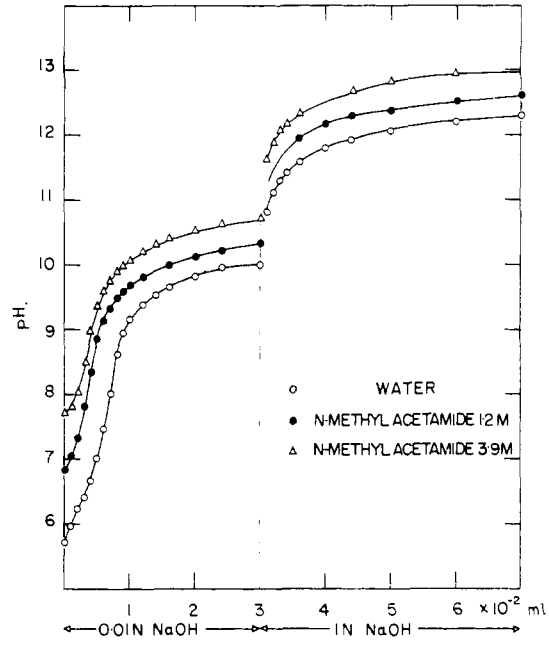


Fig. 6.—Potentiometric titration curves of aqueous N-methylacetamide solutions, basic range.

tional to the respective proton fractions. We have accordingly

$$R \equiv \frac{1}{\tau_A} = \frac{1}{\tau_W}\frac{2[H_2O]}{[\text{amide}]} \qquad (2)$$

The validity of the assumption that only transfers between amide and water occur can be tested by comparing the rate obtained from the N-methyl doublet with that obtained from the water line. These two values are given in Table I as R and R', respectively, for those solutions in which the broadening of the water line was sufficiently large to obtain a rate determination. It is seen that their ratio, $p = R'/R$, is, within experimental accuracy, equal to unity. These results are consistent with the equations

$$CH_3CONHCH_3 + OH^- \underset{}{\overset{k_1}{\rightleftharpoons}} CH_3CON^-CH_3 + H_2O \quad (I)$$

$$CH_3CONHCH_3 + H_3O^+ \overset{k_2}{\rightleftharpoons} CH_3CON^+H_2CH_3 + H_2O \quad (II)$$

Equation I describes the dominant reaction in the basic range and (II) the dominant reaction in the acidic range. From the slopes of the lines in Figs. 7 and 8 these rate constants are obtained

$k_1 = (5.2 \pm 1.0) \times 10^6$ l. mole^{-1} sec.$^{-1}$ at 21 ± 1°

$k_2 = (3.8 \pm 0.4) \times 10^2$ l. mole^{-1} sec.$^{-1}$ at 23 ± 2°

A third possible exchange reaction can be represented by the equation

$$CH_3CONH^*CH_3 + HOH \overset{k_3}{\rightleftharpoons} CH_3CONHCH_3 + H^*OH \quad (III)$$

The contribution from this reaction is small, as follows from the absence, within experimental accuracy, of an intercept in Figs. 7 and 8, and from an experiment with a solution in D_2O, described in Appendix B. From this experiment the following upper limit can be given

$$k_3 < 2 \times 10^{-3} \text{ sec.}^{-1}$$

A number of spectra in very acidic solutions in the presence of dioxane were also recorded. Figure 9 gives three of these spectra. The compositions of the solutions are given in the figure caption. The point of interest in these figures is the doublet

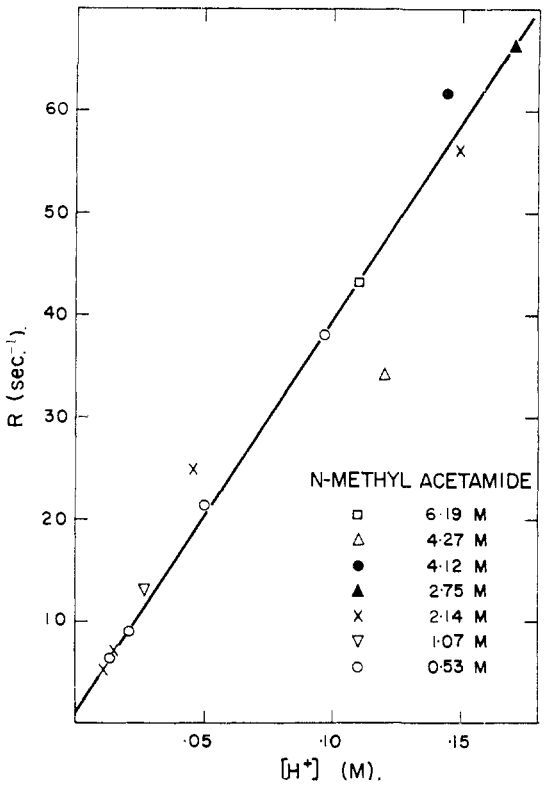


Fig. 7.—Specific rate of hydrogen exchange, as defined in eq. 1, as function of hydrogen ion concentration; acidic range.

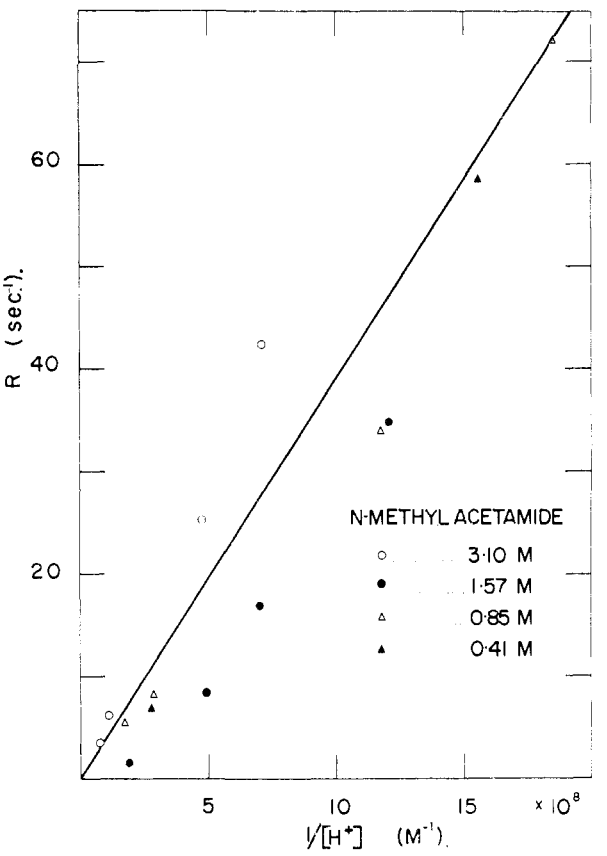


Fig. 8.—Specific rate of hydrogen exchange as function of the reciprocal of the hydrogen ion concentration; basic range.

structure of the N-methyl resonance, which proves that the protonation takes place on the oxygen. Only by the addition of dioxane could the reaction be slowed down sufficiently for the doublet to appear.

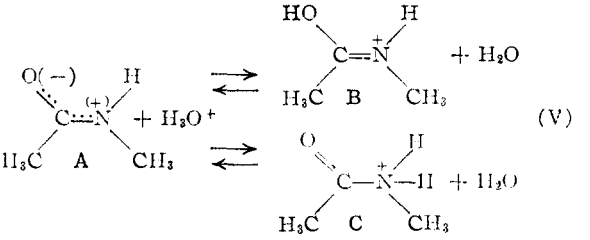
Discussion

Equation I, which applies to the exchange reaction in the basic range, can be considered to indicate the actual reaction mechanism. There seems to be no other possibility of forming a negative amide ion, and the magnitude of the rate constant, k_1, is reasonable when compared to other exchange reactions.[12,13] On the other hand, several facts seem to indicate that equation II does not describe the detailed reaction mechanism in the acidic range: (1) the rate constant k_2 is several orders of magnitude smaller than k_1. (2) As shown in Fig. 5, the amide is already ionized to a considerable extent at pH values where the exchange is still relatively slow. It is hard to understand why the bimolecular reaction

$$CH_3CONHCH_3 + CH_3CON^+H_2CH_3 \rightleftharpoons CH_3CON^+H_2CH_3 + CH_3CONHCH_3 \quad (IV)$$

would be so slow as to be undetected. (3) In very acidic solutions the amide is almost completely ionized. If the exchange could be slowed down sufficiently, the $CH_3CON^+H_2CH_3$ ion would show a triplet for the N-methyl resonance. Indeed, as shown in Fig. 9, the exchange becomes slow again in extremely acid solutions, but a doublet for the N-methyl resonance is observed. This shows that the alternative ionic species $CH_3C(OH)=N^+HCH_3$ is the dominant one.

All the observed facts are explained by assuming the equilibrium in acidic solution



In the pH range between 0.8 and 2, where the rate of exchange was investigated, most of the amide is in the non-ionized state (A). The equilibrium A $\rightleftarrows$ B, which exists to an appreciable extent, does not contribute to the observable hydrogen exchange, and the latter is exclusively due to the equilibrium A $\rightleftarrows$ C. The low value of the observed rate constant k_2 is the result of the low probability of the reaction A $\rightleftarrows$ C, which causes observable hydrogen exchange, as compared to the reaction A $\rightleftarrows$ B, which does not. The fact that ion C is present only in small concentration explains why a second-order exchange between A and C, involving the equilibrium A + C $\rightleftarrows$ C + A, is not observed.

In the very acidic solutions, the dominant species is B, as seen from the doublet structure of the N-methyl resonance. The only way in which species B can exchange its hydrogen is its conversion into C, either through the tautomeric equilibrium

B $\rightleftarrows$ C or, more probably, *via* the intermediate form A, by way of the reactions B $\rightleftarrows$ A $\rightleftarrows$ C.

A set of observations on N,N-dimethylacetamide lends strong support to the proposed mechanism of ionization. As has been shown by Phillips and by Gutowsky and Holm,[11] this compound shows a doublet for the N-methyl resonance, due to the non-equivalence of the two N-methyl groups as a result of the double bond character of the C–N linkage. Onset of free rotation around the C–N bond will result in a collapse of this doublet into a single line. We found that in acidic solution this collapse occurs at nearly the same *p*H values as the collapse of the spin–spin doublet in N-methylacetamide. (The doublet persists even in very basic solution.) Even more striking is the fact that in this case also the doublet reappears under strongly acid conditions.[14] In general the behavior of the chemical shift doublet in N,N-dimethylacetamide is very similar to that of the spin–spin doublet of N-methylacetamide. The conclusion that the same mechanism is responsible for the hydrogen exchange in one case, and for the free rotation in the other, is obvious. A set of equilibria analogous to eq. V can be written for dimethylacetamide. In this case only the N-protonated form, C, allows free rotation. The transformation of A or B into C provides the occasion for a switch of the methyl groups in the same way as the corresponding transformation in N-methylacetamide provides the N-hydrogen exchange.

Appendix A

As mentioned in the body of this paper, each of the components of the N-methyl doublet actually consists of a closely spaced quadruplet. Nevertheless, equations for the exchange broadening of a doublet were used, and the quadruplet splitting was taken into account by the introduction of an effective natural line width of the magnitude of the width of the quadruplet. This procedure was thought justified, as the rate determinations were only made when the exchange broadening was so large that the quadruplet structure was completely washed out. As an estimate of the effective line width to be introduced in order to account for the quadruplet structure, we have taken the width of a Lorentian line forming the envelope of the quadruplet. Admittedly, this is somewhat an arbitrary assumption, and we cannot offer a formal justification. That this correction is essentially of the right magnitude follows from the fact that the plot of rate *versus* hydrogen ion concentration in the acidic range (Fig. 7) shows, within accuracy of the measurements, no intercept when extrapolated to zero hydrogen ion concentration. If the same plot is drawn without applying the above correction, an appreciable intercept results. This intercept cannot be interpreted as a direct water reaction, as the experiments with heavy water (see Appendix B) show that this reaction is very slow. The same applies for the plot in the basic range (Fig. 8).

(14) It could be argued that in this case the doublet is possibly a spin–spin doublet caused by the protonation at the nitrogen atom. That this is not so can be concluded from the fact that the doublet is not symmetric, one component being broader and correspondingly lower than the other. The same fact is observed in neutral solution. The reason is that one of the N-methyl groups has appreciable spin-spin interaction with the C-methyl group and is actually an unresolved quadruplet. (This interaction is similar to the one observed in N-methylacetamide.) The interaction is absent (or much smaller) for the other N-methyl group.

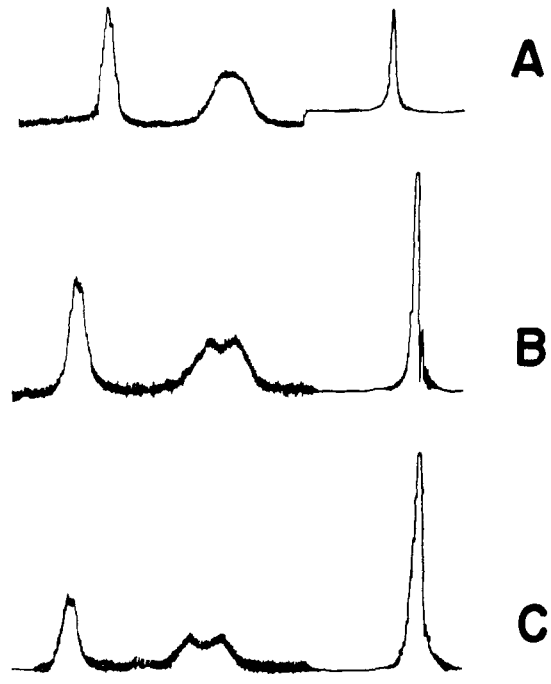


Fig. 9.—N.m.r. spectra of N-methylacetamide in very acidic solution. The composition of the solutions were as follows: (A) 4 ml. of 72% $HClO_4$, 1 ml. of amide, 1 ml. of dioxane; (B) 4.25 ml. of 72% $HClO_4$, 0.5 ml. of amide, 1.25 ml. of dioxane; (C) 4.25 ml. of 72% $HClO_4$, 0.25 ml. of amide, 1.25 ml. of dioxane. The resonances are, from left to right: C-methyl, N-methyl (doublet) and dioxane. The water line, which is much further to the right, is not shown

Appendix B

An upper limit for the rate constant of the direct exchange between amide and water (reaction III) was determined as follows. N-Methylacetamide was dissolved in D_2O (>99.6%). N.m.r. spectra of this solution were recorded at intervals of about one minute, the first spectrum being taken immediately after mixing. The extent of deuteration of the N-methylacetamide could be followed by the gradual decrease in intensity of the N-methyl doublet and the simultaneous growth of a single line at its center. This line is due to the deuterated compound, the spin–spin interaction with the deuterium being averaged out because of fast quadrupole relaxation of the latter. The substitution reaches completion after about 15 minutes, and a half time of about 5 minutes is estimated. This lifetime is about five times longer than that calculated from the rate constants of I and II by extrapolation to the *p*H which gives minimum total rate. This difference probably should be attributed to isotope effects. It can be concluded that reaction III has a half time longer than five minutes.

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