Direct Evidence for the Imidic Acid Mechanism of Proton Exchange in *N*-Methyl Amides

Wei-Hsien WANG* and Hsing-Ching HSIEH
Department of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan 80424, R.O.C.
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Proton exchange of *N*-methyl amides with general acid catalysis in aqueous solution was studied by NMR. Rate coefficients were determined by NMR lineshape analysis of the *N*-methyl protons. The imidic acid mechanism is favored by amides with electron-withdrawing substituents. Only the imidic acid mechanism is expected to show general acid catalysis, and this mechanism was observed with Brønsted $\alpha=0.16$ for the *Z* proton of *N*-methylformamide. No general acid catalysis was observed for *N*-methyl-2-chloroacetamide and *N*,*N*′-dimethylmalonamide which exchange protons via the imidic acid mechanism. This result is attributed to their *Z* conformations.

Proton transfer[1] is a fundamental chemical reaction. Proton exchange in amides is of interest because it provides information about questions of biochemical significance.[2—13]

The proton exchange reaction in *N*-methylacetamide was studied by Berger et al. with NMR,[14] who found that the reaction is catalyzed by both acid and base. They concluded that acid-catalyzed exchange occurs by direct protonation on the amide nitrogen;

$$\mathrm{RCONHR'} + \mathrm{H^+} \rightleftharpoons \mathrm{RCONH_2R'^+} \qquad (1)$$

and this mechanism has been generally accepted. Evidence to favor this mechanism includes the observation that electron-withdrawing substituents retard the reaction,[15,16] the rate of acid-catalyzed exchanged of H_E is greater than that of H_Z in primary amides,[17] and these rates and the rates of acid-catalyzed rotation in *N*,*N*′-dimethyl amides are similar.[14,18]

The evidence for this mechanism is also consistent with an alternative mechanism, proposed by Martin,[19,20] proceeding by *O*-protonation and followed by deprotonation to the imidic acid tautomer.

$$\begin{aligned}\mathrm{RCONHR'} + \mathrm{H^+} &\rightleftharpoons \mathrm{RC(OH){=}NHR'^+} \\ &\rightleftharpoons \mathrm{RC(OH){=}NR'} + \mathrm{H^+}\end{aligned} \qquad (2)$$

This mechanism, although more circuitous, is plausible, especially in view of the well known fact that the amide oxygen is about 10^7 as basic as nitrogen.[21] Bovey and Tiers[22] favored the imidic acid mechanism based on NMR evidence that indicates that rotation of the C–N bond does not occur synchronously with proton exchange for aqueous polyacrylamide solutions.

Perrin and Johnston[23] used saturation-transfer techniques to investigate the reaction mechanisms of acid-catalyzed proton exchange. By comparing intramolecular exchange to intermolecular exchange, they concluded that the *N*-protonation mechanism is operative in many primary amides. They found that the intramolecular exchange in amides with electron-withdrawing substituents is significantly slower than the intermolecular exchange, they interpreted this result as the first unambiguous evidence for the imidic acid mechanism.

Despite the fact that primary amides are certainly of interest, proteins and peptides are structurally more similar to *N*-alkyl amides, of which the mechanism of acid-catalyzed proton exchange ought to be elucidated.[24] Most *N*-alkyl amides exist almost exclusively as the *Z* stereoisomer, which has the *N*-alkyl group cis to the carbonyl oxygen. The saturation-transfer techniques and comparison of intermolecular exchange with intramolecular exchange are therefore inapplicable to *N*-alkyl amides. Perrin and Arrhenius[4] reported a correlation between $\log k_H$ for substituted *N*-methylacetamides and pK_a of the corresponding RCOOH. A slope variation from 0.43 for amides with electron-withdrawing substituents to about 1.84 for amides with electron-donating substituents was observed. This alteration was taken as evidence for a change from the imidic acid mechanism to the *N*-protonation mechanism. However, such arguments based on substituent effects rely on analogy and cannot be definitive. We therefore seek unambiguous evidence.

Our approach to this mechanistic problem rests on the expectation that general acid catalysis can be diagnostic for the imidic acid mechanism, whereas specific acid catalysis is expected to be observed for the *N*-protonation mechanism. The imidic acid pathway required protonation on oxygen, followed by removal of a proton to form the imidic acid tautomer. However, proton exchange is incomplete at this stage. To fulfill this process, this imidic acid intermediate must be protonated by a new proton source and reverts to the amide form by losing the oxygen proton with a rate controlled by diffusion.[25] According to the principle of microscopic reversibility,[26] in a reversible reaction, if a certain fraction of the molecules follow one path in the forward direction, the same fraction follow that path in the reverse direction. Therefore, we simply focus on the reverse process of this proton exchange reaction. pK_a of the N–H proton in the protonated amide is about 7. A general acid with $pK_a<7$ is therefore capable of protonating the imidic acid in a diffusion-controlled process.

When such an acid is added as a new proton source, the rate of protonation of the imidic acid is expected to be enhanced. As a result, the rate of the proton exchange is increased. Such acceleration is not expected for the *N*-protonation mechanism, as the concentration of the *N*-protonated amide, which resembles the transition structure, depends only on pH. Therefore, general acid catalysis may enable one to distinguish the mechanisms of proton exchange in *N*-methyl amides.

Moreover, the Brønsted α,[27] which is the slope of the plot of $\log k_{BH}$ vs. $\log K_a$ of BH, is expected to be about zero for these general acid-catalyzed reactions due to the transfer of a proton, controlled by diffusion, from general acids to the imidic acid. This general acid-catalyzed proton exchange in amide is a process of specific acid-general base catalysis in the forward direction.

N-Methylformamide is a compound of special interest, as it exists as a mixture of both *E* and *Z* stereoisomers, for which both mechanisms are operative.[3] The *N*-protonation mechanism permits not only proton exchange but also *E*–*Z* interconversion (Scheme 1). For the imidic acid mechanism, no *E*–*Z* interconversion is allowed because of configurational stability of the imidic acid intermediates (see Scheme 2). Hence, general acid catalysis, which occurs via the imidic acid mechanism, enhances intermolecular proton exchange of both the *E* and *Z* stereoisomers without affecting the *E*–*Z* interconversion.

Experimental

Chemicals and Sample Preparation. Amides were either commercially available or synthesized as described below. *N*-Methylacetamide and *N*-methylformamide (Aldrich) were used without further purification. *N*-Methyl-2-chloroacetamide and *N*,*N*′-dimethylmalonamide were synthesized by addition of excess methylamine in aqueous solution to the corresponding ethyl ester.

Buffer solutions were prepared by adding hydrochloric acid to the base or sodium hydroxide solution to the acid. The concentration of buffer components were either calculated stoichiometrically or determined by titration if necessary.

Exchange sample solutions were prepared by dissolving the same amount of sample in buffer solutions with varied concentrations of buffer. The range of buffer concentrations was varied about 10 fold. Sodium chloride was added to maintain constant ionic strength in the sample solutions. Small discrepancies of pH between sample solutions with various buffer concentrations were adjusted by adding microliter quantities of hydrochloric acid and/or sodium hydroxide solution. pH measurements were made with a pH meter (Corning Model 125) connected to a combination pH electrode (Orion or Ingold). The meter was calibrated with standard buffer solutions (Merck, Germany). Electrodes were rinsed with deionized water and dried before each measurement.

Kinetics. The exchange rate coefficients were estimated from analysis of the NMR lineshape of the *N*-methyl doublet.[28] NMR spectra were recorded on a 90-MHz NMR spectrometer (Varian EM 390). Samples for kinetic measurement were allowed to equilibrate for 15 min to the probe temperature 34 °C which was measured with a neat ethylene glycol sample.[29] *t*-Butyl alcohol (1%,v/v) was invariably included to test the field homogeneity.

Results and Discussion

General acid catalysis was studied through four *N*-methyl amides. As both the specific acid H^+ and the general acid BH can catalyze the proton exchange, the pseudo-first-order rate coefficient is expressed as

$$k_{obsd} = k_H[H^+] + k_{BH}[BH], \quad (3)$$

in which k_H is the second-order rate coefficient of specific acid catalysis and k_{BH} is the second-order rate coefficient of general acid catalysis. At constant pH, the first term in Eq. 3 is constant. Therefore, k_{BH} can be determined by plotting k_{obsd} vs. [BH] and k_H obtained from the ordinate intercept. Rate coefficients for general acid catalyzes of the amides are listed in Table 1. First-order rate coefficients observed from analysis of the NMR lineshape are reproducible; the errors of reproducibility are less than 5%. Errors listed in the data table are standard deviations from least-square fitting. Second-order rate coefficients generally have a standard deviation in the range 5—10%. Therefore, the general acid catalyzes that we observed are well beyond experimental error. The *Z* form of *N*-methylformamide has the *N*-methyl group cis to the carbonyl oxygen. In the *E* form, the *N*-methyl group is trans to the carbonyl oxygen. The *E*:*Z* population ratio is 1:13.5. Kinetic data of both isomers were measured and are listed in Table 1.

The rate coefficients and pK_a of conjugate acids were corrected for statistical effects, which result from the fact that in some acids and bases there may be more than a single site which can donate or accept a proton.[30,31] The intrinsic acidic strength pK_a^0 of the acidic functional group in a general acid can be expressed as

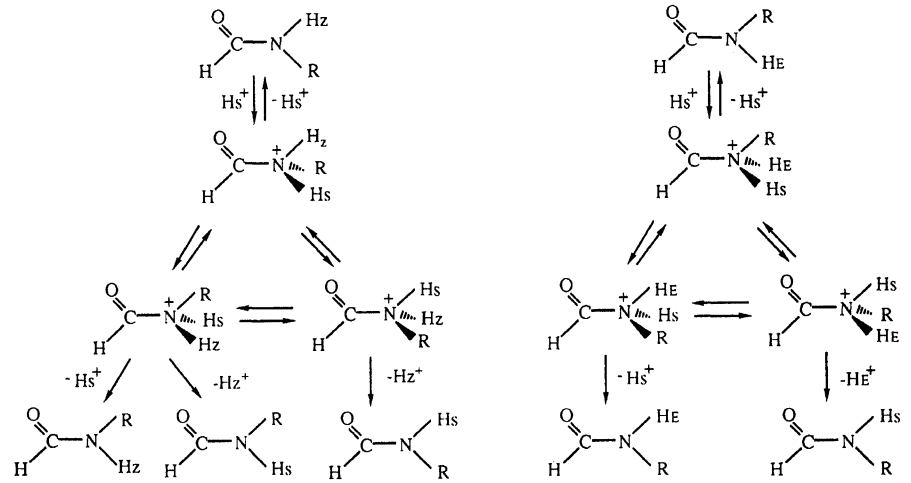
$$pK_a^0 = pK_a^0 + \log \frac{p}{q}, \quad (4)$$

in which p is the number of equivalent protons that can be transferred from the conjugate acid and q is the number of sites that can accept a proton in the conjugate base.[32] Modified Brønsted expressions are given in

$$\log k_{BH}^0 = \log \frac{k_{BH}}{p} = C_{BH} - \alpha(pK_a^0 + \log \frac{p}{q}), \quad (5)$$

in which C_{BH} is a parameter. In Eq. 5, the general acid-catalyzed rate coefficient k_{BH} is divided by the statistical factor p due to the probability effect. α is obtained as the slope of the plot of $\log k_{BH}^0$ vs. pK_a^0, for which k_{BH}^0 and K_a^0 are statistically corrected values.

In general, the *N*-methyl amide $RCONHCH_3$ with a superior electron-donating substituent R has a large acid-catalyzed rate coefficient k_H and the values for k_H

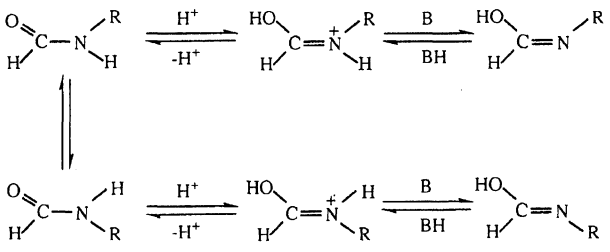


Scheme 1. *N*-Protonation mechanism for exchange in *N*-methylformamide, including competition between deprotonation and rotation about the C–N single bond. (R=CH_3).

Table 1. Results for General Acid Catalysis of $RCONHCH_3$ with Various Substituents R

R	BH	pK_a	k_{BH}/$M^{-1}s^{-1}$	k_H/$M^{-1}s^{-1}$
CH_3	3-Cyanopyridinium	1.45	ND[a)]	1350±40
	3-Chloropyridinium	2.84	ND	1270±50
H(E isomer)	H_3PO_4	2.12	10.3±2.0	
	H_3PO_4	2.30[b)]	3.4±0.7[b)]	
	3-Cyanopyridinium	1.45	4.28±0.40	212±3
	4-Cyanopyridinium	1.90	3.84±0.21	217±12
	3-Chloropyridinium	2.84	2.53±0.30	177±3
H(Z isomer)	H_3PO_4	2.12	1.52	61.2
		2.30[b)]	0.51[b)]	
$CH_3NHCOCH_2$	H_3PO_4	2.12	ND	105±2
	$NCCH_2COOH$	2.45	ND	110±2
	$ClCH_2COOH$	2.85	ND	93±2
$ClCH_2$	$CHCl_2COOH$	1.29	ND	43.0±0.7
	3-Cyanopyridinium	1.45	ND	43.4±1.7

a) ND: not detected. b) Corrected with statistical factor.



Scheme 2. Imidic acid medchanism for proton exchange in *N*-methylformamide. (R=CH_3).

are in good agreement with those reported.[4)] For each $RCONHCH_3$, k_H observed in various buffers are consistent. For instance, the values of k_H for $CH_3CONHCH_3$ are 1350±40 $M^{-1}s^{-1}$ and 1270±50 $M^{-1}s^{-1}$ in 3-cyanopyridine and 3-chloropyridine buffers. (1 M = 1 mol dm^{-3}) However, there is no significant general acid catalysis observed in buffered solutions of 3-cyanopyridine or 3-chloropyridine samples within experimental errors. Similar results were observed in other amides, except in the *E*, *Z* isomers of *N*-methylformamide. Direct proof is exhibited in Fig. 1, in which the *E* methyl peaks are coalesced in the phosphate buffered sample where as the unbuffered sample at identical pH shows a well resolved doublet. The *Z* methyl peaks in Fig. 1 are off scale, but a small increase of the ratio of valley to peak of the *Z* methyl doublet was observed for the phosphate-buffered sample. The small peaks at greater chemical shift than the *E* methyl peaks are spinning side band of the huge *Z* methyl peaks. The ratios of valley to peak of the valley between *E* and *Z* methyl peaks are comparable for these two samples. Therefore the rate of *E*–*Z* isomerization, which affects the depth of the valley between *E* and *Z* methyl peaks, depended on only pH. The general acid H_3PO_4 has no effect on this ratio. Other evidence of general acid catalysis for the *E* isomer of *N*-methylformamide is presented in Fig. 2

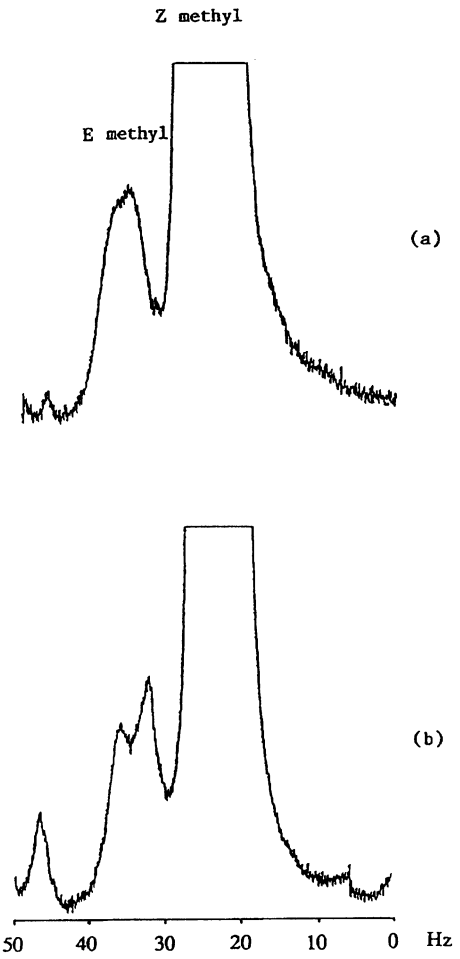


Fig. 1. Expanded spectra of *N*-methyl regions of *N*-methylformamide in general acid catalysis. (a) phosphate buffered sample, pH 1.17. (b) unbuffered sample, pH 1.17. Plot widths are 50 Hz.

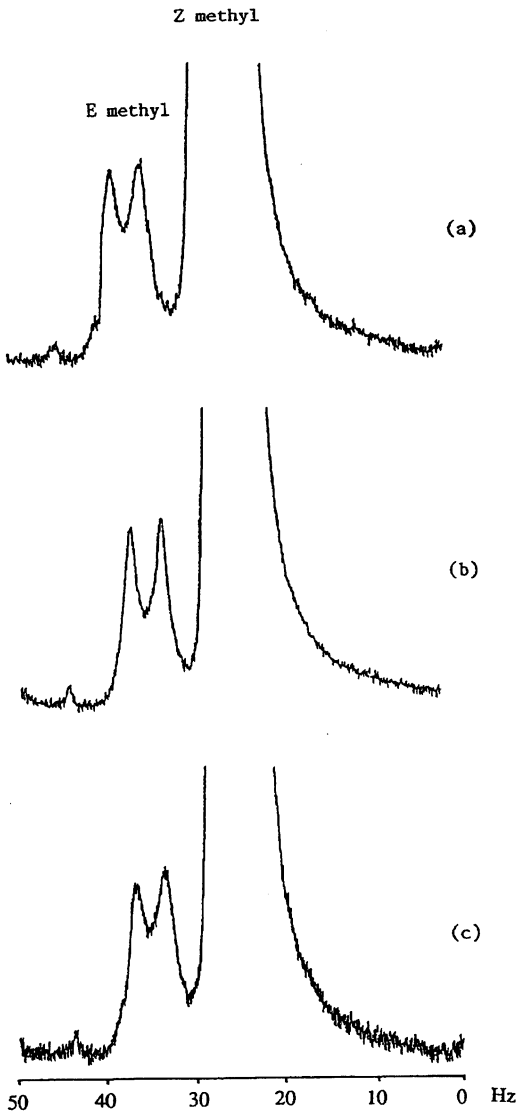


Fig. 2. Expanded spectra of *N*-methyl regions of *N*-methylformamide in general acid catalysis. (a) 4-cyanopyridinium (0.73 M), pH 1.51. (b) 4-cyanopyridinium (0.04 M), pH 1.51. (c) 4-cyanopyridinium (0.04 M), pH 1.35. Plot widths are 50 Hz.

where 4-cyanopyridinium also shows a catalytic effect in proton exchange. The ratio of valley to peak of the *E* methyl peaks of the sample of 4-cyanopyridinium (0.73 M) is greater than that of the sample with 4-cyanopyridinium (0.04 M), as demonstrated in Figs. 2(a) and 2(b), although the pH of both solutions is 1.51. The *E*–*Z* isomerization is not affected by a general acid, as judged by the comparable ratios of valley to peak between *E* and *Z* methyl doublets in 2(a) and (b). In order to increase the ratio of exchange of the sample with 4-cyanopyridinium (0.04 M), further hydrochloric acid was added. As shown in Fig. 2(c), this solution was adjusted to pH 1.35 to produce a comparable rate of exchange of the *Z* proton (ratio of valley to peak of the *E* methyl doublet) to Fig. 2(a). However, there is greater isomerization in Fig. 2(c), judged from the greater ratio of valley to peak between the two doublets. Therefore, only specific acid H^+ catalyzes isomerization, whereas a general acid catalyzes proton exchange without isomerization.

A Brønsted plot with statistically corrected data from Table 1 is shown in Fig. 3. An α value 0.16 ± 0.02 is observed. This α value is consistent with the imidic acid mechanism, which is expected to have α value nearly zero due to proton transfer controlled by diffusion from general acids to the imidic acid intermediate. Hence, the general acid catalysis of *N*-methylformamide occurs via the imidic acid mechanism, for which the *E*–*Z* isomerization is prohibited.

Reprotonations of both imidic acid tautomers of *N*-methylformamide by general acid are controlled by diffusion. By assuming that the coefficients of these diffusion-controlled rates are equal, we derive the relative stability of these two imidic acids from the general acid-catalyzed rate coefficients of *E* and *Z* isomers. The equilibrium quotient K_e^{IA} for the imidic acids is

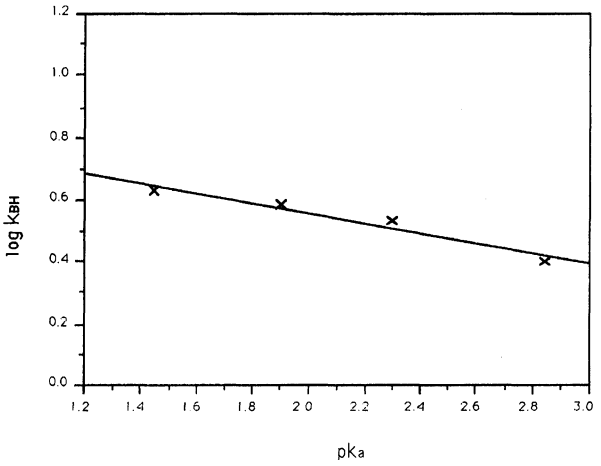


Fig. 3. Brønsted plot of *N*-methylformamide (*E* isomer) with statistically corrected pK_a and log k_{BH}.

$$K_e^{IA} = \frac{[Z\text{-HC(OH)=NCH}_3]}{[E\text{-HC(OH)=NCH}_3]} = K_e \frac{k_{ES}^{IA}}{k_{ZS}^{IA}}, \quad (6)$$

in which k_{ES}^{IA} and k_{ZS}^{IA} are the coefficients for the rate of exchange via imidic acid mechanisms only, and K_e is the equilibrium quotient between *Z* and *E* isomers of *N*-methylformamide. There was detectable general acid catalysis for the *Z* isomer only with H_3PO_4, for which the statistical factor of 3 for H_3PO_4 advances the general acid catalysis; a value of 1.52 $M^{-1}s^{-1}$ (see Table 1) was obtained from analysis of spectral lineshape. $K_e = 13.5$ and $k_{H_3PO_4}$ of *E* and *Z* *N*-methylformamide from Table 1 were inserted into Eq. 6 to yield $K_e^{IA} = 2.0$. Therefore, the *Z* imidic acid is more stable than the *E* imidic acid, but the stability difference is quite small (about 2.0 kJ mol^{-1}) as presented in Fig. 4. By comparison with the *E* imidic acid, the *Z* imidic acid has additional destabilization not found in the amides. The origin of the stability difference in the parent amides is unclear. An aromatic six-pi system[33] and dipole-induced dipole interactions[34] have been invoked to resolve this question. We therefore propose an explanation that whatever stabilizes the *Z* amide is reduced in the imidic acid. This idea is reasonable as the imidic acid is less delocalized and less polarizable than the amide; hence aromaticity or dipole-induced dipole interaction would be reduced.

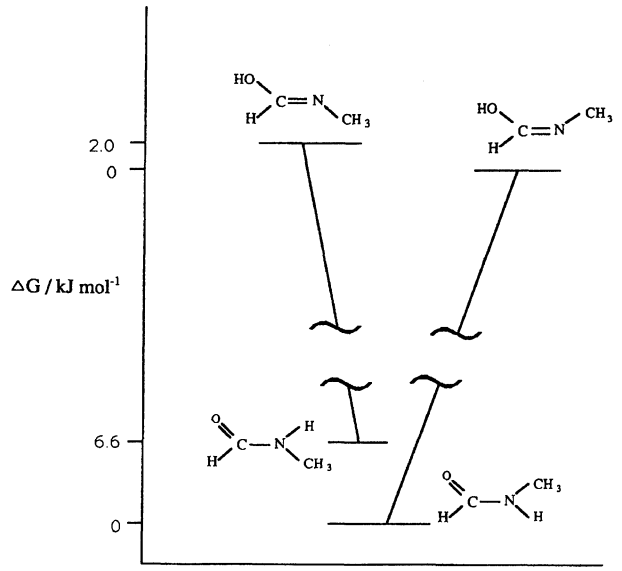


Fig. 4. Relative energies of the imidic acids of *N*-methylformamide.

The equilibrium quotient for the amide/imidic acid tautomerization is smaller for the *Z* conformer than in the *E* conformer in the case of *N*-methylformamide. The existence of the imidic acid mechanism is explained by the substituent effect. The transition structure of the *N*-protonation mechanism, which resembles $RCONH_2CH_3^+$, is strongly destabilized by electron-withdrawing substituents R. As a result, the *N*-protonation mechanism is retarded and the imidic acid mechanism becomes dominant. The fact that no general acid catalysis was observed in *N*-methylchloroacetamide and *N*,*N*'-dimethylmalonamide might result from their *Z* conformation.

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