Mechanisms of Acid-Catalyzed Proton Exchange in Amides

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Abstract: The kinetics of acid-catalyzed proton exchange in a series of primary amides were studied by NMR. The saturation-transfer method measures independently all six rate constants for exchange among sites H_E, H_Z, and solvent OH (usually ethylene glycol). Some additional rate ratios were determined by line broadening. For many amides intramolecular exchange of H_Z occurs at the same rate as intermolecular exchange, and this is taken as definitive evidence for the N-protonation mechanism. For these amides H_E exchanges faster than H_Z, according to both line broadening and saturation transfer. Therefore the intermediate, $RCONH_3^+$, is so strong an acid that its deprotonation is competitive with rotation about the C–N single bond. It is further concluded that the rate of this rotation is independent of viscosity and that the $-NH_3^+$ rotates in picoseconds within its solvation shell. For amides with electron-withdrawing substituents, intramolecular exchange is slower than intermolecular exchange, and either H_E or H_Z may be the faster to exchange. These amides exchange predominantly via the imidic acid, and the change of mechanism can be rationalized in terms of substituent effects.

Proton exchange in amides has long been of interest; many studies have been cited.[1] In the earliest NMR study[2] it was observed that the reaction is subject not only to base catalysis but also to acid catalysis. The mechanism of the acid-catalyzed reaction was proposed[2] to involve N-protonation:

$$RCONHR' + H^+ \underset{k_d}{\overset{k_p}{\rightleftharpoons}} RCONH_2^+R' \qquad (1)$$

Although the most basic site of an amide is oxygen and reexamination has been suggested,[3] this mechanism seems to have been generally accepted.[4] It is not an impossible one, since acid also catalyzes rotation about the C–N bond of tertiary amides, $RCONR'_2$,[2,5] and this must be due to N-protonation.[5d] The similarity of the rates of these two acid-catalyzed processes, in either aqueous HCl[2] or CF_3COOH–$CDCl_3$,[5c] was adduced as evidence for the N-protonation mechanism. Other evidence claimed to suport this mechanism was the fact that the second-order rate constant for this acid-catalyzed reaction is low, less than that for the base-catalyzed reaction (since most protonations are at oxygen, and therefore unproductive),[2] and the observation that electron-withdrawing substituents retard the reaction.[6] Nevertheless, this evidence is also quite consistent with an alternative mechanism involving O-protonation, to acidify the NH proton, and proceeding via the imidic acid:

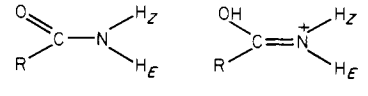
$$RCONHR' + H^+ \underset{K_a^o}{\rightleftharpoons}$$

$$RC(OH)=NHR'^+ \underset{k_i}{\overset{k_{-i}}{\rightleftharpoons}} RC(OH)=NR' + H^+ \qquad (2)$$

Since the second step would be the rate-limiting one, most protonations would still be unproductive. Also, electron-withdrawing substituents would still retard the reaction, inasmuch as it is acid catalyzed. This mechanism, although more circuitous, is more attractive, since it avoids protonating the amide on nitrogen, which is hardly basic.

Martin[7] has favored the imidic acid mechanism on the basis of a discrepancy between the rate of acid-catalyzed exchange in $RCONHCH_3$ and the rate of acid-catalyzed rotation in $RCON(CH_3)_2$. By using the rate of the latter reaction and correcting for the different basicities of secondary and tertiary amides, Martin estimated the rate constant for exchange via N-protonation. Since this was less than the observed rate constant, he concluded that 94, 68, and 93% of the exchange, for R = H, CH_3, and phenyl, respectively, occurs via the imidic acid (eq 2). However, this method involves the implicit assumption that the tautomeric equilibrium constant for N- vs. O-protonation is the same for a secondary amide as for the corresponding tertiary amide, and this is unwarranted, as judged from the tendency[8] of ureas and protonated ureas to undergo protonation at the less substituted nitrogen. Indeed, the rate discrepancy between secondary and tertiary amides increases with increasing steric hindrance in a series of benzamides,[9] and although this is consistent with exchange via the imidic acid, it is most easily interpreted in terms of N-protonation.

We sought to elucidate the mechanism by investigating the exchange of primary amides (**1**), where there are chemically



1 **2**

inequivalent protons, H_E and H_Z, which may be expected to undergo base-catalyzed exchange at different rates. They remain inequivalent in the conjugate acid (**2**), so that if exchange proceeds via the imidic acid (eq 2), they may again be expected to exchange at different rates. In contrast, N-protonation, to form $RCONH_3^+$, was expected to render the protons equivalent, so that they would exchange at identical rates. In a preliminary communication[10] we reported that H_E of five primary amides exchanges faster than H_Z, not only in base but also in acid. Likewise, the faster acid-catalyzed exchange of H_E of NAD^+ was reported.[11a] but without

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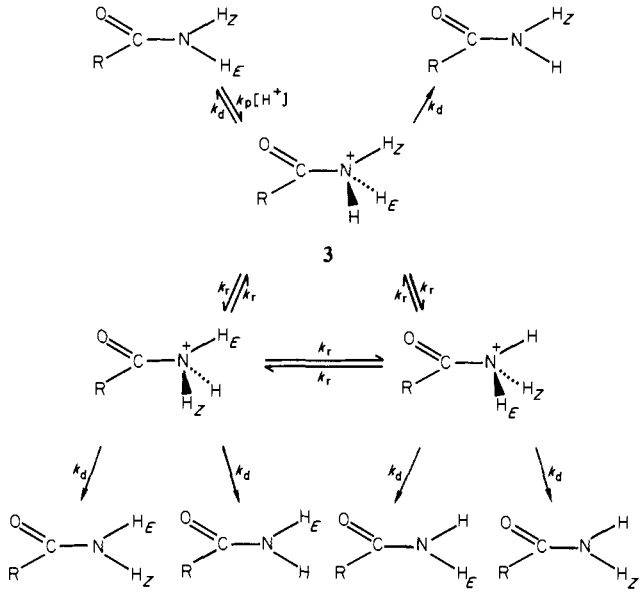
Scheme I. N-Protonation Mechanism for Exchange in Primary Amides, Including Competition between Deprotonation and Rotation about the C–N Sinele Bond



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comment. More recently, H_E of the three primary amide residues of oxytocin was shown by saturation transfer to exchange faster than H_Z.[11b] Initially this reactivity difference seemed to support the imidic acid mechanism, except that analogy to imidate esters[12] and MO calculations on HC(OH)=NH[13] suggested that H_Z would be the faster to exchange. We therefore rejected the imidic acid mechanism and returned to the N-protonation mechanism, but with the additional features that the intermediate is formed in the preferred[14] conformation **3** and that this intermediate is such a strong acid that diffusion-controlled deprotonation[15] is competitive with rotation about the C–N single bond. As a result, H_E exchanges faster, since exchange of H_Z requires rotation. This mechanism is detailed in Scheme I, where k_p, k_d, and k_r are rate constants for protonation, deprotonation, and rotation, respectively. The labeling of the protons in **3** must be as shown,[16] since the observed rate of rotation[17] about the C–N bond of an amide is too slow to permit H_E or H (from solvent) to enter the site syn to oxygen.

As evidence for these features we demonstrated[16,18] that H_E of amidinium ions, $ArC(NH_2)_2^+$, undergoes acid-catalyzed exchange faster than H_Z. Here there is no mechanistic ambiguity. The result shows that the intermediate, $ArC(=NH_2^+)NH_3^+$, is formed in a conformation analogous to **3** and that deprotonation is competitive with rotation about the C–N single bond. However, this conclusion may still not be applicable to amides. We therefore sought an independent method for elucidating the mechanism, one that would not depend on differential broadenings of NH resonances or analogies to imidate esters.

It has been claimed[19] that the two mechanisms are kinetically equivalent and cannot be distinguished. However, a further distinction between them is that N-protonation leads also to intramolecular exchange, whereas the imidic acid mechanism involves exchange of H_E and H_Z independently into solvent, but not with each other. Analysis of Scheme I by the steady-state approximation leads to

$$k_{ZS} = k_p[H^+]k_r/(3k_r + 2k_d) = k_{ZE} = k_{EZ} \quad (3)$$

$$k_{ES} = k_p[H^+](k_r + k_d)/(3k_r + 2k_d) = k_{ZS}[1 + (k_d/k_r)] \quad (4)$$

where k_{ZS} and k_{ZE} are pseudo-first-order rate constants for exchange of H_Z into solvent and into the H_E site, respectively, and k_{ES} and k_{EZ} are analogous. Equation 4 reflects the previous conclusion that H_E exchanges faster. Equation 3 arises because exchange of H_Z requires rotation, whereupon there is equal likelihood of exchange into solvent and the H_E site; the last part of the equation is merely the requirement that forward and reverse rates of intramolecular exchange be equal. In contrast to eq 3, the imidic acid mechanism requires that

$$k_{ZE} = 0 = k_{EZ} \quad (5)$$

Thus the two mechanisms may be distinguished by comparing k_{ZE} and k_{EZ} with k_{ZS}. This comparison is the same as Martin's,[7] but it is made on the same primary amide and needs no assumption concerning pK_a's.

How can these rate constants be measured? In principle, line shape analysis of NMR spectra can separate intramolecular exchange from intermolecular, but in practice this is extremely difficult. Thus Bovey and Tiers[20] suggested that acid-catalyzed proton exchange in polyacrylamide occurs without C–N rotation, since they could detect no coalescence of the NH peaks with each other (at 40 MHz). However, we have simulated the spectra to be expected from a combination of intermolecular and intramolecular exchange, and we conclude that at the exchange rates necessary to produce detectable coalescence of the NH peaks, they would be so exchange-broadened as to be lost in the solvent peak.

To determine these rate constants, we have extended[21] the NMR saturation-transfer method of Forsén and Hoffman.[22] The method involves measurement of steady-state intensities and spin–lattice relaxation times under conditions of selective saturation. In particular, we measure six values of $t_i(j)$, the transfer of saturation[16] from each site j (H_E, H_Z, $H_{Solvent}$) to each other site i, or the fractional loss of intensity at site i, I_i, on saturating site j.

$$t_i(j) = \frac{I_i^0 - I_i(j)}{I_i^0} \quad (6)$$

We also measure $M_S(E,Z)$, the apparent spin–lattice relaxation rate constant ($=1/T_{1S}^{app}$) of solvent protons, obtained in an inversion-recovery experiment under conditions that both H_E and H_Z are saturated, as well as $M_E(S)$ and $M_Z(S)$, apparent spin–lattice relaxation rate constants of H_E and H_Z under conditions of solvent saturation. Pulsed Fourier transform instrumentation makes such measurements quite convenient. We can thereby determine independently all six rate constants—the four in eq 3–5, plus k_{SE} and k_{SZ}—in this three-site system. The equations for evaluating the rate constants are given in the accompanying paper.[1] In a preliminary communication[23] we reported that the method is valid, that acid-catalyzed proton exchange in two primary amides satisfies eq 3 and 4, so that they exchange predominantly via N-protonation, but that two other amides with electron-withdrawing substituents exchange predominantly via the imidic acid. Similar results, with a significantly different interpretation, have been presented by Redfield and Waelder.[24]

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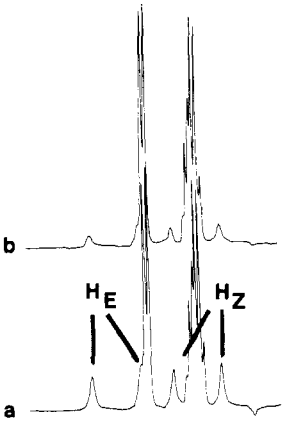

Figure 1. Saturation-transfer experiment on 0.99 M benzamide-^{15}N in ethylene glycol at pH 2.0 (NH and aromatic CH region (plot width 500 Hz)): (a) off-resonance spectrum; (b) with saturation of solvent OH (offscale upfield).

Experimental Section

Sample preparation, instrumentation, signal assignments, methods for line-shape analysis and saturation-transfer measurements, and error analysis have been described.[1,16,21] 3,5-Dimethylbenzamide was prepared from the acid via the acid chloride: mp 137–137.5 °C (lit.[25] 135–135.5 °C). Sulfuric acid or aqueous hydrochloric acid was used as the acid catalyst, except for formamide-*d*, where trichloroacetate buffers were used to reduce pH drift due to hydrolysis. Line-broadening measurements on acrylamide in ethylene glycol showed that the rate ratio k_E/k_Z was the same, within experimental error, from 0.25 to 2.7 M, so that aggregation is again not significant.[1]

Results

Figure 1 shows a representative saturation-transfer experiment on benzamide-^{15}N; the greater transfer of saturation from solvent to H_E is apparent from the significantly greater reduction of intensity of the downfield peak in the top spectrum. Complete saturation-transfer data for acid-catalyzed exchange of several amides are given in Table I, and the kinetic results calculated therefrom are in Table II. Rate constants for uncatalyzed rotation, as well as any contribution from cross-relaxation,[1] have been subtracted from the entries for k_{EZ} and k_{ZE}, so that these entries are rate constants purely for acid-catalyzed intramolecular exchange. Table III lists more detailed saturation-transfer data for benzamide-^{15}N. Table IV lists some additional line-broadening and line shape data on acid-catalyzed exchange; our line widths in the absence of exchange have been reported.[1] Table V summarizes the *relative* rates of acid-catalyzed exchange of H_E and H_Z. For comparison of saturation-transfer data with line-broadening data, all rate ratios in Table V are given as

$$\frac{k_E}{k_Z} = \frac{k_{ES} + k_{EZ}}{k_{ZS} + k_{ZE}} \tag{7}$$

Where rate ratios were determined by both methods, they agree well. Some of these ratios differ slightly from those previously reported,[10] which were less accurate. No error estimates are provided for this ratio as determined by saturation transfer, because errors given in Table II are not independent.[1]

The reliability of the kinetic data in Table II may be judged by several criteria. (1) Within experimental error rates of forward and reverse reactions are equal: $p_i k_{ij} = p_j k_{ji}$, where p_i is the relative population of site *i*. (2) Rate ratios for acetamide-^{14}N and -^{15}N are the same, although the rate constants do vary with pH. (3) The inherent spin–lattice relaxation rate of solvent OH, M_S, determined according to eq 4 of ref 21a from all the k_{SZ}, k_{SE}, and $M_S(E,Z)$ values in Tables I and II and in Tables III and IV of ref 1, is 1.95 ± 0.4 s^{-1}, which is reasonably constant and independent of amide. (4) For acetamide-^{15}N the apparent relaxation rates under conditions of selective saturation are equal to the sum

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Table I. Saturation-Transfer Data for Acid-Catalyzed Proton Exchange of Primary Amides in Ethylene Glycol

	$p_S/p_{E,Z}$[a]	$t_E(Z)$	$t_Z(E)$	$t_S(E)$	$t_S(Z)$	$t_E(S)$	$t_Z(S)$	$M_E(S)$, s^{-1}	$M_Z(S)$, s^{-1}	$M_S(E,Z)$, s^{-1}
formamide-d_1	11.31	0.30 ± 0.02	0.30 ± 0.02	0.09 ± 0.005	0.08 ± 0.005	0.46 ± 0.03	0.42 ± 0.03	2.80 ± 0.10	2.73 ± 0.11	1.81 ± 0.07
acetamide	13.05	0.35 ± 0.02	0.39 ± 0.02	0.158 ± 0.009	0.134 ± 0.007	0.605 ± 0.035	0.56 ± 0.035	7.16 ± 0.28	6.66 ± 0.25	2.48 ± 0.10
acetamide-^{15}N	14.33	0.283 ± 0.016	0.308 ± 0.018	0.083 ± 0.005	0.075 ± 0.004	0.404 ± 0.024	0.375 ± 0.020	5.1 ± 0.2	4.8 ± 0.2	2.20 ± 0.09
acetamide-^{15}N	14.33	0.355 ± 0.02	0.364 ± 0.02	0.128 ± 0.007	0.118 ± 0.007	0.53 ± 0.03	0.51 ± 0.03	6.53 ± 0.25	6.17 ± 0.24	2.14 ± 0.08
acrylamide	10.90	0.41 ± 0.024	0.43 ± 0.025	0.24 ± 0.01	0.20 ± 0.01	0.57 ± 0.03	0.535 ± 0.03	7.95 ± 0.3	7.2 ± 0.3	2.32 ± 0.09
methacrylamide	12.17	0.55 ± 0.03	0.57 ± 0.3	0.26 ± 0.015	0.23 ± 0.015	0.54 ± 0.03	0.50 ± 0.03	9.6 ± 0.4	8.9 ± 0.35	2.65 ± 0.10
benzamide-^{15}N	33.0	0.41 ± 0.03	0.42 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.63 ± 0.04	0.525 ± 0.03	18.0 ± 0.7	15.3 ± 0.6	2.13 ± 0.08
benzamide-^{15}N[b]	150.0	0.40	0.61	0.040	0.023	0.74	0.65	1.65[c]	1.50[c]	1.15
3,5-dimethylbenzamide	40	0.31	0.33	0.074	0.058	0.52	0.43			
cyanoacetamide	27.49	0.235 ± 0.014	0.234 ± 0.015	0.096 ± 0.006	0.090 ± 0.005	0.67 ± 0.04	0.63 ± 0.04	10.1 ± 0.4	9.6 ± 0.4	3.6 ± 0.14
malonamide	56.0	0.18 ± 0.01	0.16 ± 0.01	0.047 ± 0.003	0.041 ± 0.003	0.47 ± 0.03	0.43 ± 0.025	8.3 ± 0.3	8.2 ± 0.3	2.00 ± 0.08
ethyl oxamate	77.9	0.094 ± 0.006	0.057 ± 0.003	0.040 ± 0.003	0.075 ± 0.004	0.48 ± 0.3	0.66 ± 0.04	9.7 ± 0.4	14.3 ± 0.6	1.98 ± 0.08
chloroacetamide	28.8	0.29 ± 0.01	0.35 ± 0.02	0.081 ± 0.005	0.064 ± 0.004	0.61 ± 0.04	0.58 ± 0.035	6.9 ± 0.3	6.1 ± 0.2	3.0 ± 0.1
dichloroacetamide	12.81	0.15 ± 0.04	0.125 ± 0.007	0.159 ± 0.009	0.185 ± 0.010	0.54 ± 0.04	0.60 ± 0.04	11.4 ± 0.4	11.4 ± 0.4	3.27 ± 0.12

[a] p_i = relative population of site *i*. These values also apply to Table II. [b] 60% aqueous methanol. [c] M_E or M_Z measured under noncxchange conditions.

Table II. Kinetic Results for Acid-Catalyzed Proton Exchange of Primary Amides in Ethylene Glycol at 23 °C

	pH	k_{ES}, s^{-1}	k_{ZS}, s^{-1}	k_{ZE}, s^{-1}	k_{EZ},[a] s^{-1}	k_{SE}, s^{-1}	k_{SZ}, s^{-1}
formamide-d_1	1.0	1.49 ± 0.14	1.19 ± 0.13	1.05 ± 0.11	1.03 ± 0.11	0.118 ± 0.01	0.097 ± 0.01
acetamide	1.7	4.0 ± 0.4	2.9 ± 0.4	2.60 ± 0.36	2.8 ± 0.4	0.34 ± 0.04	0.27 ± 0.03
acetamide-^{15}N	1.9	2.07 ± 0.20	1.78 ± 0.19	1.38 ± 0.18	1.60 ± 0.20	0.147 ± 0.01	0.122 ± 0.01
acetamide-^{15}N	1.8	3.0 ± 0.3	2.6 ± 0.3	2.55 ± 0.34	2.57 ± 0.34	0.245 ± 0.03	0.215 ± 0.02
acrylamide	1.72	4.6 ± 0.5	3.14 ± 0.5	2.5 ± 0.5	2.5 ± 0.55	0.43 ± 0.05	0.34 ± 0.05
methacrylamide[b]	1.7	5.9 ± 1.0	4.2 ± 0.9	4.1 ± 1.5	4.3 ± 1.7	0.455 ± 0.08	0.34 ± 0.07
benzamide-^{15}N	2.0	10.0 ± 1.1	6.4 ± 1.0	6.6 ± 1.3	6.2 ± 1.2	0.38 ± 0.04	0.21 ± 0.03
benzamide-^{15}N	2.3[c]	5.9	1.6	1.8	1.1	0.044[d]	0.011[d]
cyanoacetamide	1.05	7.8 ± 0.7	7.0 ± 0.7	1.3 ± 0.3	1.06 ± 0.26	0.25 ± 0.02	0.215 ± 0.02
malonamide	1.58	4.7 ± 0.4	3.75 ± 0.3	1.63 ± 0.15	1.34 ± 0.12	0.071 ± 0.006	0.063 ± 0.005
ethyl oxamate	0.8	5.5 ± 0.5	11.05 ± 0.8	1.38 ± 0.10	1.10 ± 0.08	0.059 ± 0.005	0.123 ± 0.009
chloroacetamide	1.20	5.6 ± 0.55	3.8 ± 0.4	1.4 ± 0.3	1.9 ± 0.3	0.147 ± 0.015	0.121 ± 0.014
dichloroacetamide	0.4	5.8 ± 0.5	6.9 ± 0.6	0.64 ± 0.2	0.31 ± 0.16	0.48 ± 0.04	0.53 ± 0.04

[a] Corrected for E-Z cross-relaxation and uncatalyzed C-N bond rotation. [b] 22 °C. [c] 60% aqueous methanol. [d] Calculated with eq 9 of ref 21.

Table III. Saturation-Transfer Results for Benzamide-^{15}N in Acidified 60% Aqueous Methanol

HCl, mM	$t_Z(E)$	$t_E(Z)$
0	0.71 ± 0.04	0.69 ± 0.03
5	0.59 ± 0.03	0.47 ± 0.03
10	0.52 ± 0.02	0.31 ± 0.04
15	0.48 ± 0.05	0.29 ± 0.05
20	0.49 ± 0.02	0.25 ± 0.07

Table IV. Line-Broadening Measurements of Rates of Acid-Catalyzed Proton Exchange

amide	pH	k_E, s^{-1}	k_Z, s^{-1}
acetamide	1.9[a]	75	69
acrylamide	1.9[a]	49	33
	1.7[b]	17.8	13.7
methacrylamide	2.35[a]	81	31
pivalamide	2.3[c]	21	19
	1.4[b]	26.4	25.8
benzamide	1.6[d]	35	19
salicylamide	2.1[b]	53	53
cyanoacetamide	0.5[a]	8.0	7.4
	0.9[b]	7.2	6.7
ethyl oxamate	−0.03[a]	13.2	12.3
	0.7[b]	5.9	11.0
trichloroacetamide	0.2[b]	8.8	13.2
trifluoroacetamide	−0.3[a]	9.7	5.8

[a] Aqueous. [b] Ethylene glycol. [c] 50% aqueous methanol. [d] 60% aqueous methanol.

of the inherent spin–lattice relaxation rate, measured under non-exchange conditions,[1] plus the rate constant(s) for exchange, as required by eq 6 and 8 of ref 21a. This justifies the use of eq 9 of ref 21a for some cases where selective saturation could not be applied to measure apparent relaxation rates.

The data in Table II show conclusively that H_E exchanges faster than H_Z for all amides except salicylamide, ethyl oxamate, dichloroacetamide, and trichloroacetamide. According to the error estimates, k_{SE} usually is significantly greater than k_{SZ}, but k_{ES} does not seem significantly greater than k_{ZS}. However, it is clear from eq 1 of ref 1 that the errors in k_{ES} and k_{ZS} are not independent, and analysis shows that k_{ES} usually is indeed significantly greater than k_{ZS}. Moreover, for many amides the greater reactivity of H_E is obtained by two independent methods, line broadening and saturation transfer. Thus these results confirm the previous ones[10] based on line broadening in CW spectra, even though a recent saturation-transfer study[24] was unable to detect the small rate differences.

Comparison of k_{ZE} and k_{ZS} in Table II shows that there are two classes of amides. For the last five amides—cyanoacetamide, malonamide, ethyl oxamate, chloroacetamide, and dichloroacetamide—k_{ZE} is not zero, but it is significantly less than k_{ZS}. For the other amides k_{ZE} and k_{ZS} are equal, within experimental error. Errors in k_{ZE} and k_{ZS} are nearly independent, so that although the value of k_{ZS} is greater than that of k_{ZE} for formamide,

Table V. Relative Rates of Acid-Catalyzed Exchange: k_E/k_Z[a]

amide	water	ethylene glycol
formamide		1.13[b,c]
acetamide	1.17 ± 0.02	1.22,[b,c] 1.12[b-d]
acrylamide	1.49 ± 0.04	1.26,[b,c] 1.30
methacrylamide	2.5 ± 0.4	1.23[b,c]
pivalamide	1.10 ± 0.06[e]	1.02
benzamide	1.97 ± 0.38[e]	1.25[b-d]
	2.34[b-e]	
	2.46[b,c,e]	
3,5-dimethylbenzamide		1.28[b]
salicylamide		1.0
cyanoacetamide	1.25 ± 0.19	1.08[b]
malonamide		1.12[b]
ethyl oxamate	0.94 ± 0.11	0.53[b]
chloroacetamide		1.42[b]
dichloroacetamide		0.81[b]
trichloroacetamide		0.67
trifluoroacetamide	1.61 ± 0.06	

[a] By line broadening except where indicated. [b] By saturation transfer. [c] Found to involve substantial intramolecular exchange. [d] ^{15}N labeled. [e] Aqueous methanol.

acetamide (some runs), and acrylamide, the difference is not statistically significant.

Discussion

Substituent Effects. As expected,[26] electron-withdrawing substituents retard this acid-catalyzed reaction. The logarithms of the second-order rate constants, calculated from the data of Tables II and IV, can be fit to equations linear in the pK_a of the corresponding carboxylic acid, RCOOH. (Salicylamide deviates markedly, since the ortho hydroxyl group is an electron-donating substituent that accelerates this acid-catalyzed exchange, but a hydrogen-bonding substituent that acidifies salicylic acid.[1]) Even when salicylamide is omitted, the correlation is still only fair, as has also been noted[6b] for three N-methylamides. The poor linearity seems to be due to a change of slope, from ca. 0.3 for amides with electron-withdrawing substituents to ca. 1 for other amides. This change is associated with a change in mechanism, as discussed below.

N-Protonation Mechanism. The equality of k_{ZE} and k_{ZS} for formamide, acetamide, acrylamide, methacrylamide, and benzamide is consistent with eq 3 derived from Scheme I. This key result is therefore strong evidence for the N-protonation mechanism (eq 1), at least in ethylene glycol, as well as for benzamide in aqueous methanol. Of course, this mechanism would also be consistent with the imidic acid mechanism (eq 2) if the imidic acid were to undergo configurational isomerization, as has been suggested.[24] However, since reprotonation of the imidic acid may be expected to be diffusion controlled,[15] its lifetime at pH 2 would be less than 10^{-8} s. This is far too short to permit any appreciable isomerization, which, by analogy to imidate esters,[27] has an ac-

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tivation barrier of ca. 15 kcal/mol. Therefore we conclude that our result is strong evidence against the imidic acid mechanism.

Might there be a combination of the two mechanisms, as suggested by Martin?[7] For methacrylamide and benzamide k_{ZS} and k_{ZE} are so nearly equal that these amides must be reacting exclusively by N-protonation, but for the first three amides listed above, the value of k_{ZS} is greater than that of k_{ZE}, as expected for a combination of mechanisms. However, the difference is not statistically significant. Therefore we can neither use it as evidence for incursion of the imidic acid mechanism nor exclude the possibility of such an incursion (to the extent of ca. 20%), but we can exclude the large proportion that was estimated by Martin.

Competition between Rotation and Deprotonation. That H_E exchanges faster than H_Z in all five of these amides, as well as in pivalamide (in aqueous methanol and perhaps in ethylene glycol), is consistent with eq 4 derived from Scheme I. The values in Table V correspond to k_d/k_r ratios from 0.2 to 3. These results are therefore strong evidence for competition between rotation and deprotonation of the intermediate $RCONH_3^+$, which is formed in conformation **3**. However, alternative mechanisms have been proposed[24] that would also be consistent with this result—(1) a concerted mechanism without any intermediate, (2) a mechanism involving a substantial sixfold barrier for rotation about the C-N bond of the intermediate, and (3) a mechanism involving protonation concerted with rotation. We have rejected[16] these alternative mechanisms for acid-catalyzed proton exchange in amidinium ions, and the arguments are equally applicable to amides. Moreover, a concerted mechanism would not be expected to show differences between water and ethylene glycol.

It is perhaps surprising that deprotonation can be competitive with rotation. Barriers to rotation about sp^2–sp^3 single bonds are generally ca. 1 kcal/mol.[14] For the isoelectronic ketone, CH_3CO–CH_3, the lifetime of a conformer[28] is only 0.5×10^{-12} s, which is exceedingly short. Ketones *are* a sufficiently close analogy, since according to MO calculations and some experimental results,[29] the barrier does not vary greatly with substitution, and replacing CH_3 by NH_3^+ is calculated to have a small effect.[29b]

Of course, deprotonation should also be exceedingly fast. The intermediate is a very strong acid, with a pK_a estimated[30] independently as −9.0. It may be expected to transfer its proton to solvent (water or ethylene glycol) at a diffusion-controlled rate.[15,31] Solvent surrounds the intermediate, so these do not need to diffuse together. The rate-limiting step for the proton transfer then is not the proton transfer itself, which is ultrafast within a hydrogen-bonded system.[15] Instead, the rate-limiting step becomes the rotation of either $RCONH_2$ or H_3O^+ within a hydrated $RCONH_3^+$.[15,16] This is still quite rapid, so that the lifetime of $RCONH_3^+$ is only 10^{-11} [32a] or 10^{-12} [32b] s. Then, inasmuch as these lifetime estimates are approximate, deprotonation *may* compete with rotation.

On the other hand, it is perhaps surprising that rotation can compete with deprotonation. Neither the analogy to ketones nor the MO calculations take account of solvation. Rotation of the NH_3^+ group requires breaking and remaking strong hydrogen bonds. Rotational barriers of NH_3^+ groups in solids[33,34] range from 0.7 to 10.6 kcal/mol, and 3.5 kcal/mol is the calculated[35] difference between linear and bifurcated hydrogen bonds in $OH_2\cdots NH_3$, where the hydrogen bond is weaker. Our simple electrostatic estimate, for protons with 0.1 electronic charge solvated by water dipoles 1.8 Å away in vacuum, suggests a rotational barrier of 6 kcal/mol. Yet even though 6 kcal/mol seems to be a reasonable estimate, our observation that rotation and deprotonation are competitive provides no experimental evidence for so large a barrier.

Perhaps rotation does not require breaking the $NH^+\cdots O$ hydrogen bonds. One possibility is that the $-NH_3^+$ is anchored in solution while the uncharged remainder of the molecule rotates. This is quite reasonable for $CH_3NH_3^+$, which shows a rotational barrier not far from that of ethane.[34] However, the values of k_E/k_Z do not correlate with the bulk or the anisotropy of the RCO group. In particular, this ratio in 3,5-dimethylbenzamide is the same as for benzamide, so the outlying methyls have no effect on the rate of rotation. Therefore we conclude that it is the $-NH_3^+$ that rotates.

An alternative possibility though is that the strong $NH^+\cdots O$ bonds need not be broken as the $-NH_3^+$ rotates. Instead the $-NH_3^+$ might carry solvent with it, and only the weaker solvent–solvent hydrogen bonds would need to be broken. This possibility may be tested by determining the viscosity dependence of the rotational rate. From eq 4 and the results for benzamide-^{15}N in Table II, k_d/k_r decreases from 2.7 in aqueous methanol to 0.56 in ethylene glycol. (A similar decrease can also be seen for methacrylamide, but the data are less accurate.) To determine the viscosity dependence of k_r alone, it is necessary to estimate the viscosity dependence of k_d. Since k_d represents a diffusion-controlled proton transfer, limited by rotations,[15,16] a closely analogous process is proton mobility, which proceeds by a Grotthus mechanism in these hydroxylic solvents. The limiting proton conductivities in these two solvents are 116[36] and 27.7[37] $cm^2/(\Omega\ mol)$, respectively. Therefore the 4^+-fold decrease in k_d/k_r is completely accounted for by the 4-fold decrease in k_d, and k_r must be nearly independent of solvent viscosity. We therefore conclude that the $-NH_3^+$ does not carry solvent with it as it rotates, since a viscous solvent would then slow the rotation. (This result is further verification that the RCO is not rotating.) A similar conclusion was reached for rotation of rhodamine 6G,[38] where the hydrogen bonds are considerably weaker. We are therefore led to the conclusion that rotation of the $-NH_3^+$ within its solvent shell occurs with a rate constant of at least $10^{11}\ s^{-1}$. It is quite unusual for NMR methods to probe such a time scale, but the competition between two such fast processes is manifested in k_E/k_Z.

We have further investigated benzamide-^{15}N in order to test some baffling results of Redfield and Waelder.[24] They observed asymmetric saturation transfer—$t_Z(E) = 0.75$ and $t_E(Z) = 0.2$—for acid-catalyzed exchange in benzamide-^{14}N (0.05 M) and a similar result for methacrylamide. Such results are not consistent with the N-protonation mechanism, for which the maximum saturation transfer is 0.5. The results are consistent with acid-catalyzed intramolecular exchange without intermolecular exchange (eq 8 of ref 24), which is incredible. We do obtain similar results, but ours do not support this additional pathway. The data for methacrylamide in Table I do show saturation transfer >0.5, but it is symmetric and due to the uncatalyzed rotation. When this rate constant ($4.9\ s^{-1}$, from Table II of ref 1) is subtracted from the observed rate constant for intramolecular exchange, acid-catalyzed intramolecular exchange is not faster than the intermolecular exchange, as the rate constants in Table II show. Likewise, the data in Table III show that there is saturation transfer >0.5 for benzamide (0.4 M in 60% aqueous methanol) at low acidities, owing to the uncatalyzed rotation, but at higher acidities $t_Z(E)$ approaches 0.5. There is asymmetric saturation transfer, but this is due only to the difference in reactivities of H_E and H_Z. We are unable to explain the discrepancy between

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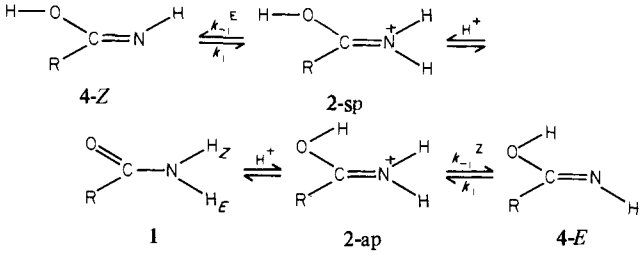
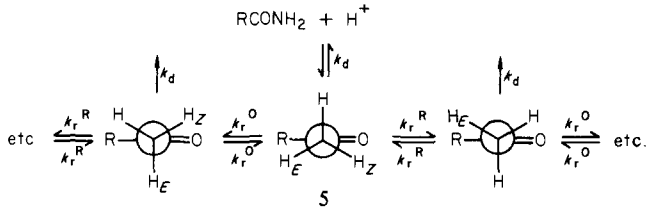
Scheme II. Imidic Acid Mechanism for Exchange in Primary Amides, via Stereoisomeric Intermediates



Scheme III. N-Protonation Mechanism for Exchange in Primary Amides, Including a Sixfold Barrier to Rotation about the C–N Single Bond



our results and those of Redfield and Waelder; it is quite unlikely to be due to the difference in solvent or amide concentration.

Imidic Acid Mechanism. For the remaining amides of Table II—cyanoacetamide, malonamide, ethyl oxamate, chloroacetamide, and dichloroacetamide—k_{ZE} is significantly less than k_{ZS}. We attribute this to a predominance of the imidic acid mechanism (eq 2), at least in ethylene glycol. However, since k_{ZE} is nonzero for each of the amides, there must be some exchange via N-protonation, to an extent ranging from 10% (dichloroacetamide) to 40% (malonamide). That these amides show a combination of mechanisms suggests that formamide, acetamide, and acrylamide do also, even though we recognized above that the data are not sufficiently precise to justify such a conclusion. For those three amides we now conclude that the imidic acid mechanism is indeed operative, to the extent of ca. 20%, and the change of mechanism is gradual.

The change in mechanism may be rationalized in terms of substituent effects. From eq 1 and 2, the ratio of rates of exchange via imidic acid and N-protonation pathways in dilute acid is

$$\frac{v_I}{v_N} = \frac{k_{-i}}{K_a^O k_p} = \frac{k_i}{k_d}\frac{K_a^N K_a^I}{K_a^O} \qquad (8)$$

where K_a^N and K_a^I are the acidity constants for proton loss from nitrogen in $RCONH_3^+$ and $RC(OH)=NH_2^+$, respectively. But k_i and k_d are rate constants for thermodynamically favorable proton transfers and thus have values ca. 10^{11} M^{-1} s^{-1} and 10^{12} s^{-1}, respectively, independent of amide. Also, K_a^N/K_a^O and K_a^I/K_a^O are tautomeric equilibrium constants for the ratio of O- and N-protonated amide and of imidic acid to amide, respectively, and these too are less dependent on amide than the acidity constants are. Then v_I/v_N is proportional to K_a^I, K_a^N, or K_a^O, with proportionality constants that are nearly independent of amide. Therefore electron-withdrawing substituents, which increase K_a^O[39a] or K_a^I,[39b] may be expected to increase v_I/v_N and thereby favor the imidic acid mechanism, as we observe.

Qualitative considerations of the transition-state structures lead to the same conclusion. Since both mechanisms involve diffusion-controlled proton transfers, the transition state for the N-protonation mechanism resembles $RCONH_3^+$, whereas that for the imidic acid mechanism resembles RC(OH)=NH. The former is more strongly destabilized by electron-withdrawing substituents. This analysis also explains the change in the slope of log k vs. the pK_a of RCOOH. Rates of exchange via N-protonation are more sensitive to substitution than rates of exchange via the imidic acid, since the latter transition state bears much less positive charge.

The results in Table V show that with few exceptions H_E exchanges faster than H_Z, not only for those amides that exchange via N-protonation, but also for those that exchange via the imidic acid. We had originally intended[10] that differential reactivity of H_E and H_Z would be diagnostic of the imidic acid mechanism (Scheme II). However, we had expected that H_Z would be the more reactive, on the basis of a chain of reasoning. Since k_i is a diffusion-controlled reprotonation, the relative magnitudes of k_{-i}^E and k_{-i}^Z are determined solely by the relative stabilities of the diastereometric imidic acids **4**-*Z* and **4**-*E*. According to both ab initio[13a] and CNDO/2[13b] MO calculations, **4**-*E* (R = H) is more stable than **4**-*Z* (R = H). Also, there is considerable firm evidence from both NMR and dipole moment measurements[12] that the *E* diastereomer of imidate esters, RC(OR′)=NR″, is the more stable. Therefore both MO calcuations and analogy to imidate esters suggested that **4**-*E* is the more stable configuration and that H_Z should exchange faster.

Thus we had rejected[10] the imidic acid mechanism on the basis of the discrepancy between the expectation that H_Z would exchange faster and the observation that for several amides it is H_E that exchanges faster. This expectation *is* borne out by ethyl oxamate, dichloroacetamide, and trichloroacetamide. If we had included them in our initial study, we would have concluded, correctly, that they exchange via the imidiç acid. It was only by chance that no amides with electron-withdrawing substituents had been included in that earlier study. Nevertheless, some amides that exchange via the imidic acid mechanism—cyanoacetamide, malonamide, chloroacetamide, and trifluoroacetamide—also show $k_E > k_Z$. We are fortunate that they were not included, for we would have concluded, incorrectly, that they exchange via N-protonation.

The error in reasoning seems to be due to neglect of solvation. The MO calculations[13] are gas-phase values. The experimental results on imidate esters[12] are chiefly from studies in nonpolar solvents. Increasing solvent polarity increases the proportion of the *Z* ester,[12,27] whose dipole moment is greater.[40] We presume that solvation effects on imidic acids are even greater than on imidate esters, which are subject to steric hindrance to solvation, and we must assume that for some imidic acids these effects are sufficient to reverse the stabilities and render the *Z* acid the more stable.

Must the amides with electron-withdrawing substituents exchange via the imidic acid, or can all the results be interpreted in terms of the N-protonation mechanism, as has been proposed?[24] Certainly the N-protonation mechanism of Scheme I cannot be operative, since the comparison of intramolecular exchange with intermolecular does not satisfy eq 3. Also, for ethyl oxamate, dichloroacetamide, and trichloroacetamide, H_Z exchanges faster than H_E, contrary to eq 4. To account for such results, it is necessary to modify the N-protonation mechanism so that the conformer initially formed is **5** (Scheme III, adapted from eq 4 of ref 24). It seems reasonable that the proton would attack perpendicular to the amide plane, to produce **5** rather than **3** as the initial intermediate. However, as we have noted,[16] **5** cannot be an intermediate since analogy to methyl rotors on a double bond indicates that it is not a minimum on a potential energy surface. For justification of Scheme III there would need to be a substantial 6-fold component to the barrier to rotation about the C–N single bond. These are generally extremely small,[14] but it is possible that solvation[41] increases this one. A further requirement though is the ad hoc assumption that the 6-fold barrier is such that **5** is a minimum rather than a maximum.

Next we may ask what restrictions do the observed rate ratios place on the rate contants of Scheme III. To account for the difference between k_{ES} and k_{ZS}, it is necessary to take two different rate constants, k_r^O and k_r^R, for rotation of a proton past O or R,

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respectively. Qualitatively this is reasonable. The transition state for k_r^O is conformer **3** of Scheme I, corresponding to a stable minimum of the threefold barrier for rotation about the C–N bond. The transition state for k_r^R is a conformer corresponding to the top of that barrier. The energy difference between these two transition states is the 3-fold component of the rotational barrier. By analogy to methyl rotors on a double bond, that energy difference may be expected to be ca. 1 kcal/mol.[14,29] However, detailed analysis[42] of Scheme III shows that our observed rate constants for cyanoacetamide, malonamide, ethyl oxamate, chloroacetamide, and dichloroacetamide require that this energy difference be only 0.6, 0.2, −0.4, 0.2, and −0.13 kcal/mol, respectively. Not only are some of these energy differences negative, corresponding to the necessity that **3** be a conformation of maximum energy, but also they are too small, appreciably less than 1 kcal/mol. To account for the observed rate ratios, it is necessary that the 3-fold component of the rotational barrier must have nearly vanished, which is unreasonable.

Furthermore, to account for the near absence of intramolecular exchange in these amides, it is necessary to assume that k_d/k_r has been increased for amides with electron-withdrawing substituents. Since k_r^O and k_r^R are required by the data to be nearly equal, they must both be quite high. Therefore the increase in k_d/k_r cannot be due to a decrease in k_r. It is necessary to conclude[24] that k_d has increased for amides with electron-withdrawing substituents, even though this is a diffusion-controlled deprotonation and should be independent of amide.

Thus invoking the N-protonation mechanism for these amides leads to three unlikely requirements—a substantial 6-fold barrier to C–N rotation, disappearance and occasional reversal of the 3-fold component of that barrier, and substituent effects on the rate constant for a diffusion-controlled deprotonation. We therefore conclude that these results for amides with electron-withdrawing substituents are consistent only with the imidic acid mechanism. Thus we have at last obtained evidence for the mechanism that we had originally though to be the more attractive one, even though we have been surprised to find that it is not the dominant mechanism for primary amides.

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Experimental Chemical Shift Correlation Maps from Heteronuclear Two-Dimensional NMR Spectroscopy. 1. Carbon-13 and Proton Chemical Shifts of Raffinose and Its Subunits

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Abstract: With the use of double Fourier transform ("2D") NMR methods it is possible to make a simultaneous measurement of proton and carbon-13 chemical shifts for each directly bonded carbon–proton pair in a molecule. Correlation of proton and carbon-13 shifts greatly increases the information capacity of NMR experiments, allowing otherwise inaccessible proton shifts to be determined and facilitating assignment. Experimental results are presented for raffinose, melibiose, sucrose, galactose, glucose, and fructose; the carbon-13 spectra of raffinose and melibiose are reassigned, and proton shifts are reported for all three significant solution forms of aqueous fructose.

Two of the fundamental problems in NMR are the measurement of NMR parameters from complex crowded spectra and the assignment of these resonances to specific nuclear sites in a molecule. Advances in instrumentation have improved sensitivity more rapidly than resolving power, with the result that the complexity of the system which may be studied by abundant spin NMR is now limited largely by the problem of resolving and identifying single resonances. Since the amount of information to be gleaned from the measurement of the chemical shift of a resonance is limited, techniques such as double-resonance and relaxation-time measurement have increasingly been adopted. By *correlating* two independent parameters such as the chemical shift of a resonance and the chemical shift of a second nucleus scalar coupled to the first (as in double resonance), the specificity with which a resonance may be characterized and hence the ease of its assignment can be greatly enhanced. Double-resonance methods such as homonuclear INDOR also attack the problem of spectral overcrowding by allowing "hidden" resonances to be detected.

In recent years a number of new techniques have been developed, based on the double Fourier transformation of NMR signals, which attack the problems of resolving individual signals and correlating NMR parameters in a very direct way. Such experiment, commonly referred to by the generic title of two-dimensional or "2D" NMR spectroscopy,[1,2] produce spectra which display signal strength as a function of two independent frequencies. The choice of which NMR parameters are responsible for spreading resonances in each frequency domain is under the control of the experimenter, through his choice of pulse sequence for the acquisition of the time domain data for double Fourier transformation.

The purpose of this paper is to illustrate the application of one 2D NMR experiment, for the correlation of proton and carbon-13

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