NMR Studies of Base-Catalyzed Proton Exchange in Amides

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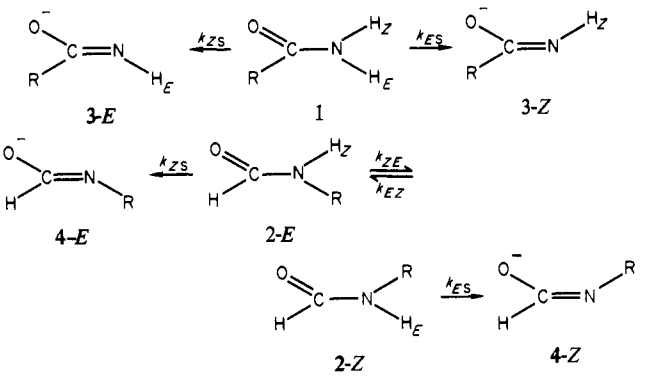
Abstract: Kinetics of proton exchange of a series of primary and secondary amides have been determined by a combination of line-shape analysis and saturation-transfer techniques. NMR signals of diastereotopic protons H_E and H_Z were assigned. Rates of interconversion, via rotation about the C–N bond, were measured. Rates of exchange of H_E and H_Z, via stereoisomeric imidates, were separately measured. For most primary amides H_E exchanges faster, as expected on the basis of lone-pair repulsions. The exception of salicylamide is discussed, and its significance. For secondary amides H_Z exchanges faster, owing to steric hindrance to solvation of the Z imidate. In less polar solvents k_E/k_Z decreases for primary amides and increases for secondary amides. The origin of this solvent effect is discussed.

Proton transfers[1] are among the most fundamental of chemical reactions. Since they can be extremely rapid, study of their kinetics has often necessitated new methods—T jump and other relaxation techniques[2] or NMR methods.[3] One early example of the NMR method was a study of proton exchange in *N*-methylacetamide, where it was observed that the reaction is subject to catalysis by both acid and base.[4] Subsequently, proton exchange in amides has been of considerable interest,[5] especially since proton-exchange kinetics in amides, peptides, and proteins can provide information about the structure of peptides and proteins in solution.[5,6] Nearly all previous studies were of secondary amides, RCONHR′, where the line shape of an adjacent CH can be analyzed to measure the exchange of the NH proton. As part of an investigation into the mechanism of exchange, we have made a detailed study of stereochemical effects, principally in primary amides, $RCONH_2$. These studies have necessitated analysis of the NH peaks themselves, which are ordinarily excessively broad, and we have utilized techniques—^{14}N decoupling, ^{15}N-labeled amides, and viscous solvents—to sharpen the peaks. Preliminary communications of some of this study have already been published.[7]

This study involves base-catalyzed exchange both in primary amides (**1**), where there are diastereotopic protons, H_E and H_Z, and in secondary formamides (**2**), where there are diastereomeric E and Z forms (Scheme I). For ease of comparison with the primary amides, the NH protons of the E and Z formamides are designated H_Z and H_E respectively. Rate constants k_{ES} and k_{ZS} are for exchange of protons H_E and H_Z, respectively, into solvent. Since H_E and H_Z, as well as **2**-Z and **2**-E, show separate NMR resonances, their separate rate constants can be determined by line broadening,[8] line shape analysis,[8] or saturation transfer,[9] despite a previous denial.[10] By saturation transfer it is also possible to determine rate constants k_{SE} and k_{SZ}, for proton exchange from solvent into sites H_E and H_Z, as well as k_{EZ} and k_{ZE}, rate constants for intramolecular proton exchange (in **1**) or isomerization (in **2**) due to rotation about the C–N bond (Scheme I).

Scheme I. Base-Catalyzed Exchange of H_E and H_Z in Amides



The base-catalyzed exchanges proceed via the imidate anions **3** and **4** (Scheme I). Since the imidates exist as E and Z diastereomers, of different energy, they may be expected to be formed at different rates. Indeed, in a preliminary communication[7a] we reported that H_E of several primary amides undergoes base-catalyzed exchange faster than H_Z. On the other hand, no such difference was observed in nicotinamide,[5k] and saturation-transfer results for oxytocin[6c] show that the downfield NH in glutamine and glycinamide residues exchanges faster whereas the upfield NH in the asparagine residue exchanges faster. Recently, saturation-transfer measurements on several amides show that H_E exchanges faster than H_Z.[5q] We here report our results concerning the stereochemical effects in the base-catalyzed exchange of a broad series of amides.

Experimental Section

Amides, $Eu(fod)_3$, and chloroform-*d* were commercial samples from Aldrich, Eastman, Fluka, or Matheson Coleman and Bell, and were used without further purification. Acetamide-^{15}N, benzamide-^{15}N, formamide-d_1, and Me_2SO-d_6 were obtained from Stohler Isotope Chemicals. Cyclohexanol was redistilled from calcium oxide; other solvents were reagent grade, from Mallinckrodt or J. T. Baker. No attempt was made to ensure anhydrous conditions, since the addition of 1% H_2O had no

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significant effect on exchange rates in ethylene glycol or on exchange ratios in cyclohexanol. A trace of hydroquinone was added to solutions of acrylamide and methacrylamide, to inhibit polymerization. The pH was adjusted to minimize exchange or to produce an exchange rate that was measurable by line shape analysis or saturation transfer. Buffers were prepared in ethylene glycol or aqueous solutions from reagent-grade components, and the pH was measured after the NMR measurements were completed. In cyclohexanol solutions tetraethylammonium hydroxide served as base.

All spectra were run on solutions that were between 0.4 and 2.7 M in amide; the exact ratio of solvent protons to NH protons was determined from the weights of the components used to prepare the solutions. Even at these high concentrations amide aggregation is not expected to be appreciable in such polar solvents as water or ethylene glycol.[11] In support of this expectation we find that the chemical shifts of formamide, *N-tert*-butylformamide, and *N*-phenylformamide, which would be sensitive to interamide hydrogen bonding,[5k,12] are independent of concentration in ethylene glycol between 0.5 and 2 M. Also, line-broadening measurements on acetamide in ethylene glycol showed that the rate ratio k_{ES}/k_{ZS} was the same, within experimental error, at both 0.25 and 2.7 M. Likewise, saturation-transfer measurements on dichloroacetamide in 1:1 cyclohexanol–dioxane showed that k_{ES}/k_{ZS} did not vary from 0.8 to 2.0 M, so that aggregation even in nonpolar solvents does not seem to be significant.

Measurements on aqueous or aqueous methanol solutions of primary amides were run on a JEOL PFT-100 NMR spectrometer equipped with a frequency synthesizer for decoupling ^{14}N, at 7 191 100 ± 50 Hz,[13] optimized for each amide. Acetone-d_6 in an external, concentric 10-mm tube served for the lock signal. The probe temperature was 27–28 °C. Measurements on ethylene glycol or cyclohexanol–dioxane solutions were run on a Varian HR-220 or 360-MHz FT spectrometer at a probe temperature of 22–23 °C. These solutions are sufficiently viscous that ^{14}N is decoupled without the necessity of heteronuclear irradiation, leading to NH line widths in the absence of exchange of 8–18 Hz. Spectra of acetamide-^{15}N and benzamide-^{15}N, which show sharp NH doublets even in nonviscous solvents, were also run on this machine. All FT spectra were processed with 2–3 Hz of additional line broadening (1 Hz for alkyl groups of secondary amides), which obscured the $^2J_{HH}$ of ca. 2 Hz[14] and permitted the use of simplified equations which neglect this coupling.

NOE[9b] experiments were performed on solutions of amides in nondeoxygenated ethylene glycol. The NH protons were separately saturated, and the intensity changes of CH protons were measured with difference spectra, obtained by subtracting the off-resonance free-induction decay (FID) from the FID under conditions of saturation.

Line-broadening measurements were usually replicates on solutions of varous pH, and k_E/k_Z was determined as a weighted average of the ratio $(\delta\nu_E - \delta\nu_E^0)/(\delta\nu_Z - \delta\nu_Z^0)$, where $\delta\nu$ is the observed line width and $\delta\nu^0$ is the line width in the absence of exchange. To compensate for variability of line broadening due to instrument inhomogeneity, we subtracted the line width of an internal *tert*-butyl alcohol standard from every NH line width. Solutions were buffered to a pH that would produce a broadening near the optimum for determining a rate constant from line broadening, which occurs when $\delta\nu = 2.1\delta\nu^0$. This result is intuitively reasonable but does not seem to have been recognized previously. It can be derived as the condition that minimizes the relative error in $\delta\nu - \delta\nu^0$, subject to the condition that the signal intensity has a constant noise background. Values were then weighted in proportion to the number of FIDs accumulated and according to how close they were to the optimum.

Saturation-transfer[9] experiments were performed with homonuclear double irradiation effected with a frequency synthesizer under control of the Nicolet computer interfaced to the HR220 FT spectrometer. Intensities for both saturation-transfer and T_1 measurements were simply peak heights, obtained from digitized spectra. The use of integrals gave the same rates, but less accurately. Further details regarding the use of 90° observation pulses, multiple saturation, and compensation for spillover have been published.[15]

Rate constants were calculated from equations adapted from Perrin and Johnston.[15a] In particular, the rate constant for exchange from site H_E into $H_{Solvent}$ is given by eq 1, and similarly for k_{ZS} and k_{SE}. Here p_i

$$k_{ES} = M_S(E,Z)\,\frac{t_S(E) - t_S(Z)t_Z(E)}{1 - t_E(S)t_S(E)}\,\frac{p_S}{p_E} \tag{1}$$

is the relative population of site i, and $t_i(j)$ is the saturation transfer[15c] from site j to site i, or the fractional loss of intensity at site i, I_i, on saturating site j (eq 2). Also, $M_S(E,Z)$ is the apparent spin–lattice

$$t_i(j) = \frac{I_i^0 - I_i(j)}{I_i^0} \tag{2}$$

relaxation rate constant ($=1/T_{1S}^{app}$) of site H_S, obtained in an inversion recovery experiment under conditions that sites H_E and H_Z are saturated. It can be derived that under these conditions, I_S approaches its steady-state value as a single exponential. Usually it proved instrumentally impossible to saturate both H_S and either H_E or H_Z, so that the other four rate constants were determined from eq 3 and 4, and similarly for

$$k_{SE} = M_E(S)\,\frac{t_E(S) - t_E(Z)t_Z(S)}{1 - t_E(Z) + t_S(Z)[t_E(S) - t_Z(S)]}\,\frac{p_E}{p_S} \tag{3}$$

$$k_{ZE} = M_E(S)\,\frac{t_E(Z) - t_E(S)t_S(Z)}{1 - t_E(Z) + t_S(Z)[t_E(S) - t_Z(S)]}\,\frac{p_E}{p_Z} \tag{4}$$

k_{SZ} and k_{EZ}. Here $M_E(S)$ is the apparent spin–lattice relaxation rate constant of site H_E, under conditions of saturating site H_S. If $M_E(S)$ and $M_Z(S)$ are nearly equal, then it can be derived that under these conditions I_E or I_Z approaches its steady-state value as a single exponential.

These complicated equations are necessary to account for the indirect transfer of saturation, from site j to site i via site k, which can arise in multisite systems. However, in the absence of acid or base catalysts, there is no exchange of H_E or H_Z with solvent OH, so that the simple two-site equations[9a] involving spin–lattice relaxation rate constants without saturation can be used:

$$k_{EZ} = M_Z\,\frac{t_Z(E)}{1 - t_Z(E)}\,\frac{p_Z}{p_E} \tag{5}$$

and similarly for k_{ZE}. Also, the rate constants in cyclohexanol-containing solvents were determined simply as

$$k_{ES} = M_E(S)t_E(S) + k_{EZ}[t_E(S) - t_Z(S)] \tag{6}$$

and similarly for k_{ZS}. This is readily derived and relies on the transferability of the rate constants from Table II.

From replicate determinations of the $t_i(j)$s and from the weighted linear least-squares[16a] determinations of the Ms, errors in all values (standard deviation of the mean) were obtained. Standard deviations in the ks were then calculated according to the propagation of errors.[16b] Although all six rate constants are determined independently, the errors are not necessarily independent. For example, errors in k_{ZE} and k_{ZS} are independent, but comparison of eq 3 and 4 shows that errors in k_{SE} and k_{ZE} are not independent.

NMR line shapes of secondary amides under exchange conditions were calculated from eq 7, applicable in the absence of strong coupling,[17]

$$I(\omega) \propto Im\{i\mathbf{1}[\mathbf{T}_2 + i\mathbf{X} - i\boldsymbol{\Omega} + \mathbf{K}]^{-1}\mathbf{p}\} = Im\{i\mathbf{1}\mathbf{S}^{-1}[\mathbf{D} + i\mathbf{X}]^{-1}\mathbf{S}\mathbf{p}\} \tag{7}$$

where $\mathbf{1}$ is a unit-row matrix, $\mathbf{T}_2 = \mathrm{diag}(1/T_{2i}) = \mathrm{diag}(\pi\delta\nu_i^0)$, $\boldsymbol{\Omega} = \mathrm{diag}(\omega_i)$, $\mathbf{K}_{ij} = k_{ji}$ $(i \neq j)$, $\mathbf{K}_{ii} = -\sum_j k_{ij}$, $\mathbf{X} = \mathrm{diag}(\omega)$, $\mathbf{p}$ is a column matrix of populations, and $\mathbf{S}$ is the transformation matrix that diagonalizes $(\mathbf{T}_2 - i\boldsymbol{\Omega} + \mathbf{K})$ to $\mathbf{D} = \mathbf{S}(\mathbf{T}_2 - i\boldsymbol{\Omega} + \mathbf{K})\mathbf{S}^{-1}$. For *N*-methylformamide the four sites are the two *N*-methyl doublets, from low-field to high-field, and for *N-tert*-butylformamide and *N*-phenylformamide there are CH and NH doublets in the *E* amide, CH and NH doublets in the *Z* amide, and the solvent site.

For secondary amides the equilibrium constant, [*Z* amide]/[*E* amide], was determined by integration. Values 13.5 (*N*-methylformamide), 2.2 (*N-tert*-butylformamide), and 2.6 (*N*-phenylformamide) are in good agreement with published values of 11.5,[18] 2.3,[19] and 2.5,[20] respectively,

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in other solvents. That the equilibrium constant for *N*-phenylformamide was independent of concentration from 0.5 to 2.0 M supports our conclusion that aggregation is insignificant in ethylene glycol or 1:1 cyclohexanol–dioxan.

Kinetics of proton exchange in secondary amides were determined by a combination of line shape analysis and saturation transfer. Rate constants for uncatalyzed isomerization in *N*-methylformamide and *N*-*tert*-butylformamide were determined by saturation transfer. For *N*-methylformamide the *E* methyl doublet was saturated and k_{ZE}, for proton exchange, calculated from eq 5. For *N*-*tert*-butylformamide no saturation transfer could be detected ($t < 0.01$). Rate constants for base-catalyzed exchange of *N*-methylformamide were determined by line shape analysis. The small interconversion rate constants (0.6 and 0.05 s^{-1}) were subsumed into $T_{2Z} = 0.23$ s and $T_{2E} = 0.31$ s, so that k_{EZ} and k_{ZE} could be set equal to zero for the computer simulations. The values for k_{ES} and k_{ZS} were then adjusted to produce the best agreement between experimental and calculated spectra. Plots of log k_{ES} and log k_{ZS} vs. pH (in aqueous solution) give second-order rate constants $k_{ES}^{OH} = 7.0 \times 10^7$ M^{-1} s^{-1} and $k_{ZS}^{OH} = 1.4 \times 10^8$ M^{-1} s^{-1}. The former value, for the dominant stereoisomer, is in excellent agreement with the 25 °C value of $(6.78 \pm 0.6) \times 10^7$ M^{-1} s^{-1} previously reported.[5g] Likewise, k_{EZ} and k_{ZE} for *N*-phenylformamide were set equal to zero, and the values for k_{ES} and k_{ZS} were adjusted to best simulate the observed spectra, with emphasis on the CH doublet of the *E* amide and the NH peak of the *Z* amide. The rate constant k_{SE} for base-catalyzed exchange in *N*-*tert*-butylformamide was measured by saturation transfer and calculated from a permutation of eq 1. Then k_{ES} was calculated as $k_{SE}p_S/p_E$. These two values, along with zero values for k_{EZ} and k_{ZE}, were then used in a computer simulation of the downfield region of the NMR spectrum of that sample, and k_{ZS} was adjusted to produce the best agreement between experimental and calculated spectra. Justification for setting k_{EZ} and k_{ZE} equal to zero in these simulations is that the two methyls and the two *tert*-butyls as well as the formyls of all these amides sharpen into separate singlets at higher pH.

Results

Signal Assignments. Usually the *E* substituent of an amide appears downfield of the *Z* substituent,[5b] but nearly all the samples are tertiary amides, and there are exceptions.[21] For secondary formamides, this generalization often holds, but *N*-*tert*-butylformamide[18] and *N*-phenylformamide[22] are exceptions. For primary amides there are only three definitive assignments, all of which obey this generalization. The NH signals of formamide[23] and acetamide[24] were assigned on the basis of $^3J_{trans} > {}^3J_{cis}$, and the NH signals of NAD were assigned[5q] on the basis of NOE measurements. For other primary amides the generalization has been claimed to hold,[25] but the evidence is less conclusive. We therefore have felt it necessary to confirm assignments for various of the amides under study here.

NOE results[15b] for *N*-methylformamide and *N*-*tert*-butylformamide in ethylene glycol confirm the previous assignments[18] and verify that the previously unseen H_Z of *N*-*tert*-butylformamide has been shifted downfield of H_E. NOE measurements on chloroacetamide and dichloroacetamide give $f_{\alpha CH}(NH_{downfield}) = 0.15$ and 0.81, respectively, $f_{\alpha-CH}(NH_{upfield}) \simeq 0.00$. NOE measurements on salicylamide give $f_{H_6}(NH_{downfield}) = 0.12$ and $f_{CH}(NH_{upfield}) < 0.12$. We therefore conclude that H_E, the proton closer to the CH, is the downfield NH in these three amides.

Primary amides without α-protons were assigned through lanthanide-induced shift (LIS) studies. In tertiary amides the *Z* substitutent shows the greater LIS[26] and we have verified, with acetamide, that this conclusion also holds for primary amides. In $CDCl_3$ we find that $Eu(fod)_3$ produces the greater downfield shift for the downfield proton of trichloroacetamide and for the upfield proton of ethyl oxamate (where LIS reagents may be expected[27] to complex preferentially at amide oxygen over ester oxygen) and iodoacetamide. However, for the first two amides (but not for acetamide or iodoacetamide) the NH chemical shifts cross on changing the solvent from $CDCl_3$ to ethylene glycol or cyclohexanol. Therefore we conclude that in the solvents of interest H_E is the downfield NH of trichloroacetamide and iodoacetamide but the upfield NH of ethyl oxamate. The reversal in the latter assignment may be attributed to the magnetic anisotropy of the additional carbonyl group.

In summary, ethyl oxamate is the only primary amide where H_Z is the downfield proton. For all other primary amides where the assignments seem secure—formamide,[23] acetamide,[24] NAD,[5q] salicylamide, iodoacetamide, and mono- di-, and trichloroacetamide—H_E is the downfield proton, and we assume that this generalization holds for all the other primary amides under study.

Exchange Rates. Figure 1 shows a representative saturation-transfer experiment on benzamide-^{15}N under nonexchange con-

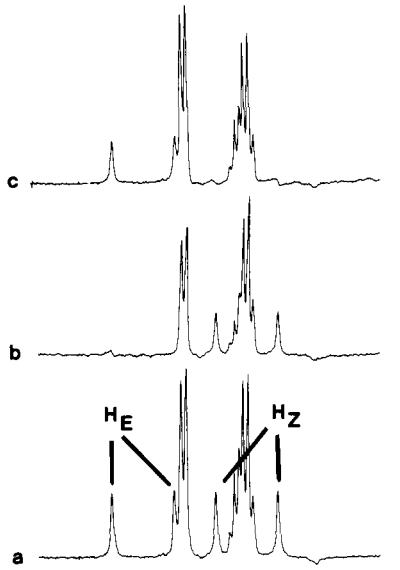

Figure 1. Saturation-transfer experiment on benzamide-^{15}N in ethylene glycol under nonexchange conditions: NH and aromatic CH region (plot width 500 Hz): (a) off-resonance spectrum; (b) with saturation of both components of the H_E doublet; (c) with saturation of H_Z.

Table II. Rate Constants for Uncatalyzed C–N Rotation of Amides in Ethylene Glycol[a]

	k_{EZ}, s^{-1}	k_{ZE}, s^{-1}
formamide-*d*	0	0
acetamide	0.40 ± 0.03	0.41 ± 0.03
acetamide-^{15}N	0.34 ± 0.03	0.40 ± 0.03
acrylamide	1.34 ± 0.13	1.44 ± 0.14
methacrylamide	5.0 ± 0.64	4.8 ± 0.60
benzamide	3.4 ± 0.3	3.4 ± 0.3
benzamide-^{15}N	3.15 ± 0.33	3.14 ± 0.30
benzamide-^{15}N[b]	3.7 ± 0.8	3.7 ± 0.7
salicylamide	21.4 ± 4.8	20.5 ± 4.5
cyanoacetamide	1.13 ± 0.10	1.00 ± 0.09
malonamide	0	0
ethyl oxamate	−0.75 ± 0.06[c]	−0.64 ± 0.05[c]
chloroacetamide	1.02 ± 0.09	1.07 ± 0.10
dichloroacetamide	0.08 ± 0.04	0.07 ± 0.04
dichloroacetamide[d]	0.68 ± 0.04	0.67 ± 0.05
trichloroacetamide[d]	1.80 ± 0.13	1.67 ± 0.13
iodoacetamide	0.69 ± 0.18	0.71 ± 0.06
N-methylformamide[e]	0.05 ± 0.01[f]	0.6 ± 0.1
N-*tert*-butylformamide	0	0

[a] At 23 °C and pH ~4.7. [b] 60% aqueous methanol. [c] A negative value arises from cross-relaxation of the spins. [d] 50% dioxan in cyclohexanol. [e] Aqueous, pH 2.3. [f] Calculated from $p_i k_{ij} = p_j k_{ji}$.

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Table IV. Kinetic Results for Base-Catalyzed Proton Exchange of Amides in Ethylene Glycol

	pH	$p_S/p_{E,Z}$	k_{ES}, s^{-1}	k_{ZS}, s^{-1}	k_{ZE},[a] s^{-1}	k_{EZ},[a] s^{-1}	k_{SE}, s^{-1}	k_{SZ}, s^{-1}
formamide-*d*	7.1	14.96	5.4 ± 0.4	3.7 ± 0.3	0.09 ± 0.11	−0.24 ± 0.07	0.40 ± 0.03	0.226 ± 0.02
acetamide	8.1	11.24	2.7 ± 0.2	1.03 ± 0.10	0.01 ± 0.07	0.04 ± 0.08	0.261 ± 0.02	0.103 ± 0.01
acrylamide	8.21	12.22	11.7 ± 1.0	5.2 ± 0.6	−0.22 ± 0.37	−0.18 ± 0.34	0.97 ± 0.08	0.39 ± 0.05
methacrylamide	8.20	13.00	5.8 ± 0.8	4.8 ± 0.7	0.14 ± 1.5	0.23 ± 1.55	0.485 ± 0.07	0.39 ± 0.06
benzamide-^{15}N	8.0	59.6	5.0 ± 0.4	2.8 ± 0.3	−0.11 ± 0.39	0.03 ± 0.36	0.103 ± 0.01	0.057 ± 0.006
salicylamide	6.95	84.8	4.9 ± 1.2	4.9 ± 1.2	−0.95 ± 5.6	−1.8 ± 5.9	0.051 ± 0.01	0.053 ± 0.01
malonamide	7.85	56.0	4.4[b]	2.0[b]	0.18	0.19	0.079[d]	0.036[d]
iodoacetamide	6.5	73	2.32 ± 0.19[b]	1.29 ± 0.18[b]	−0.33 ± 0.30	−0.29 ± 0.20	0.032 ± 0.003	0.018 ± 0.002
N-*tert*-butylformamide	8.4	13.6, 30	1.50 ± 0.15[b]	12 ± 2[c]	0	0	0.11 ± 0.01	0.4 ± 0.07[b]

[a] Corrected to exclude *E*–*Z* cross-relaxation and C–N bond rotation. [b] Calculated from $p_i k_{ij} = p_j k_{ji}$. [c] Obtained by line-shape analysis. [d] Using eq 9 of ref 15a.

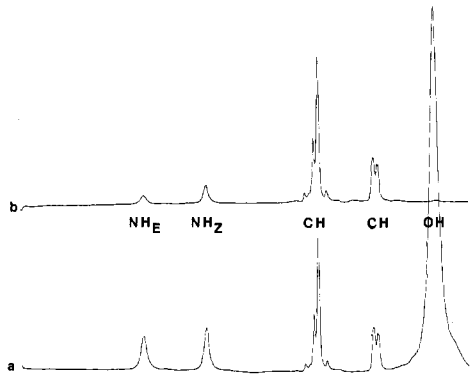


Figure 2. Saturation-transfer experiment on 2.5 M acrylamide in ethylene glycol at pH 8.21 (plot width 900 Hz (solvent CH_2 offscale to right)): (a) off-resonance spectrum; (b) with saturation of solvent OH.

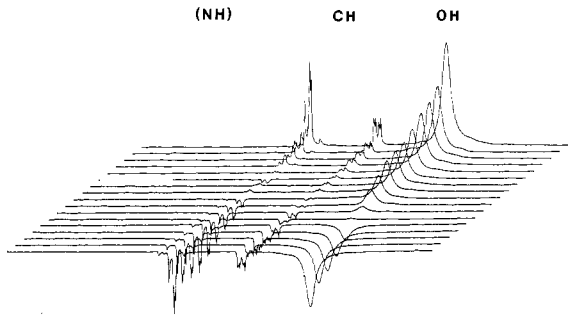


Figure 3. Saturation-transfer experiment on 2.5 M acrylamide in ethylene glycol at pH 8.21 (plot width 500 Hz). Inversion-recovery sequence of (vinyl and) solvent OH signals while H_E and H_Z are saturated. Delay times, from front to rear, are 0.04, 0.06, 0.08, 0.10, 0.12, 0.16, 0.18, 0.22, 0.24, 0.26, 0.28, 0.30, 0.34, 0.38, 0.42, 0.48, and 3.00 s.

ditions. Saturation-transfer data for uncatalyzed rotation about the C–N bond of several amides are given in Table I, and the rate constants calculated therefrom are in Table II. In the region of the rate minimum saturation transfer to or from solvent was undetectable or negligible ($t < 0.02$) in all cases, so we cannot detect any water-catalyzed exchange.

A value reported in Table II as k_{EZ} or k_{ZE} is strictly the difference between that rate constant and a term involving the cross-relaxation of the two spins.[9b] Therefore the actual rate constant is greater than what is reported. However, since that difference is zero (<0.1 s^{-1}) for formamide-*d*, *N*-*tert*-butylformamide, and malonamide, and only −0.7 s^{-1} for ethyl oxamate, where cross-relaxation is expected to be more important (since there are no other protons nearby), it is unlikely that the actual rate constants exceed the values reported in Table II by more than 0.1 s^{-1}.

Figures 2 and 3 show representative saturation-transfer and T_1 experiments for base-catalyzed exchange in acrylamide; the greater transfer of saturation from solvent to H_E is apparent from the significantly greater reduction of its intensity in the top spectrum. Saturation-transfer data for base-catalyzed exchange of several amides are given in Table III, and the kinetic results calculated therefrom are given in Table IV. Since all six rate constants are determined independently, the requirement of equilibrium, $p_i k_{ij} = p_j k_{ji}$, serves as a check on the data, and this *is* satisfied, within experimental error, by the data in Table II and IV. Table V lists some additional line-broadening and line shape data on base-catalyzed exchange. (Data were taken at several pH values, but only one is given in the table.) Figure 4 shows a comparison between measured and calculated spectra for *N*-methylformamide. Table VI summarizes the *relative* rates of

Table V. Line-Broadening or Line-Shape Measurements of Rates of Base-Catalyzed Proton Exchange

amide	$\Delta\nu_{E,Z}^0$, Hz	pH	k_E, s^{-1}	k_Z, s^{-1}
acetamide	2.86, 2.99[a]	7.7	26.4	3.64
acrylamide	3.68[a]	7.4	40	10.2
	10.5[b]	8.2	30	9.6
methacrylamide	6.42[a]	7.5	33	19
pivalamide	3.43[c]	9.2	45	28
benzamide	4.54[d]	8.2	48	17.6
	10.2[b]	8.6	27.5	7.7
salicylamide	14.5[b]	7.0	6.3	6.3
cyanoacetamide	2.09[a]	6.25	27	7
	9.3[b]	7.2	26	19
ethyl oxamate	1.98[a]	5.8	38	22
	8.7[b]	7.0	19	14.7
trifluoroacetamide	0.65, 3.88[a]	4.8	18.5	8.1
chloroacetamide	11.2[b]	7.4	21	27
dichloroacetamide	10.5[b]	7.0	32.5	29.6
trichloroacetamide	10.8[b]	6.5	22	47
N-methylformamide	1.0,[e] 1.4[e]	7.3[a]	13	26
		7.15[b]	6.3	19.6
N-phenylformamide	7, 3[e]	6.15[b]	6.3	39.3

[a] Aqueous. [b] Ethylene glycol. [c] 50% aqueous methanol. [d] 60% aqueous methanol. [e] Component of CH_3 (or CH) doublet.

Table VI. Relative Rates of Base-Catalyzed Exchange: k_E/k_Z[a]

	water	ethylene glycol
formamide-*d*		1.56[b]
acetamide	7.5 ± 0.9	2.6[b]
acrylamide	4.0 ± 0.4	2.46,[b] 2.25
methacrylamide	1.75 ± 0.02	1.22[b]
pivalamide	1.5 ± 0.3[c]	
benzamide-^{15}N	3.57	1.79[b]
salicylamide		1.0,[b] 1.0
cyanoacetamide	3.6 ± 0.6	1.40
malonamide		2.2[b]
ethyl oxamate	1.75 ± 0.02	1.29
trifluoroacetamide	2.1 ± 0.25	
chloroacetamide	1.32 ± 0.05	0.78, 0.84[b]
dichloroacetamide	1.14 ± 0.02	1.10
trichloroacetamide	0.53 ± 0.03[c]	0.46
iodoacetamide		1.80[b]
N-methylformamide	0.50	0.58
N-*tert*-butylformamide		0.125
N-phenylformamide		0.16

[a] By line broadening or line-shape analysis. [b] k_{ES}/k_{ZS}, by saturation transfer. [c] Aqueous methanol.

Table VII. Relative Rates of Base-Catalyzed Exchange in Less Polar Solvents: k_{ES}/k_{ZS}[a]

amide	cyclohexanol	50% dioxan in cyclohexanol
acetamide	2.58[b]	1.37[c]
chloroacetamide		0.37
dichloroacetamide		0.16 ± 0.03
trichloroacetamide	1.08[d]	0.28 ± 0.02
N-methylformamide	0.65[e]	
N-phenylformamide	0.20	0.25[f]

[a] By saturation transfer. [b] 1.62 with Na^+ counterion. [c] In 40% dioxan; 2.53 in 20%, 1.19 in 60%, 1.12 in 75%. [d] 0.45 with Na^+ counterion. [e] In *tert*-butyl alcohol. [f] 0.29 in 75% dioxan.

base-catalyzed exchange of H_E and H_Z. Some of these ratios differ slightly from those previously reported,[7a] which were less accurate. Table VII lists additional rate ratios in some less polar solvents.

Discussion

Uncatalyzed Rotation about the C–N Bond. The reliability of the rate constants in Table II may be judged by several criteria. Rate constants for acetamide and benzamide are the same for both ^{14}N and ^{15}N forms, even though the ^{14}NH peaks are subject to quadrupole broadening. The rate constant for benzamide is the same, within experimental error, in ethylene glycol as in 60% aqueous methanol, even though the decreased viscosity of the latter solvent decreases the spin–lattice relaxation rate, which enters into eq 5. The rate constant for benzamide—3.4 s^{-1}—differs slightly from the value 10 s^{-1} recently reported.[28] The rate constants for acetamide and benzamide—0.4 and 3.4 s^{-1}, respectively—are significantly lower than values 3 and 19 s^{-1}, respectively, extrapolated from line shape measurements at higher temperatures in aprotic solvents.[29] However, the effects of protic solvents may be expected[25c] to retard the reaction, so we conclude that the agreement is good.

Substituent effects on the rotational rates in Table II are qualitatively as expected.[30] The rate is increased by electron-donating substituents, which stabilize the transition state's carbonyl group. The rapid rotation in salicylamide is especially noteworthy; an even more marked acceleration has been observed[28] in NADH, which is a vinylogous urea. The rate is also increased by bulky substituents, which sterically destabilize the planar form; thus rotation in trichloroacetamide is faster than in dichloroacetamide, and rotation in methacrylamide is faster than in acrylamide. These effects have also been seen in *N,N*-dimethylamides,[31] where steric effects are larger.[32] However, correlation with inductive[33] and steric[34] parameters is poor.

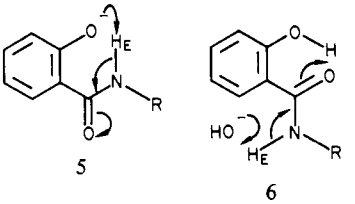
Base-Catalyzed Exchange in Primary Amides. The data in Table IV show that there is no *base-catalyzed* intramolecular exchange. For some amides there is intramolecular exchange, owing to uncatalyzed rotation, but the rate constants from Table II have been subtracted from k_{EZ} and k_{ZE} in Table IV, and these values are zero, within experimental error. Of course, they are expected to be zero, since the intermediate $RC(=NH)O^-$ is a strong base,[5g] stronger than OH^- or $HOCH_2CH_2O^-$, so that reprotonation by solvent is diffusion controlled.[2] The lifetime of the intermediate is thus $<10^{-11}$ s,[35] far too short to permit rotation or inversion.[36]

As observed previously with secondary amides,[37] the rate of this base-catalyzed reaction is increased by electron-withdrawing substituents. The second-order rate constants, calculated from the data of Tables IV and V, do increase with decreasing pK_a of the corresponding carboxylic acid,[33] but the correlation is nonlinear, even with inclusion of steric parameters.[34] Such curvature has been attributed[5g] to the approach to diffusion control, which renders rate constants insensitive to substitution. This insensitivity is more marked in ethylene glycol, where the greater viscosity may make diffusion control more significant.

Of course, the absolute rate constants are not the principal objective of this study, and we now turn to the relative rates of exchange of diastereotopic protons. To simplify the following discussion, we shall assume that since the imidate is a stronger base than OH^- or $HOCH_2CH_2O^-$, its reprotonation is diffusion controlled,[2] with the same rate constant for both stereoisomers. Alternatively, according to the Swain–Grunwald mechanism,[38] the rate-limiting step for proton exchange is the breaking of a hydrogen bond in the imidate hydrate (or solvate), so that the transition state greatly resembles the imidate. (This conclusion holds even for amides such as trifluoroacetamide, which are slightly more acidic than the solvent.) Thus we may substitute imidate for transition state in considerations of relative stability.

As expected, H_E and H_Z exchange at different rates, since they are diastereotopic. Moreover, H_E generally exchanges faster, so these saturation-transfer results confirm what was previously claimed from line-broadening measurements.[7a] The greater reactivity of H_E had been expected, on the basis of a greater stability expected for (*Z*)-$RC(=NH)O^-$ (**3**-*Z*). Imidate configurations have not been determined but may be judged by analogy with carboxylic acids and esters,[39] which are antiperiplanar, and by MO calculations that OH^- preferentially hydrogen bonds to H_E of formamide[40] and that the more stable configuration of the isoelectronic HCF=NH is *Z*.[41] The lesser stability of the *E* imidate may be attributed to repulsion of lone pairs on N and O^-.[42] We attribute the exceptions, chloroacetamide (in ethylene glycol) and trichloroacetamide, to repulsion between nitrogen and chlorine lone pairs in the *Z* imidate. However, the contrast between trichloroacetamide and trifluoroacetamide shows that there is a delicate balance between nitrogen–oxygen and nitrogen–halogen lone-pair interactions. Steric effects seem to be operative as well, but correlation of rate ratios with steric parameters[34] is poor.

The results for salicylamide answer a question posed by Menger and Saito.[5j] They observed that *N*-methylsalicylamide undergoes exceptionally rapid base-catalyzed exchange. They formulated two mechanisms—intramolecular general-base catalysis (**5**, R = CH_3) and specific-base intermolecular general-acid catalysis (**6**,



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R = CH_3)—but they could not distinguish between them. Primary amides permit a distinction, since in the first mechanism (**5**, R = H_Z) only H_E should show the acceleration, whereas in the second mechanism (**6**, R = H_Z) exchange of both H_E and H_Z should be

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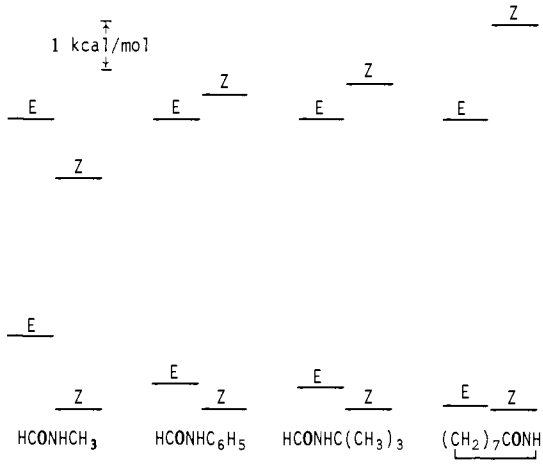

Figure 5. Relative energies of stereoisomeric amides (bottom) and imidates or transition states (top, energy gap arbitrary).

accelerated. The data of Tables IV and V show that salicylamide, just as *N*-methylsalicylamide, does show accelerated base-catalyzed proton exchange. Moreover, exchange of both H_E and H_Z is accelerated. Therefore we conclude that the ortho hydroxyl acts as an intramolecular general-acid catalyst, to acidify both NH protons and render them more rapidly removed by base (**6**).

Base-Catalyzed Exchange in Secondary Amides. Just as primary amides show no base-catalyzed intramolecular exchange, secondary amides show no base-catalyzed *E*/*Z* isomerization. Of course no such isomerization is to be expected, since the lifetime of the intermediate, $HC(=NR)O^-$, is again too short[2,35] to permit rotation or inversion.[36] Also, except for *N*-phenylformamide, where the phenyl accelerates the reaction, the secondary amides exchange at nearly the same rate as formamide, so that the alkyl group does not seem to make much difference. Nevertheless, in contrast to nearly all the primary amides, secondary amides show $k_E/k_Z < 1$. A similar result has been obtained for 2-azacyclononanone.[5o]

Any discussion of this result is complicated by the fact that each *E* amide is itself the less stable stereoisomer. Unfortunately the origin of the stability difference in the reactant amides is obscure,[43] having been ascribed to an aromatic 6-π system[44] and to dipole-induced dipole interactions.[45] The energy relationships of stereoisomeric reactants and transition states or imidates are shown in Figure 5. For *N*-methylformamide the *E* imidate is the less stable, as expected, but for *N*-phenylformamide, *N*-*tert*-butylformamide, and 2-azacyclononanone it is the more stable. Relative to the *E* imidates, the *Z* imidates have an additional destabilization not found in the amides of 0.32, 1.07, 1.22, and 1.89 kcal/mol, respectively. This is a surprising result, since the analogy to carboxylic esters[39] and the MO calculations[40,41] still suggest that an *E* imidate ought to be destabilized by lone-pair repulsions and ought to be formed more slowly. The destabilization of the *Z* imidate from azacyclononanone was interpreted[5o] by analogy to cyclononene, but this cannot be valid for the acyclic amides.

An alternative explanation invokes a reduction or elimination in the imidate of whatever[43-45] stabilizes the *Z* amide. However, such an explanation seems unreasonable, since the imidate is more delocalized and more polarizable than the amide, so aromaticity[44] or dipole-induced dipole interactions[45] would be enhanced. We therefore propose that the destabilization of the *Z* imidate is due to steric hindrance to solvation at the oxyanion. Molecular models suggest that the hindrance increases in the order methyl < phenyl < *tert*-butyl < bridging heptamethylene, which is the order of destabilization observed.

These observations clarify the comparison of lactams with acyclic amides. It had been observed[5d,e,r] that lactams undergo base-catalyzed NH proton exchange at rates comparable to those of acyclic amides and that γ-butyrolactam even exchanges 4–20 times as rapidly as *N*-methylacetamide. Since the proton of a lactam is H_Z, our results with primary amides, and the considerations of lone-pair repulsions, had led us to expect lactams to be retarded. One could question the previous observations,[5d,e] which depended on the transferability of pH meter readings in 0.4–1.0 M solutions of different amides, but we have verified[46] by NMR measurements in a common solution that γ-butyrolactam undergoes base-catalyzed proton exchange 11.5 times as fast as *N*-methylacetamide. On the basis of our results for secondary formamides we conclude that steric hindrance to solvation in acyclic secondary amides retards their exchange, and as a result lactams and secondary amides are of similar reactivity. However, these are small effects, and we are still unable to rationalize the reactivities in detail.

Solvent Effects. Small but significant solvent effects on k_E/k_Z are apparent in Tables VI and VII. We had expected that decreasing the solvent polarity would intensify the lone-pair repulsions that destabilize the *E* imidate and would thereby increase k_E/k_Z. Alternatively, we might have expected a loss of positional selectivity as the acidity of the amide approaches the acidity of the solvent and the reaction approaches diffusion control. This latter seemed to be the case for acetamide, but diffusion control should be reached earlier for the more acidic chloroacetamides, and they, as all the primary amides, show a consistent decrease in k_E/k_Z. This cannot be due to dimerization in the less polar solvents, since this would hydrogen bond H_Z and increase[2] k_E/k_Z. We therefore attribute this solvent effect to the preference, in less polar solvents, for proximity of the countercation to oxygens of both the lyate and imidate, and the corresponding destabilization of the transition state for removal of H_E. In support, we note that k_E/k_Z for acetamide and trichloroacetamide in cyclohexanol is lower when the counterion is Na^+, for which proximity is more important than for Et_4N^+.

The secondary amides show the opposite solvent effect. Above we have attributed the greater reactivity of H_Z to steric hindrance to solvation in the transition state for removal of H_E. If solvation becomes less effective in less polar solvents, then H_E would be subject to a lesser retardation, as observed.

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Supplementary Material Available: Tables I and III of saturation-transfer data and Figure 4, showing experimental and simulated NMR spectra of *N*-methylformamide between pH 6.9 and 7.55 (3 pages). Ordering information is given on any current masthead page.

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