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Water Solvent Exchange Rates of Primary Amides. Acid-Catalyzed NMR Saturation Transfer as an Indicator of Rotation and Structure of the Protonated Form

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Abstract: The nitrogen proton resonances of several primary amides have been studied by Fourier transform NMR. The generally accepted assignment of these resonances has been confirmed by nuclear Overhauser effect. Solvent exchange rates have been measured for the amide protons of propionamide, α- and β-chloropropionamide, oxidized and reduced nicotinamide adenine dinucleotide, and benzamide by means of pulsed NMR saturation recovery and solvent saturation transfer, and roughly estimated for other compounds by NMR saturation. Base catalysis of exchange for the proton cis to the carbonyl oxygen is roughly twice that previously observed for analogous *N*-methylamides. Base catalysis for the proton trans to the carbonyl oxygen is roughly three times more rapid than for the cis proton. Acid catalysis rates are the same for cis and trans protons for all saturated amides, and are roughly ten times faster than has been reported for analogous *N*-methylamides. Rotation of the amide nitrogen has been estimated by means of saturation transfer between cis and trans resonances. There is a pH-independent rotation at a rate of roughly 1 s^{-1} at 22 °C, and no base-catalyzed rotation. There is definite acid-catalyzed rotation resulting in 20–40% saturation transfer for saturated amides at low pH. These data are interpreted in terms of N-protonation and rotational diffusion of the protonated form at a rate comparable to deprotonation, for the saturated amides. There is asymmetric saturation transfer for unsaturated compounds; the acid-catalyzed trans to cis saturation transfer is 75% for benzamide and methacrylamide, but much smaller from cis to trans. In these compounds the trans proton shows more rapid solvent exchange than the cis proton. The kinetic behavior for the unsaturated amides is generally consistent with the model of Perrin, but the strong trans–cis saturation transfer indicates the existence of some other, unknown, acid-catalyzed rotation mechanism not involving nitrogen protonation.

I. Introduction

We report NMR measurements of solvent exchange rates and rotation of the nitrogen protons of several primary amides in aqueous solution as a function of pH. We were interested in these measurements because these protons are observable and identifiable in the NMR spectrum of small peptides[2-4] and their exchange rates may help provide a picture of conformational dynamics as they do in other cases.[5] We were also attracted by the readily observable amide protons[6] of nicotinamide adenine dinucleotide (NAD), and felt that observation of their exchange rates might be of interest. An unexpected result was observation of saturation transfer between the two amide nitrogen protons, extending without diminution into the low pH range where acid-catalyzed exchange occurs, indicating a rotation event associated with exchange and, most likely, indicative of dynamics of the protonated amide. Finally, we were able to confirm the generally accepted assignment[7] of the amide resonances by nuclear Overhauser effect (NOE) of NAD.

There have been previous surveys of exchange rates of secondary amides,[8,9] but only limited and fragmentary studies of primary amides in small molecules[10] and peptides. There is not universal agreement on the mechanism of acid-catalyzed exchange, e.g., whether or not O-protonation plays a role in the pathway of exchange.[10,11]

This work was made possible in part by unique instrumentation built by us for pulsed ^1H NMR in protonated water.[12] It has also benefited by our specialization in double-resonance techniques. To some extent it was stimulated by the incorrect and oft-repeated statement by others that our methods are not applicable to NMR lines close to the water proton resonance. Small amides, with their broad lines less than 2 ppm from water, are a considerable challenge to any spectrometer, especially at the relatively low concentrations which are desirable to avoid concentration effects.

Our data and our general theoretical approach to this problem are similar to those of Perrin and Johnston,[10] but the model which we use to interpret our data differs from theirs somewhat, especially for the saturated amides.

Acid-catalyzed amide-nitrogen rotation has been observed previously, for several N-substituted amides.[11] The rotation rates observed for dimethylamides have been compared with proton exchange rates for monomethylamides, and used[11a] to argue that an O-protonation pathway contributes to exchange. The observations reported below permit this comparison to be made on the same compound, and the results are similar. However, we interpret our data in terms of N-protonation alone, primarily because the two amide nitrogen protons show equal solvent exchange rates as well as acid-catalyzed rotation for all the saturated amides that we studied. Unfortunately,

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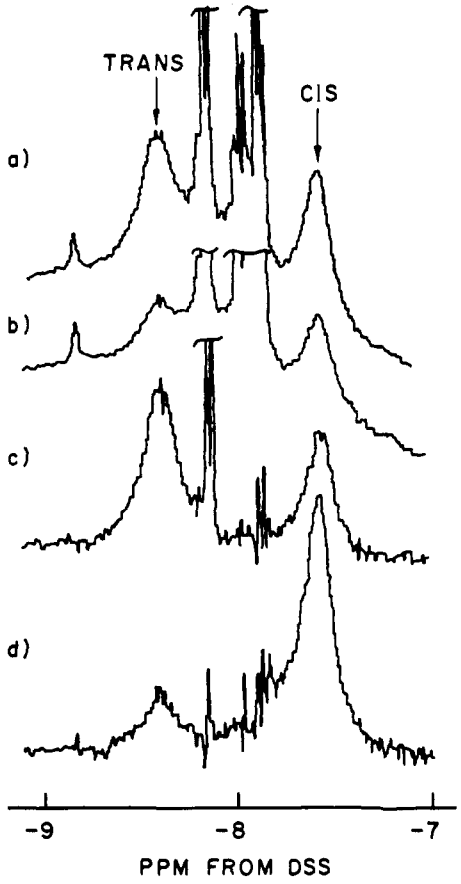


Figure 1. (a) Control spectrum of benzamide, 50 mM at pH 1.8, with preirradiation at a point (−6.7 ppm) where saturation is negligible. (b) Preirradiation applied at the trans peak to produce partial saturation of it. (c) Difference between (a) and (b), multiplied by 2. (d) A similar difference spectrum showing cis to trans saturation transfer. The reported saturation transfer is the ratio of the unirradiated to the irradiated peak amplitude in such a difference spectrum. For most cases it is more nearly equal (cis-to-trans compared to trans-to-cis) than observed in this case.

these observations do not prove that N-protonation is the dominant mechanism, although they do strongly suggest it.

II. Materials and Methods

Chemicals were purchased from Sigma and Aldrich and used without further purification. Amides were dissolved to a concentration of 20–100 mM (usually 50 mM) in 95% H_2O–5% D_2O. Buffers used were 20 mM phosphate pH 6.0–7.5, 20 mM acetate pH 4.2–6.0, 20 mM glycine pH 2.0–3.0, 20 mM borate pH 7.5–8.5, and unbuffered from pH 0 to 2.0. The solution pH was adjusted with dilute KOH or HCl and measured with a Radiometer GK2321 electrode before each run, and in many cases checked afterward. Temperature was 22 ± 2 °C.

NMR data were obtained at 270 MHz using techniques previously[13,14] described: a preirradiation pulse of about 0.5-s length was applied at a frequency f_2 to selectively saturate a peak in the spectrum. It was usually followed by a 2-ms homogeneity spoil pulse and/or was turned off slowly in 5–10 ms, to reduce preirradiation-induced H_2O signals.[15] After a recovery time of at least 2 ms, to allow the homogeneity to recover, and in some cases a variable delay time τ, a 2I4 observation pulse[15] was applied. To measure solvent exchange rates, the first-order recovery rate of the signal, as τ was increased, was measured to obtain an apparent relaxation rate, and this rate was multiplied by the degree to which saturation of the amide was observed when H_2O was presaturated. Details of the methods, and justification for neglecting solvent–amide NOE in a small molecule, are given elsewhere.[13,14] At extremes of pH the solvent exchange rate was also estimated from the extra broadening of the resonances. Typical relaxation runs consisted of about ten spectra obtained at different delay times, each taking a few minutes.

Intramolecular NOE and saturation transfer were observed with the same sequence but with preirradiation long compared to T_1's of observed protons. In most cases saturation transfer was observed as a difference between two runs. If, for example, the proton trans to the carbonyl oxygen was irradiated at f_t and the cis proton resonance at f_c observed, then we took the difference spectrum (Figure 1) obtained by subtracting a run (Figure 1b), taken with $f_2 = f_t$, from a nearly unperturbed spectrum (Figure 1a) with irradiation at the point symmetric about f_c, namely, $f_2 = f_c - (f_t - f_c)$ or $2f_c - f_t$. This was designed to cancel direct off-resonant saturation (or "spillover") by the preirradiation, at least when saturation transfer was small. Selectivity was also improved by saturating less than fully, so as to saturate the directly irradiated peak by only 50–80%. Saturation transfer as reported herein is the ratio of the indirectly to the directly saturated peak heights as obtained in difference spectra like those in Figures 1c or 1d. At pH extremes, where the unsaturated peak heights were sometimes unequal because of chemical exchange broadening, a correction was made for this inequality.

Chemical shifts were measured relative to water, and then corrected to 3-(trimethylethylsilyl) propanesulfonate (DSS) reference by adding 4.81 ppm.

Table I. Chemical Shift Data for Compounds Studied[a]

compd	cis	trans
acetamide	6.79	7.54
trimethylacetamide	6.72	7.24
butyramide	6.87	7.54
propionamide	6.79	7.50
β-chloropropionamide	7.02	7.72
α-chloropropionamide	7.27	7.87
isonipecotamide	6.95	7.58
NAD	7.76	8.72[c]
NADH[b]	6.55	6.55
nicotinamide[d]	7.61	8.35
acrylamide	7.02	7.65
methacrylamide	6.92	7.53
benzamide	7.57	8.43

[a] In parts per million downfield from DSS. Except as noted, shifts were pH independent over the range studied. Probable error ±0.03 ppm. [b] Chemical shifts averaged by rotation. Unpublished data at 22 °C, obtained by J. Tropp, private communication. [c] Upfield shift below pH 1, by 0.06 ppm at pH 0.5, relative to other peaks. [d] pH 0.5; resonances titrate at higher pH.

III. Spectra and Assignment

Spectra of the region downfield from the H_2O resonance for benzamide and NAD are shown in Figures 1 and 2. Resonance positions for the molecules studied are given in Table I. Figures 1 and 2 were taken at pH values where solvent exchange does not contribute to the line width. Line widths are presumably due to spin–spin coupling between ^{14}N and the protons, which is averaged by ^{14}N quadrupolar relaxation. This relaxation is faster, and its effect is averaged less completely, the smaller the molecule, giving the broadest lines for the smallest molecules.

An NOE experiment on NAD is shown in Figure 2. Saturating either amide resonance produces a roughly 50% kinetic transfer of saturation to the other amide resonance at low pH. However, irradiation of the downfield peak produces a twofold larger NOE on the nicotinamide C2 protons than does irradiating the upfield peak. This identifies the downfield peak as the proton trans to the carbonyl oxygen since the trans proton is much closer to the ring protons than is the cis proton. This identification can probably be generalized to all the other primary amides.

With the aid of a space-filling model, we estimate that the distances of the cis and trans protons from the ring center are 5 and 3.8 Å, respectively, yielding ring-current shifts of about 0.25 and 0.5 ppm, assuming shifts similar to those for benzene.[16] The downfield shift observed for benzamide is considerably less than for NAD, presumably because of strong inductive shifts in the latter. Also, the observed shift difference

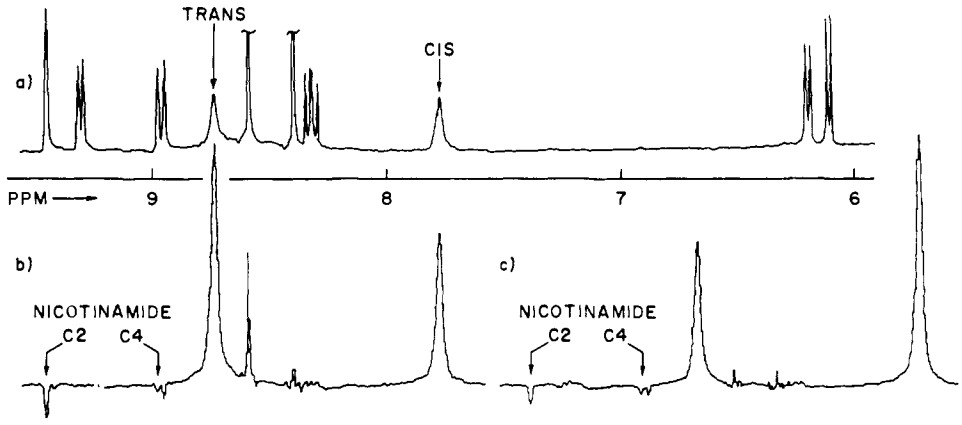


Figure 2. (a) NAD spectrum with no saturation. (b) Difference spectrum, showing the effect of irradiation at the trans resonance. (c) Difference spectrum showing the effect of irradiation of the cis resonance. In (b), the amplitude of the NOE observed for the nicotinamide C4 resonance is artifactually affected by direct saturation, as are the adenine C2 and C8 protons. However, the proportionality of the NOE found for the nicotinamide C4 proton to the direct (b) or indirect (c) saturation of the trans resonance is meaningful and demonstrates the correctness of the cis/trans assignment. The roughly equal NOEs observed for the nicotinamide C2 and C4 protons in (c) show that there is no strongly preferred orientation about the ring–carbonyl bond. All NOEs are about 5%.

between the two peaks in benzamide is only about 0.1 ppm more than in propionamide, compared to 0.25 ppm expected as estimated above from ring currents. On the other hand, the shift difference for nicotinamide is comparable to those of aliphatic compounds. Thus it seems that the source and variation in the shifts of these peaks are not understood, and it is remotely possible that they change places in different compounds. The assignment given here is the same one which has been generally accepted, based on NOE of dimethylformamide[7b] and on spin–spin splitting in formamide,[7a] and we will assume that it is correct throughout the remainder of this paper.

As has been previously noted,[16] the relative shifts of the N protons are opposite from that which is expected from magnetic deshielding by the carbonyl double bond, and may be a consequence of perturbation of the electron density on the nitrogen, or of the position of the cis proton, by the partial negative charge on the oxygen.

Approximately equal NOE is observed between the amide protons and the nicotinamide C2 and C4 protons, showing that the two planar orientations about the C3 to amide-carbon bond are roughly equally populated. Both orientations of the amide group are found in X-ray studies of nicotinamide compounds.[17]

Transition between the two amide orientations about the ring–carbonyl bond presumably occurs very rapidly on the NMR time scale in typical primary amides. A possible exception is reduced NAD (NADH), which shows a single broad amide peak at room temperature. This molecule has been studied extensively at lower temperatures in our laboratory.[18] The amide resonance can be distinguished from the amino resonance because the position of the latter is pH dependent. It splits into a doublet around 0 °C; the splitting seems too small (80 Hz) to be explained by freezing out of C–N rotation, and we conjecture that it is due to slowing of rotation about the ring–carbonyl bond. This work will be reported elsewhere.

The shifts and data reported here are in general agreement with an earlier study[6] of nicotinamide at 1.33 M concentration by Bridsall et al. They found concentration-dependent shifts and exchange rates at concentrations higher than 1 M, and suggested that interamide hydrogen bonding was occurring at these concentrations.

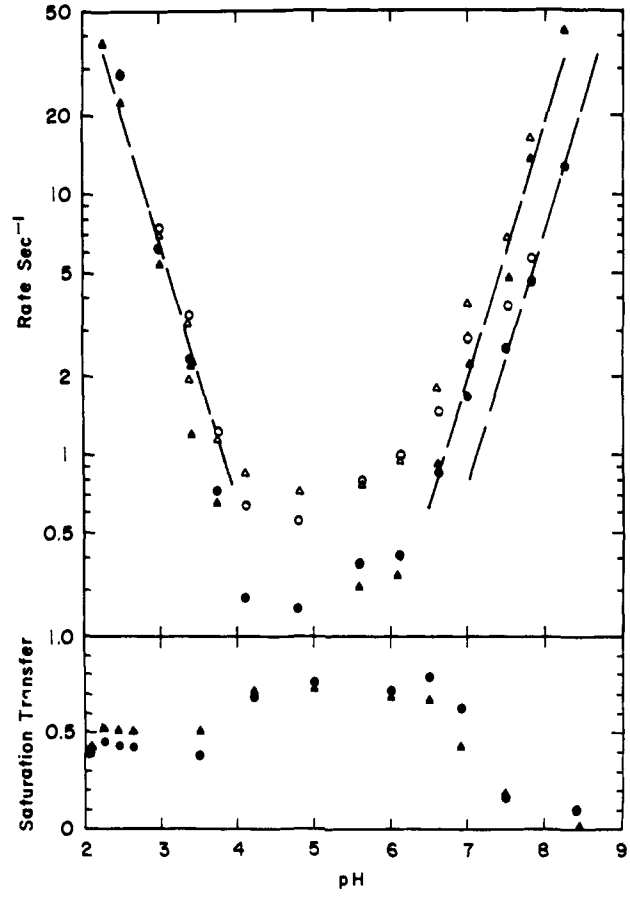


Figure 3. Exchange-rate data (top) and cis–trans saturation transfer (bottom) for propionamide. The data for the cis peak are the circles, and for the trans peak are triangles. The observed NMR saturation-recovery rates are the open circles or triangles and the solid points are inferred solvent-exchange rates, corrected to subtract contributions from magnetic relaxation. This correction consists of multiplying the observed rate by the fractional saturation transfer which is observed when the *solvent* resonance is saturated (data not shown). The saturation-transfer data at the bottom are the ratio of indirect cis saturation to direct trans saturation (circles) and the ratio of indirect trans saturation to direct cis saturation (triangles) when either amide resonance is saturated.

IV. Kinetic Measurements

Typical kinetic data are shown in Figures 3–5. The observed relaxation rate is the first-order rate constant for recovery of the intensity of a peak after it is selectively saturated. At the extremes of pH this is equal to the exchange rate but near the pH minimum it is dominated by magnetic relaxation, most likely by the nitrogen spin.[13,14] Near the pH minimum, saturating the H_2O resonance (by moving f_2 to the H_2O frequency)

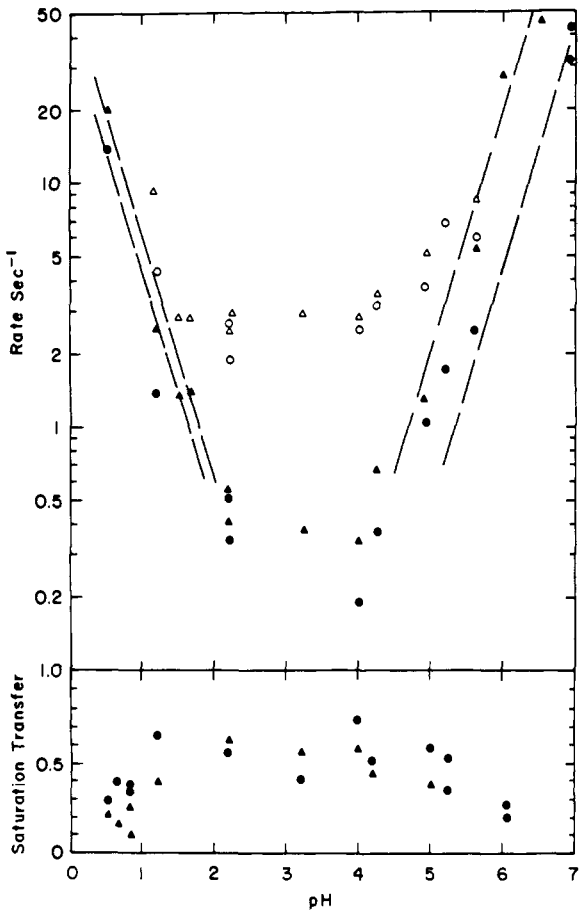


Figure 4. Exchange rate and transfer of saturation data for NAD. Symbols are the same as in Figure 3.

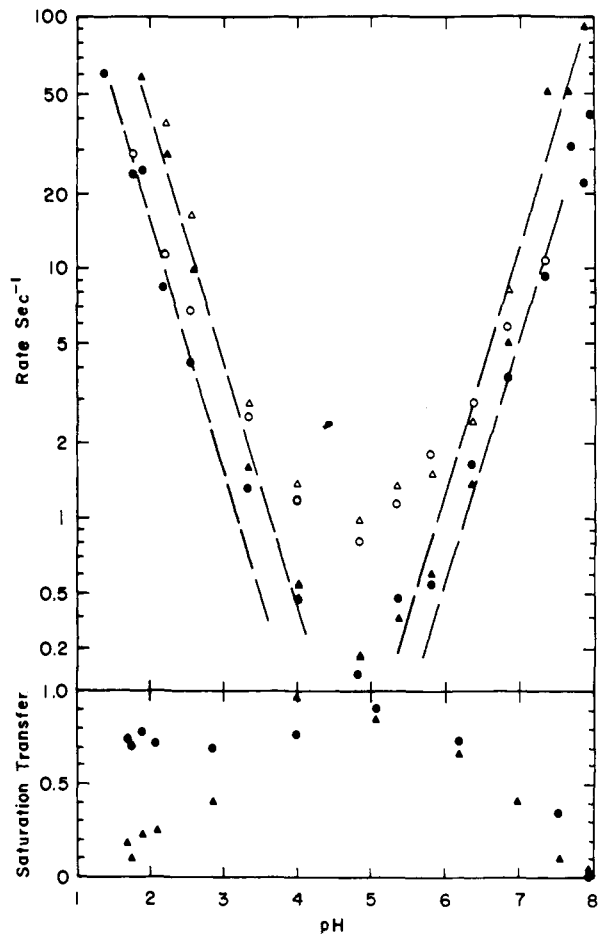


Figure 5. Exchange rate and transfer-of-saturation data for benzamide. Notation is the same as in Figure 3. In this case the plotted acid-catalyzed cis rates (solid circles below pH 3.5), corrected for magnetic relaxation as described in the caption for Figure 3, probably do not represent solvent exchange rates but are dominated by acid-catalyzed cis–trans interchange (see text).

produces only partial transfer of saturation to the amide resonances, whereas at the pH extremes this transfer is complete. The solvent-exchange rate is inferred with reasonable accuracy from these measurements by multiplying the observed relaxation rate by the fractional change in the amide resonance intensity when H_2O is completely saturated.[13] Rates converted in this way are also plotted in Figures 3–5 (solid circles and triangles).

In the case of the benzamide cis proton at low pH, the rate calculated in this way (Figure 5, solid circles) is not an accurate estimate of the direct solvent exchange rate, as will be discussed later. In this case it appears that the cis-to-solvent exchange pathway occurs predominantly by cis–trans interchange followed by solvent exchange from the trans position. Direct evidence for this pathway is the unusually large (75%) trans-to-cis saturation transfer observed below pH 3 for this compound.

For the most part the rates inferred in this way are the sum of acid- and base-catalyzed rates,[19] and the data were analyzed by hand drawing the best straight line (rate proportional to hydroxide or hydronium concentration) through the data (see Figures 3–5). The results are summarized in Table II in which we give the pH values, pH_a and pH_b, at which these lines cross the rate value of 1 s^{-1}. The acid- and base-catalyzed exchange rate constants can be obtained from these values by the formulas $\log k_H = pH_a$ and $\log k_{OH} = 14 - pH_b$.

In the case of NADH, the data were difficult to obtain. We were not able to verify that the rates were strictly proportional to $[H^+]$ or $[OH^-]$ and the values given in Table II are only rough estimates. They are in reasonable agreement with ultraviolet stopped-flow studies by Cross and Fisher.[20] Acid-catalyzed rates could be esimated very roughly for those species not studied by means of a relaxation rate measurement, from the degree of saturation in a saturation-transfer experiment (below). Acrylamide, methacrylamide, trimethylacetamide, and isonipecotamide (hexahydroisonicotinamide) show acid catalysis similar to the other aliphatic compounds, and nicotinamide was similar to NAD.

In most cases where rotation of the nitrogen protons (see below) was small or zero, we observed definite differences in the rates for the two amide protons. The base-catalyzed exchange rate is greater for the trans than for the cis proton in every case. A similar difference, in the same direction, holds for acid catalysis of the two unsaturated amides which were studied most thoroughly, benzamide and NAD, but not for the aliphatic amides acetamide, butyramide, propionamide, and α- and β-chloropropionamide. From the magnitude of direct saturation observed during saturation-transfer runs for several other compounds, it appears that this may be a general trend; there was a difference between the saturation behavior of the cis and trans proton for nicotinamide, acrylamide, and methacrylamide, but not for trimethylacetamide and isonipecotamide.

These differences in rates are consistent with those reported previously by Perrin,[10a] except that we could not confirm the small (<30%) rate difference between cis and trans protons which he reported for acid catalysis in trimethylacetamide and acetamide from our saturation measurements (we did not do relaxation measurements on these species). Our solvent and concentration conditions were considerably different from his.

Table II. Acid and Base Catalytic Constants[a]

species	pK_a (carbox)[b]	pH_a	pH_b	$k_H \times 10^{-2}$	$k_{OH} \times 10^{-7}$
propionamide cis	4.9	3.8	7.1	65	0.8
trans		3.8	6.7	65	2
β-chloropropionamide cis	4.0	3.2	6.4	16	4
trans		3.2	5.6	16	25
α-chloropropionamide cis	2.9	2.6	5.5	4	32
trans		2.6	5	4	100
benzamide cis	4.2	3.2	6.3	16[c]	5
trans		3.6	5.9	40	13
NAD cis		1.6	5.3	0.4	50
trans		1.8	4.7	0.65	200
NADH cis and trans		~6.5	~7.5	~30 000	~0.3

[a] These represent the best agreement with experiment for the rate law $k_H[H^+] + k_{OH}[OH^+]$. The quantitites pH_a and pH_b are the pH values for which the extrapolated acid and base catalysis rate is 1 s^{-1}. The first column is the pK_a of the corresponding carboxylic acid. [b] pK_a of carboxylic acids from "Handbook of Biochemistry and Molecular Biology", Vol. 1, 3rd ed., G. D. Fasman, Ed., CRC Press, Cleveland, 1976. [c] Observed rate represents predominantly acid-catalyzed rotation (see Discussion section).

Table III. Transfer-of-Saturation Data for Compounds Studied[a]

species	acid catalyzed		at pH min
	cis to trans	trans to cis	
acetamide	0.4	0.4	
butyramide	0.4	0.4	
propionamide	0.4	0.4	0.75
β-chloropropionamide	0.3	0.3	0.65
α-chloropropionamide	0.25	0.25	0.6
NAD	0.2	0.35	0.6
nicotinamide[b]	0.35	0.45	
benzamide	0.2	0.75	0.9
acrylamide	0.4	0.5	
methacrylamide[b]	0.45	0.75	
isonipecotamide[b]	0.3	0.3	
trimethylacetamide[b]	0.4	0.4	

[a] The first column is the value of indirect saturation of the trans resonance divided by the direct saturation of the cis resonance, and the second column is the corresponding ratio for the reverse experiment. Near the pH minimum, saturation transfer was roughly symmetric between the two resonances for all the compounds studied, and is given in the third column. [b] Limited data.

Propionamide appears to have measurable pH-independent solvent exchange at a rate of 0.15 s^{-1}, in addition to acid- and base-catalyzed exchange. For the other samples this rate is less than 0.2 s^{-1} (except for β-chloropropionamide, for which we did not take adequate solvent transfer-of-saturation data).

Another kinetic process which is readily apparent from the data (Figures 1-5) is rotation about the amide nitrogen–carbonyl bond, evidenced by transfer of saturation between the two amide peaks. Data are summarized in Table III. In principle there could also be a NOE between the two amide protons, but this appears to be small relative to saturation transfer due to rotation since nearly full transfer of saturation between the two peaks is observed near the pH minimum, where chemical transfer is slowest. Such an NOE would lead to an underestimate of the rotation rate since the saturation transfer is expected to be opposite in sign for NOE relative to chemical exchange in a small molecule (see, for example, Figure 2).

Again, the saturation-transfer data suggest a possible difference between saturated and unsaturated compounds for acid catalysis. The saturated compounds show trans–cis saturation transfer that is equal to cis–trans saturation transfer, while for the olefinic amides trans–cis transfer is always greater.

It is straightforward to write and solve general coupled relaxation equations for the cis and trans magnetizations which include a term to represent the selective saturation of one resonance, and to use these equations in conjunction with the data to estimate the rates of rotation and of proton exchange separately. We do not do so at this point for several reasons. First, when rotation is negligible, as seems to be true at high pH, the observed rates, corrected as described above for magnetic relaxation, give exchange rates directly. Second, when the cis and trans protons show symmetric saturation-transfer and recovery behavior (as in propionamide and its derivatives), then, although biphasic relaxation may occur in principle, it should be a small effect and the rates as described here should be a good approximation to the exchange rate. Only in the case of compounds showing strongly asymmetric behavior for the two protons (benzamide) would a complete kinetic analysis be useful; in this case the experimental strategy described by Perrin and Johnston[10b] would also be preferable. However, the measurements we have made are sufficient for us to make interesting tests of microscopic models, as will be seen later. Finally, and most important, the fractional transfer of saturation is readily extracted directly from most theoretical models without the extra step of calculating rate constants to compare with experimental rate constants. Thus it is this quantity, the fractional transfer of saturation between cis and trans protons, that we compare directly with theory.

V. Discussion

After a brief and conventional discussion of the solvent-exchange rates, we will consider possible quantitative explanations for the observed saturation transfer between the cis and trans protons. We will develop a model for the N-protonation exchange mechanism assuming a rapid rotation rate, in the protonated state, compared to the deprotonation rate, and find that this model does not quantitatively explain the observed saturation-transfer data. We then modify the N-protonation model by assuming a rotation rate comparable to the deprotonation rate, and also strongly hindered rotation in the case of the aromatic amides. In the case of the saturated amides this analysis permits a comparison of the rates of deprotonation and of rotation of the protonated nitrogen. In the case of benzamide, which we studied carefully, we show that no obvious theory can explain our low-pH saturation-transfer data; there appears to be acid-catalyzed interchange of the cis and trans protons which is not directly associated with protonation of the nitrogen. We conclude the section with a brief consideration of the O-protonation pathway and other alternatives.

Thus, our discussion is biased toward the N-protonation pathway. We do not claim to have proven that this is the dominant pathway, but we have developed it as a convenient framework for unifying most of our observations. We feel that more work needs to be done to determine when, or if, this is in fact the major pathway of proton exchange.

A. Acid and Base Catalysis of Exchange. If the rates of deprotonation and reprotonation of the conjugate acids and bases

of these amides are assumed to be diffusion limited and equal to 5×10^{11} s^{-1}, then their pK_as and the pK_as of their conjugate acids can be estimated;[19] the former is $pH_b + \log(5 \times 10^{11})$ and the latter is $pH_a - \log(5 \times 10^{11})$, where pH_a and pH_b are given in Table II. Thus the amide moiety of NAD is estimated to have a pK_a of 16.5, and that of NADH to have pK_a = 19. The difference in pK_a between NAD and NADH is expected qualitatively because of the greater electron-donating capacity of the reduced species. A similar pK_a difference can be inferred for the conjugate acid species from the value of pH_a; the protonated amide is estimated to have a pK_a of −10 for NAD and −5 for NADH. These pK_a values are, of course, estimates based on an assumed, but uncertain, rate of protonation or deprotonation by water of 5×10^{11} s^{-1}. They are also based on the assumption that each protonation or deprotonation leads to the exchange of a single proton. In the case of acid catalysis, this is unlikely to be a valid assumption since protonation at the carbonyl oxygen will not necessarily lead to any exchange. Thus these pK_as refer to the equilibrium for deprotonation of the N-protonated species. Fersht[21] has estimated protonated amide nitrogen pK_as in a different way to be −8.

There is a fair correlation between the values of pH_a and pH_b and the pK_a of the corresponding carboxylic acids. The value of pH_a varies by about ½ unit for each unit variation of pK_a of the carboxylic acid, while pH_b varies by nearly twice as much.

These measurements can be compared with those of Molday and Kallen[9] on a similar group of *N*-methylamides. When our data are added to their plot of log k_{OH} vs. pK_a of the carboxylic acid, the cis proton rates are less than a factor of 2 greater than corresponding *N*-methylamide rates. The values we find for k_{II} are at least tenfold higher than those found by Molday and Kallen, for similar values of the carboxylic pK_a.

B. Lack of Base-Catalyzed Rotation. We find no positive evidence for base-catalyzed interchange of cis and trans protons; we believe that there is none occurring at a rate competitive with exchange. Generally, cis to trans saturation transfer is unobservable and thus less than 10% at the highest pH values where measurements were made. Trans–cis transfer was as large as 20% at these pH values, and could be explained fairly well as the residual effect of pH-independent rotation.

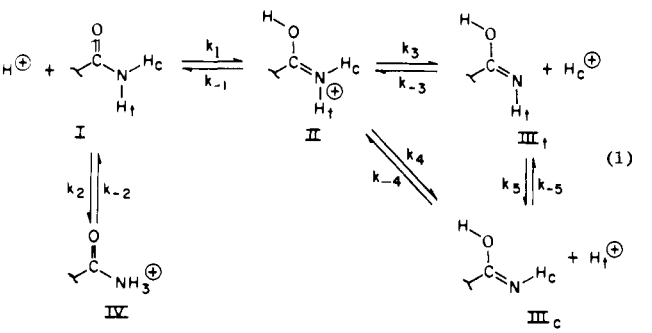
Presumably hydroxide removes a proton leaving a lone proton on the nitrogen. This reaction would reverse itself by diffusion-controlled extraction of a proton from water in roughly 10^{-11} s and the lone proton would have to migrate between trans and cis positions in this time in order to produce base-catalyzed saturation transfer. The lack of such transfer shows that such migration occurs at a rate of less than about 10^{10} s^{-1}. The greater exchange rate consistently seen for the trans proton could mean that the species with a lone proton at the cis position is the most stable deprotonated species. This rate difference has been noted previously.[10a]

C. Uncatalyzed Amide Rotation. In those compounds for which it was studied (Table III), saturation transfer is generally observed to be a maximum at the pH minimum for the exchange rate, consistent with a pH-independent rotation rate of about 1–10 s^{-1}. The pH-independent isomerization of proline peptides has been studied previously[11c] and is much slower than this.

D. Acid-Catalyzed Rotation. Rapid Rotation of the Protonated Nitrogen. As the pH is lowered, saturation transfer generally decreases, but in most cases it levels off at a value of at least 20% transfer over a pH range of at least one unit, while the exchange rates are strongly increasing. This means that the rate of cis–trans interchange remains comparable to the exchange rate in the acid-catalyzed regime.

Such acid-catalyzed rotation has been observed previously[11a,b] in small secondary amides, where its rate is only slightly less than in primary amides, and in proline-containing peptides,[11c] where it is much slower. The present observation of partial saturation transfer that is constant in the acid-catalyzed pH range is especially useful because it provides a relative measure of processes which exchange protons, and processes which interchange protons, in the same compound. Thus, it is possible to test various proposed mechanisms for exchange in detail.

Obvious pathways for acid-catalyzed exchange are summarized in eq 1.



(1)

We first consider the N-protonation pathway assuming that the protonated nitrogen will rotate many times before deprotonation. Such a model is suggested both by the observation of acid-catalyzed saturation transfer and by the symmetric behavior of the two amide protons for the saturated compounds. The latter observation suggests that the two protons become equivalent in some sense, during catalysis, as they would in a rapidly rotating sp^3 nitrogen.

In our model, we assume that N-protonation occurs by donation of a proton perpendicular to the amide plane, resulting in formation of an sp^3 configuration. Subsequently the $-NH_3^+$ group can rotate so that one of the former cis or trans protons is in a favorable position to be extracted approximately perpendicular to the amide plane. As long as the sense of rotation after protonation is not hindered or selected (as it may be for the aromatic compounds; see below), such a model leads a priori to equal exchange rates for cis and trans protons.

We consider the origin of a cis proton which resides on a neutral amide that has just returned from the protonated state. There is an equal probability that it originally was the proton that added to the nitrogen to initiate the exchange event, or that it was a trans proton on the amide before exchange, or a cis proton. It is most convenient in what follows to write equations for the magnetizations per mol of cis, trans, and solvent protons, m_c, m_t, and m_s, rather than equations for total magnetization. The rate of production of the protonated compound IV, per mol of unprotonated amide I, is $k_2[H_3O^+]$, and the rate of return to compound I from compound IV with, say, a cis proton replaced by either a trans or a solvent proton is one-third this rate. Thus the contribution to the rate of change of cis magnetization per mol, dm_c/dt, from influx of solvent and of trans magnetization is $+\frac{1}{3}k_2[H_3O^+]$ times m_s and m_t, respectively. Protonation and deprotonation that do not change the identity of a cis proton as compared to the initial molecule also occur at the same rate but can be ignored since there is no change. A complete kinetic equation must also consider loss of cis magnetization by the same routes. At equilibrium, $m_c = m_t = m_s$ and these efflux rates must be equal to the influx rates. They are therefore equal to two-thirds of $k_2[H_3O^+]m_c$. Combining these three contributions we conclude that

$$dm_c/dt = k_2[H_3O^+][\tfrac{1}{3}(m_s - m_c) + \tfrac{1}{3}(m_t - m_c)] \quad (2a)$$

A similar equation applies to the trans magnetization:

$$dm_t/dt = k_2[H_3O^+][\tfrac{1}{3}(m_s - m_t) + \tfrac{1}{3}(m_c - m_t)] \quad (2b)$$

Here we have ignored magnetic relaxation since it occurs at a negligible rate compared with the chemical exchange in the

strongly acid-catalyzed regime. Relaxation ultimately occurs through the solvent pool whose magnetization is m_s. The roughly 1000-fold larger size of the solvent pool means that m_s remains within 1% of its equilibrium value, which we denote by m_0 and which is the same for the three classes of protons, cis, trans, and solvent. Thus we can approximate $m_s = m_0$ in eq 2 unless, of course, solvent has been intentionally directly saturated as it was to correct the observed saturation-recovery rate for the contribution of magnetic relaxation, described at the beginning of section IV above.

We now evaluate the expected transfer of saturation when, say, the trans resonance is partially saturated by direct irradiation at the trans resonance frequency. In principle, it is necessary to augment eq 2, which governs the longitudinal magnetizations of the three classes of protons, with equations for their transverse magnetizations and to include the effect of the saturating rf field in these coupled Bloch equations. In practice, within the accuracy needed for subsequent discussion, we can ignore the direct effect of the saturating radio-frequency field on the cis and solvent protons when the trans proton is saturated, and only consider the indirect effect of the last term in eq 2a on m_c. Support for this approximation comes experimentally from observation of a near lack of spillover of saturation under conditions where kinetic transfer is small (high pH), and from direct observation of the solvent resonance after saturation. Theoretically, direct spillover of saturation is expected to be small because the cis resonance line, though broad, has a negligible intensity roughly 200 Hz from its center, at the trans frequency (with the expections of acrylamide and acetamide, for which broadening due to the nitrogen spin–spin coupling was not as effectively narrowed as for the larger molecules). Methodologically, direct spillover was canceled to first order by using a control frequency symmetrically placed with respect to the unirradiated resonance (see section II above).

Thus to evaluate trans-to-cis saturation transfer we ne only consider eq 2a and assume that m_t differs from its equilibrium value m_0 by a finite amount, that the solvent magnetization remains equal to m_0 because of the large solvent pool size, and that steady state has been achieved so that $dm_c/dt = 0$. Substituting these values in eq 2a, we obtain for the transfer of saturation T_{tc}

$$T_{tc} = (m_0 - m_c)/(m_0 - m_t) = 1/2 \qquad (3)$$

Likewise the reverse transfer of saturation T_{ct} is predicted to be one-half.

This prediction of symmetric 50% transfer of saturation between cis and trans protons is most nearly successful for the unsubstituted saturated amides, but saturation transfer in these compounds is consistently slightly smaller (~40%) than this prediction (Table III).

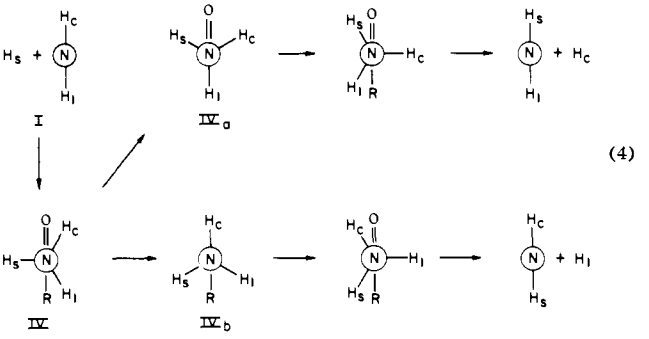
We have presented eq 2 and their solutions in considerable detail because we will make similar assumptions, except as explicitly stated, in solving equations derived from other models below. One correction which we will ignore is to take account of the likelihood that a proton which has just left a previously protonated amide has a reasonable chance to reprotonate the same molecule before diffusing far away from it. These multiple events probably tend to reduce the proton exchange rate relative to the cis to trans interchange rate (for a discussion, see, for example, ref 22 and references cited therein). We do not attempt this correction because we cannot evaluate it precisely and because we think it likely to be small. The number of multiple protonations by the same proton is likely to be a small (~10%) fraction of single ones. Also, such multiple events will probably not explain most of the discrepancies between models we develop and the experimental results, although we have not evaluated their possible effect.

We conclude by briefly considering the transient solution of eq 2 after a saturating pulse is applied to one resonance. It is easy to show that there are two normal exponential modes of decay, one with $m_c = m_t$ and rate $1/3k_2[H_3O^+]$ and the other one with a rate three times this. The second mode represents decay of the magnetization difference $m_c - m_t$. In a saturation-recovery experiment under our conditions the amplitude of this mode will be small, and we do not believe that the existence of this decay mode affects our results by more than 10%. Thus the acid-catalyzed rates presented in Figures 3 and 4 are $1/3k_2[H_3O^+]$ and the acid catalytic rate constant for propionamide in Table II (6.5×10^3) is $1/3k_2$. The factor of $1/3$ which appears here may change the estimated pK_a of the protonated amide (section VA) by 1/2 pH unit but this change is insignificant compared to other likely errors in that estimate; for example, one could argue that k_{-2} (eq 1) is 1.5×10^{12} rather than 5×10^{11} s^{-1} because any one of the three amide protons can be removed at a diffusion-controlled rate.

E. Saturated Aliphatic Amides. Intermediate Rotation Rate of the Protonated Nitrogen. Although acetamide, propionamide, and butyramide are in fair agreement with the N-protonation model, they show slightly lower transfer of saturation than it predicts, about 0.4 instead of 1/2. The situation is worse for isonipecotamide and the chloropropionamides, especially α-chloropropionamide, which shows exchange rates for the two protons which are very nearly equal at low pH, but low transfer of saturation, about 0.2 (Table II). This result was carefully rechecked over the low pH range.

We explain the discrepancy between predicted and experimental saturation transfer by making the obvious and plausible assumption[10] that the deprotonation rate is comparable to, but not much faster than, the rate of rotation of the protonated nitrogen. That is, we retain the essential features of the N-protonation model, namely, that a well-defined and long-lived sp^3 configuration is formed at the nitrogen, so that the former cis and trans protons become kinetically similar, and rotation of either one can take place to a position where extraction by solvent is favorable. We simply drop the assumption that rotation is rapid compared to proton extraction.

Such a model is developed in Appendix A, assuming diffusive rotation for the protonated nitrogen. We assume that the solvent proton H_s attacks perpendicular to the amide plane and that, by detailed balance, deprotonation will be favorable only for the proton which is roughly perpendicular to the N–C–O plane (eq 4).



(4)

In this model it will be seen that rotation by at least one-third revolution is required for exchange to occur; furthermore, rotation by more than one-third revolution is required to get any cis–trans proton interchange since one-third rotation does not produce interchange (eq 4). For the sake of mathematical simplicity (Appendix A), it is assumed that the system exists stably in one of the six possible conformers with one of the three nitrogen protons perpendicular to the N–C–O plane. Transitions between these conformers are assumed to occur at a rate k_d, and deprotonation for the perpendicular proton only is assumed at a rate k_{-2}. When k_d is not large compared to k_{-2}, then the probability of either solvent replacement of a cis

proton or of its replacement by a trans proton is smaller than one-third for a given protonation event, as we have just seen.

The amount of this decrease for each type of replacement is complicated (see eq A5), but the predicted expression for saturation transfer is simple. It is

$$T_{tc} = T_{ct} = 0.5k_d/(k_d + k_{-2}) \quad (5)$$

It is also shown in Appendix A that k_d is identical with the rotational diffusion constant of the protonated nitrogen within this model. Thus the magnitude of the transfer of saturation is a direct measure of the relative rates of deprotonation and diffusion, if this model is correct and complete.

Before discussing experimental results we focus on the assumptions made and ask which are essential. We have made two assumptions, namely, that protonation and deprotonation occur only perpendicular to the amide plane and that the rotational potential for the protonated nitrogen has sixfold symmetry. The result will most likely be similar if the amide can rotate freely, as long as directional selectivity for proton transfer is retained. If the rotation of the amide nitrogen is not viscously damped within 1 rad of rotation, the mathematics would have to be modified but the result would be qualitatively the same. The rotational diffusion constant would then most likely be replaced by the root mean thermal rotation rate as predicted by setting the rotational energy equal to $\frac{1}{2}kT$. This limit is about 10^{13} s^{-1}.

This model will not be valid if the transition states, IVa or IVb (eq 4), are appreciably different in energy. If they are different and if $k_d \gtrsim k_{-2}$, then the two kinetic branches of Figure 4 will not be equally favorable and the cis and trans protons will exchange at different rates. This behavior might be expected for a moderately bulky R group.

It is also possible that one of the "transition" states, IVa or IVb, is appreciably more stable than the states of sixfold symmetry. This might be expected if R is a bulky group which prefers to be coplanar with the amide, as in benzamide. In that case IVb sould be the most stable conformer and IVa the least stable. Such a model is considered in the next section, and a similar one was proposed previously by Perrin.[10]

Turning to the experimental data (Table III), all the saturated amides show behavior consistent with the model of the present section, with k_d/k_{-2} ranging from 4 (unbranched hydrocarbon side chains) to 0.7 (α-chloropropionamide). It is of interest to ask whether the decrease in this ratio occurs predominantly because of a variation in k_d or in k_{-2}. If k_d decreased upon substitution because of increased steric interaction between an eclipsing proton in state IVa and the R group, then the trans proton should exchange more slowly than the cis proton, according to the argument given in the paragraph before last, in those compounds showing low saturation transfer.

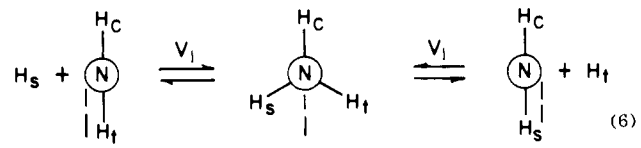
This point was checked carefully for α-chloropropionamide, by line width, by saturation recovery, by studying saturation as a function of power, and by looking for asymmetry of saturation transfer. No asymmetry was found so we conclude that steric hindrance is not a factor in determining the variation of k_d/k_{-2}.

Thus we are led to the tentative conclusion that the deprotonation rate k_{-2} is different for the different compounds studied, and is affected inductively by substitutions on the side chain, by a factor of at least 4. This is surprising because this deprotonation is strongly driven energetically. The estimated pK_a of the protonated nitrogen is several units lower than that of hydronium, so that the reaction is expected to be diffusion limited. However, this is no more surprising than is the conclusion that the rate of rotational diffusion k_d must be several times greater than the deprotonation rate k_{-2}, for such a strongly driven reaction.

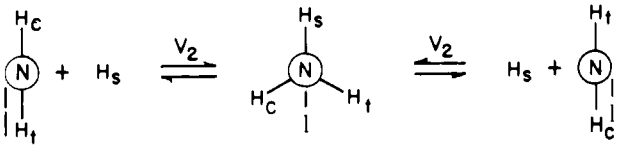
There does not seem to be any good way to estimate either k_d or k_{-2} directly. Methyl rotational diffusion might remotely resemble that of a protonated amide nitrogen. For methyl groups of acetyl and propionyl esters, k_d is about 10^{10} s^{-1} as determined[23] by NMR. If this number were correct for the protonated nitrogen, then our analysis predicts that the deprotonation rate k_{-2} is as slow as 10^{10} s^{-1}, much less than the accepted diffusion-limited rate. If, on the other hand, k_d approaches its thermal maximum value of 10^{13} s^{-1}, then k_{-2} is predicted to be comparable to the accepted diffusion-limited rate.

F. Unsaturated Amides. Hindered Rotation of the Protonated Nitrogen. All the unsaturated amides show unequal cis and trans acid-catalyzed exchange rates. This inequality is particularly strong in the case of benzamide, which we studied painstakingly and repeatedly. In that case there is also a striking asymmetry in transfer of saturation ($T_{tc} \sim 0.75$, $T_{ct} \sim 0.2$) in the acid-catalyzed regime. We now consider whether these results can be explained by an N-protonation model in which we assume strongly hindered rotation for the protonated nitrogen. A space-filling model for benzamide shows some hindrance between the trans proton and the ring protons ortho to the amide, and there is X-ray evidence for a slight rotation of the ring–carbonyl bond away from planarity to accommodate this hindrance.[24] Thus there may exist two stereoisomers of benzamide, differing in which way the amide plane is tilted away from the benzene plane about the ring–carbonyl bond. Presumably, interconversion between these two isomers is rapid at room temperature on the NMR time scale. On the other hand, this interconversion may well be slow on the presumed time scale of deprotonation ($\sim 10^{-11}$ s). We will now assume this to be the case, and also assume that rotation of the protonated nitrogen is likewise hindered.

In Appendix B we develop a model for exchange assuming such hindered rotation. There are two parameters in such a theory, namely, the pseudo-first-order rates V_1 and V_2 for proton attack, and departure, via the two nonequivalent faces of the amide (eq 6 and 7). The vertical dotted line in eq 6 and



(6)



(7)

7 symbolizes the plane of the ring, and the N-carbonyl bond is assumed perpendicular to the paper with the carbonyl oxygen pointing up.

The pathway of eq 6 is essentially that proposed by Perrin[10] except that he did not explicitly invoke the likelihood of steric hindrance for the unprotonated amide. He argued that the pathway of eq 7 was highly unfavorable because the nitrogen protons would have to rotate far out of the amide plane before the solvent proton could attack the group. We include this pathway partly for completeness and to try to explain our data, but also because it seems possible that the solvent proton could attack the nitrogen before much rotation occurs, first forming the sp^3 configuration, and that the rotation could occur thereafter, or concertedly during protonation.

The analysis of Appendix B shows that $T_{ct} = V_2/(V_1 + V_2)$ and $T_{tc} = (V_1 + V_2)/(V_2 + 4V_1)$. This result is in definite disagreement with the results for benzamide, at least. It is impossible to find a ratio of the rates V_1 and V_2 for which T_{tc} is large (~0.7) and T_{ct} small (~0.2) as is observed for the acid-catalyzed regime for benzamide. It does appear that the data for NAD could be fit to such a model with a ratio V_1/V_2 of roughly 3.

The reason that such a model does not explain the data for benzamide is easy to see. From the strong trans-cis saturation transfer in this compound and the weak reverse effect, and from the more rapid exchange of the trans proton, it appears that direct cis exchange is slow and that the dominant pathway for cis exchange is transfer to a trans site, followed by direct exchange with solvent. Mechanism 6 provides a natural way to rationalize direct trans exchange, and at first sight eq 7 provides a rationale for cis-trans interchange without solvent exchange. Unfortunately eq 6 and 7 use a chemically equivalent intermediate, so that pathways involving protonation by eq 6 and deprotonation by eq 7, and the reverse, must also be considered. Such pathways can lead to a solvent cis exchange without interchange, thereby decreasing trans-cis saturation transfer by competing with the pure interchange produced by eq 7 alone.

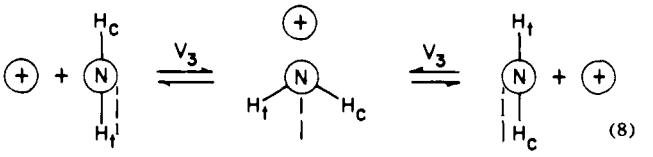
The same definite discrepancy between this model and experiment is observed for methacrylamide. Nicotinamide and acrylamide also show definite asymmetric saturation transfer between the two resonances but they do not appear to exceed 50% acid-catalyzed saturation transfer as do benzamide and methacrylamide.

We next consider the possibility that rotation of the protonated sp^3 nitrogen occurs before deprotonation and that such rotation could explain the benzamide results. It is conceivable that steric hindrance is relatively small for the protonated nitrogen. If so, and if rotation is rapid before deprotonation then we have exactly the model of section VD, with predicted saturation transfer of 50% in both directions, contrary to experiment. It is also simple to make predictions from a model in which there is a small probability of a single rotation by one-third revolution before deprotonation, and in which protonation occurs only via the pathway of eq 6 and not eq 7. In this case cis-trans saturation transfer may be small but trans-cis saturation is 50%, independent of the ratios of the rates involved, and smaller than observed for benzamide. Finally, it is straightforward though tedious to treat (unpublished) a model in which both limited rotation and the pathway of eq 7 occur. Since such rotation seems unlikely sterically and since it adds another unknown parameter to the model, we will not consider it further.

Finally, therefore, we are led to conclude that there is some other way that acid catalysis can lead to rotation without the exchange that is predicted by straightforward nitrogen protonation as considered above.

The essence of a model incorporating such a mechanism must be that there are two distinct protonated intermediates which lead to exchange or interchange. These intermediates are also distanct from the presumed unproductive O-protonated amide (below). For one of them, in eq 6 and 7, the nitrogen is presumed to have accepted a third proton. For the other intermediate, the nitrogen is presumed to possess two protons while the third is retained on a water molecule or elsewhere and may simply electrostatically attract electrons toward the nitrogen to form an sp^3 configuration and promote acid-catalyzed rotation. We do not view this as a complete microscopic description of what happens; it is little more than a phenomenological description of the experimental data.

This acid-catalyzed rotation mechanism can be symbolized by eq 8. The plus charge inside the circle is supposed to represent, for example, a hydronium ion shared, perhaps, between



the nitrogen and the carbonyl oxygen in such a way as to form a sp^3 electronic configuration at the nitrogen without an actual proton transfer to the nitrogen. From examination of eq B9, B10, and B11 we conclude that, to fit the observations in benzamide, that is, $T_{ct} \approx 0.2$ and $T_{tc} \approx 0.75$, we must have $V_3 \approx 0.3V_1$ and $V_2 \approx 0.03V_1$. In other words, attack of the proton from the same side as the ring, leading to expulsion of only the trans proton, occurs most often. Hydronium-catalyzed rotation of the amide without exchange occurs about one-third as often, and attack of a proton with proton exchange from the face of the amide opposite the ring is a rare event which occurs only a few percent of the time.

In the case of methacrylamide, which also shows a large asymmetric saturation transfer, the same analysis leads us to conclude that $V_3 \sim V_1$ and $V_2 \sim 0.1V_1$, and for nicotinamide $V_2 \sim V_3 \sim 0.3V_1$.

It is also possible to explain our data without the mechanism of eq 7 provided that we assume that there is some rotation of the protonated sp^3 nitrogen in eq 6 and that the mechanism of eq 8 also applies. Analysis (unpublished) of such a model is similar to Appendix B and fits the benzamide data if it is assumed that $V_3 = V_1/12$ and that there is also a total probability of $^1/_{12}$ that the protonated nitrogen rotates in either direction by one-third revolution before deprotonation.

There is evidence from reaction studies that olefins can be transiently protonated.[25] Such protonation could conceivably rationalize the mechanism of eq 8 if it could occur at a point on the side chain physically close to the amide nitrogen. The proton might then be able to polarize the amide electrostatically, to reduce its double-bond character and promote its rotation.

G. Alternate Models and Inconsistencies. Up to this point we have tried to fit our data with variations on an N-protonation model; we now consider alternatives. The most obvious is O-protonation. Much of the earlier literature on this question has been reviewed by Perrin.[10] At first sight it may appear that the observation of cis-trans saturation transfer is a priori indicative of N-protonation, and that lack of saturation transfer indicates O-protonation. Unfortunately, neither criterion is conclusive. We have already seen that, if the rate of rotation of the protonated nitrogen is small compared to the rate of deprotonation, so that rotation by more than one-sixth revolution is highly improbable, and if assumptions are made concerning directional selectivity of protonation, then (eq 4) it is possible that exchange will occur with negligible cis-trans interchange. Furthermore, the exchange rates will be different for the two protons if the transition states for the two eclipsed transition states (IVa and IVb) are of unequal energy. Lack of saturation transfer and unequal exchange rate have been suggested as criteria for the O-protonation pathway,[10c] but these criteria are not rigorous.

On the other hand, it is possible to construct an O-protonation model in which there is cis-trans saturation transfer. If, for example, the isomerization rates k_5 and k_{-5} in eq 1 are comparable to the protonation rates, k_{-3} and k_{-4}, then there will be observable saturation transfer. It is simplest to consider the case $k_5, k_{-5} \gg k_{-3}, k_{-4}$. In that case the protons labeled H_c and H_t in compounds III_c and III_t will become scrambled, and upon return via step 3 the proton labeled H_t will have a probability $k_3/(k_4 + k_3)$ of being a former trans proton, leading to no change in the trans proton of the product I, and a probability $k_4/(k_3 + k_4)$ of being a former cis proton. Step 1 is presumably a preequilibrium step with a pK_a similar to that

of water (−1 to −2) and II is present in concentration which is a fraction $F_2 = [H_3O^+]k_1/k_{-1} \ll 1$ of the unprotonated amide concentration. The pseudo-first-order rate constant for replacement of the trans proton by a cis proton is thus $F_2k_3[k_4/(k_3 + k_4)]$, and that for its replacement by a solvent proton is F_2k_4; to get these expressions we invoke the fact that at equilibrium, by microscopic reversibility, the number of molecules per second undergoing steps 3 or 4 equals the number for the reverse step. Proceeding by analogy with the derivation of eq 2, we can get corresponding equations for this model, and derive expressions for saturation transfer:

$$T_{ct} = k_3/(2k_3 + k_4) \quad (9)$$

$$T_{tc} = k_4/(k_3 + 2k_4) \quad (10)$$

For the saturated amides we find symmetric experimental behavior requiring that $k_3 = k_4$, and saturation transfer is predicted to be 33%, less than we find for unsubstituted compounds. The maximum saturation transfer that this model predicts is 50%, which is less than we find for benzamide. Thus, O-protonation alone cannot explain our data unless we invoke several assumptions: an extra isomerization mechanism, as we did at the end of the last section, for some compounds (e.g., benzamide and propionamide) as well as an unlikely equality of rates ($k_3 = k_4$) for all the saturated amides that we studied, and a limited isomerization rate ($k_5 = k_{-5} \approx k_{-4}, k_{-3}$) for the chloropropionamides.

The symmetric cis–trans exchange behavior that we observe could also be explained by assuming that $k_3, k_4 \gg k_{-1}$ so that O-protonation is rate limiting. In order to explain cis–trans saturation transfer it is again necessary to invoke a mechanism like that at the end of the last section for all these compounds. However, it seems highly unlikely that $k_{-1} \ll k_3, k_4$ since k_{-1} is a nearly diffusion-limited deprotonation by water.

Bovey and Tiers[26] argued in favor of O-protonation on the basis of their study of the amide resonances in polyacrylamide, as a function of pH. They found that the two resonances broadened without noticeable coalescence and concluded that the interchange of the cis and trans protons was not acid catalyzed at a rate comparable with exchange. We have estimated the frequency shift to be expected for their spectra assuming that the rate of rotation equals the rate of exchange, using the appropriate theory,[27] and we feel that the expected shift (~2 Hz) is too small to be resolved in their spectra. Further, we have just seen that lack of saturation transfer does not in itself rule out the N-protonation mechanism. Thus we disagree with the interpretation of Bovey and Tiers, both with respect to their conclusion concerning a lack of significant acid-catalyzed cis–trans interchange and also their further conclusion that this lack shows that O-protonation is the dominant exchange pathway.

Before leaving the question of O-protonation we point out the possibility that N-protonation does not occur directly as pictured in eq 1, but occurs via O-protonation. This seems likely because of the much higher pK_a of the imidic acid (≈−1) compared to the protonated nitrogen (≈−8) and the possibility that compound IV can be easily generated directly from II by participation of a water molecule (or a chain of water molecules) which could accept a proton from the oxygen and concertedly donate a proton to the nitrogen.[28]

Perrin[10] proposed an N-protonation pathway which is essentially that of eq 6, together with limited rotation of the protonated nitrogen. We have seen that our observations for benzamide are inconsistent with this mechanism alone, and appear to require an additional unknown pathway symbolized by eq 8. Our data for the saturated compounds are also inconsistent with Perrin's proposed mechanism in that it predicts either nonsymmetric cis–trans saturation behavior (when rotation of the protonated form is limited) or 50% saturation transfer (when protonated rotation is rapid).

However, our data for these compounds could be explained by a model somewhat akin to Perrin's in which the eclipsed forms IVa and IVb, eq 4, are the most stable and have equal energy. The predictions of such a theory are similar to, but not identical with, those deduced in section VE, and there does not seem to be any experimental reason to favor one model over the other.

Finally, there is an important self-inconsistency in the models we have presented. Since we see acid-catalyzed rotation without exchange in benzamide and some other compounds, the saturation transfer which we see in the saturated compounds could be almost entirely due to the same mechanism, whatever that might be. The apparent acid-catalyzed exchange rate of the cis proton of benzamide is determined by such rotation without exchange, and this rate is comparable to the acid-catalyzed exchange rate of propionamide and its derivatives (Table II). Thus such a contribution to saturation transfer may be important for saturated compounds but is impossible to separate from the other rates, which is why we did not include it in our analysis for them. We hope that the rotation-without-exchange mechanism is unique to unsaturated and/or aromatic amides, but this point is not proven. Support for this hope is provided by (1) the equality of the cis and trans acid-catalyzed rates for saturated amides, which could be coincidental but is explained most naturally, in our opinion, by N-protonation and rotation; (2) the fact that saturation transfer is smallest for saturated compounds having the slowest acid-catalyzed exchange, which means that any acid-catalyzed rotation-without-exchange mechanism must at least be strongly influenced by the nature of the side chain; (3) possible rationalization of the mechanism as a result of side-chain protonation.[25]

VI. Conclusion

The assumption of N-protonation as the major pathway for proton exchange for primary amides is consistent with experimental data provided that two assumptions are made: first, that rotational diffusion of the protonated amide occurs at a rate comparable to the deprotonation rate, and second, that there is some mechanism whereby the two amide protons undergo acid-catalyzed interchange without involvement of a protonated nitrogen intermediate. If the latter mechanism is also assumed to be important only for unsaturated amides, then the data permit an estimate of the relative rates of rotational diffusion compared to deprotonation of the protonated nitrogen for saturated amides. The relative rates depend on substituent, suggesting not only that deprotonation is relatively slow despite its strongly favorable free-energy change, but also that it is influenced inductively by substituents.

Acknowledgment. We thank W. P. Jencks and E. Grunwald for many informative discussions, and C. L. Perrin for his comments and for sending preprints of his work. This work was partially supported by U.S. Public Health Service Grant GM20168 and by the Research Corporation. A. G. Redfield is also at the Physics Department and the Rosenstiel Basic Medical Sciences Research Center of Brandeis University.

Appendix A. Transfer of Saturation for N-Protonation When Rotation Is Not Infinitely Rapid

There will be six equivalent rotational states of an N-protonated amide (compound IV) in which one proton is nearly perpendicular to the plane of the amide. Three of these are shown in eq 4. This proton will be exactly perpendicular to the plane if the carbonyl group interacts with the N protons in the same way that the aliphatic side chain does. Insofar as these interact differently with the amide protons, there will be a different barrier to rotation through the angle where the carbonyl oxygen is eclipsed by a proton compared to the barrier where the side group is eclipsed. In Appendix B we consider

the case where this difference is so great that the six rotational energy minima coalesce into three minima. Here we consider the case where this difference is negligible so that the six minima differ in angle by 60° and so that the energy barrier to rotation from a given state is essentially the same for rotation, in either direction, to the next stable state.

Thus, if the N protons are imagined to be labeled, we can define six distinct rotational isomers and we assume that exchange occurs to and from the proton site that is perpendicular to the N-C-O plane and never from the other two sites. Define C_0, C_1, C_2, and C_3 as the probabilities that deprotonation occurs after a net rotation of the amide group of zero, one-sixth, one-third, or one-half revolution in either direction from the position it had at the time of protonation from solvent. There is no exchange or interchange associated with zero net rotation since the same proton will be removed, according to our assumption. If there is rotation by one-half revolution, there is also no exchange but there is definitely cis-trans interchange. For one-sixth rotation, one proton is exchanged while the other is unchanged, and for one-third rotation there is exchange of one proton and a definite interchange in position for the other. Thus, for each protonation there is a probability $\frac{1}{2}C_1 + \frac{1}{2}C_2$ that a cis proton is replaced by a solvent proton, and a probability $\frac{1}{2}C_2 + C_3$ that it is replaced by the former trans proton. In this way we deduce that

$$dm_c/dt = k_2[H_3O^+][(\tfrac{1}{2}C_1 + \tfrac{1}{2}C_2)(m_s - m_c) + (\tfrac{1}{2}C_2 + C_3)(m_t - m_c)] \quad (A1)$$

Proceeding as before, we find transfer of saturation from trans to cis of

$$T_{tc} = (\tfrac{1}{2}C_2 + C_3)/(\tfrac{1}{2}C_1 + C_2 + C_3) \quad (A2)$$

This leads to the same answer as before in the appropriate limit of rapid rotation and random departure angle, when (see below) $C_1 = C_2 = 2C_0 = 2C_3$, namely, $T_{tc} = \frac{1}{2}$.

In order to calculate the probabilities C_0 to C_3, we replace the actual problem with an equivalent one in which we imagine an ensemble of amide groups fixed in space and suppose that protonation always occurs at a single site followed by rotational diffusion between rotamers and by deprotonation from the proton site perpendicular to the N-C-O plane. Within the steady state of this model, the probabilities C_0 to C_3 above will be proportional to the numbers N_0 to N_3 of amides in which the solvent proton has rotated by the corresponding net angle before deprotonation. Let k_d be the rate at which transitions occur from one rotamer to either neighboring one. The equations governing the numbers of rotamers are

$$dN_0/dt = k_2[H_3O^+] - (k_{-2} + k_d)N_0 + \tfrac{1}{2}k_dN_1 \quad (A3a)$$

$$dN_1/dt = -(k_{-2} + k_d)N_1 + k_dN_0 + \tfrac{1}{2}k_dN_2 \quad (A3b)$$

$$dN_2/dt = -(k_{-2} + k_d)N_2 + \tfrac{1}{2}k_dN_1 + k_dN_3 \quad (A3c)$$

$$dN_3/dt = -(k_{-2} + k_d)N_3 + \tfrac{1}{2}k_dN_2 \quad (A3d)$$

where k_2 and k_{-2} are defined in eq 1.

If there is no exchange (k_2, $k_{-2} = 0$) these equations describe discrete rotational diffusion. At equilibrium, in that case, $N_1 = N_2 = 2N_0 = 2N_3$ because there are two ways to get rotamers where the solvent proton has rotated $\frac{1}{6}$ or $\frac{1}{3}$ revolution. It is easy to show that the lowest order normal decay mode of (A3), with k_2 and $k_{-2} = 0$, decays at rate k_d s^{-1}. Thus k_d is identified as the rotational diffusion constant in rad^2 s^{-1} since the lowest order solution to the rotational diffusion equation $df(\theta)/dt = k_d\, d^2f/d\theta^2$ also decays with rate constant k_d.

The steady-state solution of (A3) in the presence of exchange is obtained by setting the time derivatives equal to zero. Then (A3d) becomes $N_3 = \frac{1}{2}N_2Q$, where $Q = k_d/(k_{-2} + k_q)$. Also (A3c) becomes $N_2/Q - N_3 = \frac{1}{2}N_1$, or, combining these equations, $N_2(2Q^{-1} - Q) = N_1$. Finally, (A2) can be evaluated by recognizing that the conditional probabilities C_i should be proportional to the N_i in the steady-state problem, so that N_2 can replace C_2 in (A2), while in the expressions just given N_1 and N_3 can replace C_1 and C_3. After some rearrangement and substitution this yields the unexpectedly simple result that

$$T_{tc} = \tfrac{1}{2}Q \quad (A3)$$

Probably this result does not depend much on the details of the model, i.e., whether one assumes a sixfold set of rotamers with all-or-nothing lability of protons or, say, a finer mesh of rotamers and a similar assumption about lability. It presumably does depend in detail on the assumption about the selectivity of the site of lability.

In general the exchange rate is less than the deprotonation rate, and is one-third the protonation and deprotonation rate in the limit $Q = 1$, or fast rotation. Within this model, only a fraction R of all nitrogen protonations lead to exchange, where

$$R = \frac{C_1 + C_2}{C_1 + C_2 + C_3 + C_4} \quad (A4)$$

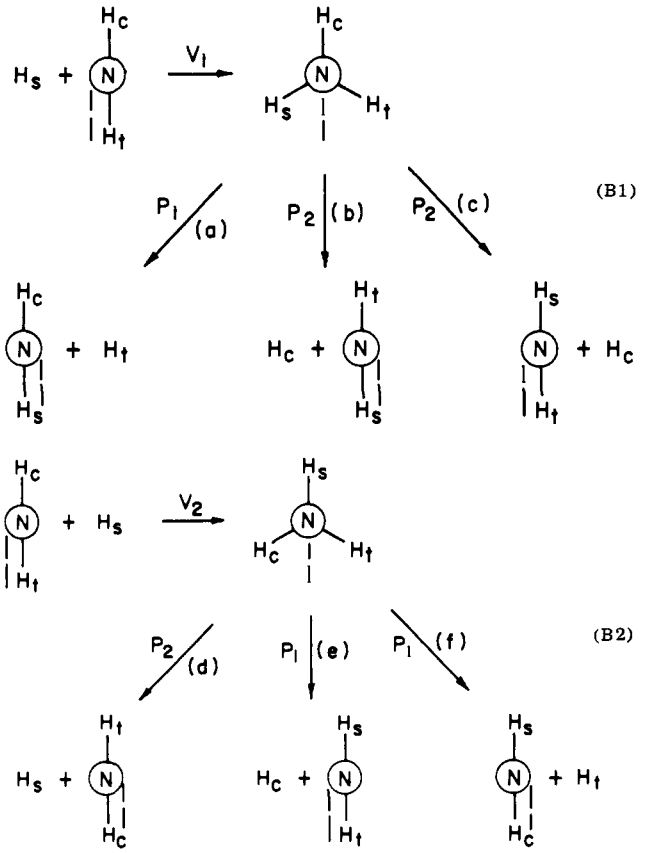
because exchange occurs only after rotation by one-third or one-sixth revolution in this model. This expression can be evaluated in the same way as (A2), using (A3b)-(A3d), and after some algebra becomes

$$R = \frac{Q(2 - Q)(1 + Q)}{Q + 2} \quad (A5)$$

The exchange rate is $\frac{1}{2}Rk_2[H_3{}^+O]$, where the factor of $\frac{1}{2}$ occurs because only one of the two nitrogen protons is exchanged at a time. The catalytic constant k_H is then $\frac{1}{2}Rk_2$ s/mol.

Appendix B. Transfer of Saturation for N-Protonation When Rotation Is Hindered

There are six distinct pathways for exchange, enumerated as (a)-(f).



The quantities p_1 and p_2 are probabilities that deprotonation will occur by each pathway and are proportional to the corresponding pseudo-first-order rates V_1 and V_2. Deprotonation can also occur unproductively by exact reversal of the protonation pathway, so the normalization equation for p_1 and p_2 is $2p_1 + 2p_2 = 1$.

The equations for the magnetizations are

$$dm_c/dt = (m_s - m_c)(V_1p_2 + 2V_2p_1) + (m_t - m_c)(V_1p_2 + V_2p_2) \quad (B3)$$

$$dm_t/dt = (m_s - m_t)(V_1p_1 + V_1p_2) + (m_c - m_t)(V_2p_2 + V_2p_1) \quad (B4)$$

The first term in eq B3 comes from paths (c), (e), and (f), and the second from (b) and (d). The first term in eq B4 comes from paths (a) and (b), and the second term comes from paths (d) and (f).

Transfer of saturation is evaluated as usual by setting the left-hand side of (B3) and (B4) equal to zero, m_s equal to the equilibrium magnetization, and either m_c or m_t equal to zero. The result is

$$T_{ct} = 1 - (V_1p_1 + V_1p_2)/(V_1p_1 + V_2p_2 + V_1p_2 + V_2p_1) \quad (B5)$$

$$T_{tc} = 1 - (V_1p_2 + 2V_2p_1)/(V_2p_2 + 2V_2p_1 + 2V_1p_2) \quad (B6)$$

If we define $a = V_2/V_1$ and note that $p_2/p_1 = a$, these become

$$T_{ct} = a/(a + 1) \quad (B7)$$

$$T_{tc} = (a + 1)/(a + 4) \quad (B8)$$

If we assume an additional mechanism which interchanges the protons without exchange, with a first-order rate constant V_p, we would add terms $\pm(m_t - m_c)V_p$ to (B3) and (B4) and deduce

$$T_{ct} = (a^2 + a + b)/(a^2 + 2a + 1 + b) \quad (B9)$$

$$T_{tc} = (a^2 + a + b)/(a^2 + 4a + b) \quad (B10)$$

where $b = V_p/V_1p_1$. Note that V_p is equal to $1/2V_3$, where V_3 is defined in eq 8, because in that equation only half of the form that is associated with the hydronium ion will dissociate to produce a rotation. Also note that $2p_1 + 2p_2 = 1$, so that the maximum value of either p_1 or p_2 is one-half. Thus, if a is small, b is approximately V_3/V_1.

Equations B9 and B10 can be inverted to yield the useful result

$$a = T_{ct}\frac{1 - T_{tc}}{3T_{tc} - T_{ct} - 2T_{ct}T_{tc}} \quad (B11)$$

This equation was used to estimate the ratios of V_1, V_2, and V_3 at the end of section VF.

Again the exchange rate is less than the protonation rate. The latter is $V_1 + V_2$. The total exchange rate, defined as the initial rate of increase of $m_c + m_t$ just after both are saturated, divided by m_0, is $\frac{1}{2}V_1p_1 + V_1p_2 + V_2p_1$. The ratio of these quantities, obtained after some substitution of formulas already given, is $(1 + 4a)/4(1 + a)^2$.

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