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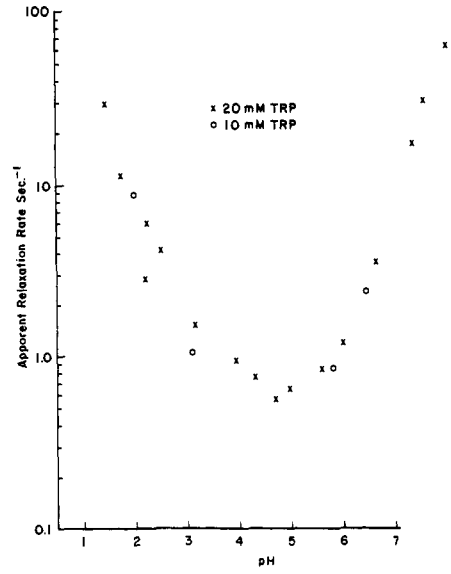

Figure 1. Apparent relaxation rates for the tryptophan indole nitrogen proton at 27°, in unbuffered aqueous (80% H_2O, 20% D_2O) solvent. As discussed in the text, the broad minimum represents primarily magnetic relaxation, while the higher rates represent chemical exchange. At acid pH the rate does not appear to be quite proportional to hydronium ion concentration. If this is not experimental error, it may be a result of minor perturbation by the carboxyl group which titrates in this range. The catalytic rate constant quoted in the text is estimated from the lowest pH points and is, therefore, that of the cation.

Nuclear Magnetic Resonance Studies of Exchangeable Protons. I. Fourier Transform Saturation-Recovery and Transfer of Saturation of the Tryptophan Indole Nitrogen Proton[1]

Sir:

Proton exchange rates between water solvent and sites on small molecules have been measured by several techniques including NMR.[2] We have measured this rate for the indole nitrogen proton of tryptophan. This proton was studied because its resonance is often visible and assignable in protein NMR spectrum and the protein exchange rate can be measured.[3]

The present measurement may also be of technical interest because it partially bridges the gap between measurements based on line broadening (restricted to rates greater than a few seconds^{-1}) and those based on the real-time observation of the change in intensity of such a resonance when the H/D ratio of the solvent is changed. We use a Fourier transform measurement based on a previously published technique[4] for observation of signals from compounds at millimolar concentration in nearly 100% H_2O, so that the concentrations we use are also unusually low.

The bulk of the measurements were of what we call the apparent relaxation rate, measured by the technique of saturation-recovery: a long (~0.1 sec) selective (a few milligauss) preirradiation pulse is applied at the exact indole NH resonance frequency, followed by a delay before the observation pulse (which flips the indole N proton by about 45° while not flipping the solvent protons[4]). Neither the preirradiation nor the observation pulse affect the water protons appreciably. Thus the water protons act as a pool of nuclear magnetization. The indole N protons recover their magnetization after preirradiation at a rate which is the sum of their magnetic spin-lattice rate and their specific chemical exchange rate with the water protons.

Except as noted, all experiments were performed on tryptophan (Sigma) (20 mM; 80% H_2O, 20% D_2O) and adjusted with minimal dilute NaOH or HCl to approximately the desired pH. Samples were placed in 10-mm NMR tubes for several hours before each measurement, and pH was measured directly in the tube prior to and usually just after each NMR run. The pH measurements were performed with a London GK2321C electrode, and we report the meter readings without correction for the 20% D_2O isotope effect. The NMR data were obtained with a Bruker WH-90 Fourier transform spectrometer connected to a data-handling and control system based on a Nova computer, built along lines described previously.[4] Saturation recovery runs usually consisted of observations at 10 to 20 delay times. In each case the data always showed a single exponential recovery when plotted. About 2 min of accumulation (32 transients) was used per point at 20 mM concentration. The peak height of the indole NH line, rather than its integral, was used in these measurements since this peak is always relatively broad (~10 Hz) and field inhomogeniety drift is not a problem.

The observed apparent rates are plotted in Figure 1. At the pH extremes this rate is much greater than the spin-lattice relaxation rate (due to dipolar interaction with other nuclei). Therefore the observed relaxation rate is the specific chemical exchange rate. The same rate can be inferred from line width measurements but the saturation-recovery method is less subject to uncertainties.

From the measurements at extreme pH values we infer hydroxide and hydronium ion catalytic constants of 8×10^7 and 8×10^2 l./(mol sec), respectively, at 300°. Crude temperature runs at pH 7.2 and 2.2 indicate that enthalpies of activation for the specific rate constants are 1 and 2 kcal/mol, respectively. These temperature measurements were performed at essentially constant pH so that the hydronium catalytic constant has an enthalpy of activation of 2 kcal/mol while the hydroxide catalytic constant is about 15 kcal/mol after correction for the enthalpy of dissociation of H_2O of about 14 kcal/mol. There was no measurable dependence on tryptophan concentration (between 10 and 20 mM).

In the central region of Figure 1 (pH ~4.7) the apparent relaxation rate is dominated by processes other than ex-

change. We showed this by transfer of saturation (Forsen-Hoffman) experiments in which preirradiation was at the water NMR frequency rather than that of the indole proton. A delay of about 0.2 sec was used between preirradiation and observation; this delay is short compared to the water T_1 but long enough to substantially decrease interference from a water signal which is stimulated by the preirradiation pulse (this water signal was also suppressed by a spoil pulse). If chemical exchange dominates the indole proton's apparent relaxation, then its resonance should disappear because the saturation of H_2O will be transmitted via exchange.[5] Furthermore, the indole resonance should reappear when the preirradiation frequency is displaced slightly from exact H_2O resonance. This was indeed observed at pH 2.2, where the apparent rate is so fast that exchange dominates. At pH 4.7, however, the indole resonance decreased by only about 20% when the water resonance was presaturated in the same way.

The observed rate R_1, of about 0.7 sec^{-1} at pH 4.7, is the sum of several rates

$$R_1 = R_x + R_n + R_s \quad (1)$$

where the right-hand terms are, respectively, contributions from chemical exchange with water, nitrogen intramolecular relaxation, and solvent proton intermolecular relaxation. We neglect relaxation due to other tryptophan protons.

For the transfer of saturation experiment, if the solvent magnetization is assumed always to be zero because of its saturation by the preirradiation, the indole NH proton magnetization M has the time derivative

$$dM/dt = -(M - M_0)R_N - MR_x - (M - 1.5M_0)R_s \quad (2)$$

Here M_0 is the equilibrium indole proton magnetization, and we assume that the Overhauser enhancement of indole NH by solvent saturation is 1.5 as expected[6] for like-nuclei in the fast correlation-time limit.

The experiment is performed by saturating the protons for a time long enough for the indole proton magnetization to reach a steady state value, for which $dM/dt = 0$. The magnitude of M shortly after such a pulse is monitored by the observation pulse and is $0.8M_0$ at pH 4.7, as mentioned above. If we set $M = 0.8M_0$ and $dM/dt = 0$ in eq 1 and 2, we obtain limits on the contribution of R_x to the total relaxation. If $R_s \ll R_N$ then $R_x \cong 0.2R_1$. If $R_s \ll R_N$ then $R_x \cong (7/15)R_1$.

A slightly better limit can be set by studying the apparent relaxation time as a function of solvent H/D ratio. If such effects as the influence of the solvent isotope ratio on either molecular tumbling or on the proton-proton exchange rate are neglected, then R_s is proportional to the H/D ratio whereas R_N is not. We compared the apparent rate R_1 in 40 and 80% H_2O at pH 5. There was no difference in R_1 within our accuracy limits (±10%), suggesting that R_1 is dominated by relaxation due to the nitrogen nucleus. This is also expected theoretically, based on reasonable estimates for internuclear distances and molecular tumbling rates. Therefore, the exchange rate at pH 4.7 is estimated to be 0.15-0.25 sec^{-1}. Of this, 0.05 sec^{-1} presumably comes from the sum of the acid and base catalyzed exchanges estimated earlier.

We hope to extend this work by studying modified tryptophan and peptides containing tryptophan in order to elucidate the mechanisms of the exchange. The present work was carried out on unbuffered solutions, but a survey of several buffers reveals no significant increase in catalysis up to 0.1 mM buffer concentration. None is expected on the basis of simple arguments based on diffusion-controlled rates of reaction and estimates of the ionization constants of the indole group. We postpone a discussion of these points to a later paper.

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Electrophilic Reactions at Single Bonds. XVIII.[1] Indication of Protosolvated de facto Substituting Agents in the Reactions of Alkanes with Acetylium and Nitronium Ions in Superacidic Media

Sir:

Brouwer and Kiffen recently reported on hydride abstraction by the acetyl cation (acetylium ion)[2] and also by protonated aldehydes and ketones,[3] from alkanes including isobutane. These reactions are of substantial interest as far as mechanistic aspects of hydrogen transfer from alkanes to electrophiles are concerned but also raise some puzzling questions.

Brouwer and Kiffen carried out their studies always using strongly acidic solvent media, generally HF-BF_3, with acetic acid serving as the source of the acetyl cation. In control experiments they proved that the alkanes studied, such as isobutane, show no reactivity with the acid solvent, which thus is not responsible for the observed hydrogen transfer. However, the reported reaction

$$\underset{152}{CH_3CO^+} + (CH_3)_3CH \longrightarrow \underset{-31.3}{CH_3CHO} + \underset{169}{(CH_3)_3C^+} \qquad \underset{-39.8}{} \quad \Delta\Delta H^\circ = 8.5 \text{ kcal/mol}$$

is endothermic by 8.5 kcal/mol (in the gas phase[4]) and thus is energetically unfavorable. Furthermore, Brouwer never observed acetaldehyde, only secondary products derived from protonated acetaldehyde, such as ethyl acetate (indicating hydride abstraction from isobutane to protonated acetaldehyde giving ethyl alcohol and esterification).

We would like to report that in our hands stable acetylium salts in aprotic media (as well as other related acylium salts, which were prepared and studied extensively in our work since 1954[5]) showed no ability to abstract hydrogen from any of the studied alkanes, including isobutane. Extensive studies were carried out with solutions of stable acyl salts, particularly acetylium hexafluoroantimonate in a va-