Effects of Carbon-Bound Deuterium on the Affinities of Acetaldehyde-*1-d* and *N*-Methylformamide-*1-d* for Solvent Water

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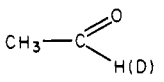
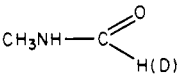
Abstract: With deuterium present in the formyl group, the equilibrium constant for transfer of *N*-methylformamide from chloroform to D_2O at 25 °C was enhanced by a factor of 3.1 ± 0.15%, as estimated independently by proton magnetic resonance and by double-labeling experiments in which ^{14}C and ^{3}H were incorporated alternatively into the methyl group. The distribution coefficient of acetaldehyde-*1-d* between D_2O and the vapor phase, on the other hand, differed from that of acetaldehyde by less than 0.5%.

Secondary deuterium isotope effects on rates and equilibria of organic reactions are often helpful in providing information concerning the probable structures of reaction intermediates and products. If solvent interactions with reactants, transition states, and products were affected to differing extents by isotopic substitution, a complicating factor would arise. Secondary isotope effects would then be observed that were not intrinsic to the reacting molecules, but depended on the surroundings in which the reaction occurred. Knowledge of the potential magnitude of isotope effects on solvation might thus be expected to enhance the usefulness of isotope effects as tests of mechanism.

Vapor pressures of water and alcohols are slightly reduced when deuterium replaces hydrogen on oxygen, whereas small increases in vapor pressure (<1% per deuterium atom) accompany replacement of hydrogen by deuterium on carbon in alcohols.[1] Most carbonyl compounds show substantial shifts in infrared C=O stretching frequencies with transfer between nonpolar environments and solvent water;[2] if adjacent bonds are rehybridized to some small extent during this process, then solvation would be expected to show some sensitivity to substitution of deuterium for hydrogen on the carbonyl group. To obtain information about effects of deuterium substitution on solvation, we have examined its influence on free energies of transfer of carbonyl compounds between nonpolar environments and dilute aqueous solution. The compounds examined are shown below.



acetaldehyde-*1-d*



N-methylformamide-*1-d*

Acetaldehyde was selected as a compound with moderate affinity (as the free aldehyde) for watery surroundings.[3] Inferences regarding the structure of complexes formed by inhibitory aldehydes with the enzyme papain have been based on the assumption that noncovalent solvation of aldehydes is not much affected by deuterium substitution,[4] and it seemed desirable to test this assumption. *N*-Methylformamide was chosen because peptides appear to represent an extreme among uncharged monofunctional compounds in their attraction by solvent water.[5] Accompanied by substantial changes in carbonyl stretching frequency when amides are transferred from the vapor phase to solvent water, this strong interaction appears to arise mainly from hydrogen bonding between the solvent and the carbonyl oxygen atom. If deuterium substitution were to affect carbonyl solvation, its influence might be relatively easy to detect in this case.

Materials and Methods

Acetaldehyde and acetaldehyde-*1-d* were purchased from Aldrich Chemical Co. and purified by fractional distillation after treatment with hydroquinone.[6]

To prepare *N*-methylformamide and *N*-methylformamide-*1-d*, methyl formate or methyl formate-*1-d* (5 g, 0.083 mol, obtained from Aldrich Chemical Co.) was stirred for 1 h at 25 °C with aqueous methylamine (40% by weight, 10 g, 0.13 mol), and the product (4 g, 82% yield) was obtained at 40 °C (0.15 mm) by fractionation on a Nester-Faust spinning-band distillation column. Isotopically labeled methylamine (either 100 μCi [^{3}H]methylamine or 100 μCi[^{14}C]methylamine, purchased from Amersham Corp.) was incorporated as a tracer. The resulting amides showed no trace of impurities by ^{13}C or ^{1}H nuclear magnetic resonance. Their distribution coefficients between water and chloroform were identical under acidic and basic conditions, indicating the absence of radiochemical impurities with basic properties, including methylamine. Before use in vapor-pressure experiments, protio and deuterio amides, alternatively labeled with ^{14}C or ^{3}H, were twice redistilled together on the spinning-band column as described above.

No trace of exchange of deuterium (<1%) from solvent into the formyl group was observed when either acetaldehyde or *N*-methylformamide was allowed to stand in D_2O for 24 h at room temperature.

Results

Deuterium Isotope Effect on the Vapor Pressure of Acetaldehyde over Water. The vapor pressure of acetaldehyde was determined by a dynamic technique similar to that applied earlier to substituted acetamides.[5] A wash bottle, filled with 100 mL of 99.8% D_2O containing acetaldehyde and acetaldehyde-*1-d* (both at a concentration of approximately 0.05 M), was placed in a water bath maintained at 25 °C. Dry nitrogen was introduced through a fritted glass disk, at a rate of approximately 3 mL/min. After bubbling through the solution at atmospheric pressure, this carrier gas passed through a ground-glass joint into three loops of glass tubing containing 2 mL of $CDCl_3$, immersed in dry ice–acetone, and then through a wet-test flow meter. Provided the rate of flow of gas did not exceed 5 mL/min, this apparatus proved capable of trapping more than 95% of the total acetaldehyde initially present. After 1 L of carrier gas had passed through, approximately 2% of the initial acetaldehyde has been transferred to the coils, and material was removed from the trap for analysis by proton magnetic resonance spectroscopy, using a Varian XL-100 spectrometer. To determine the deuterium enrichment of the acetaldehyde trapped in this way, the integrated intensity of the methyl protons (δ 2.24 ppm) was compared with that of the aldehyde proton (δ 9.5 ppm). A similar integration was performed on the D_2O solution originally present in the wash bottle.

Three separate experiments revealed that acetaldehyde transferred to the trap did not differ significantly in isotopic

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enrichment from material present in the pot. In no case did enrichment in the trapped material differ from that in the pot by more than 0.5%, placing this upper limit (approximately equal to the estimated error of the experiment) on the deuterium isotope effect on solvation of acetaldehyde.

The present analysis was confined to the free (not covalently hydrated) aldehyde, in water and in the vapor phase above it. Earlier analyses have shown that deuteration enhances the equilibrium constant for hydration of acetaldehyde, in dilute aqueous solution, by a factor of 1.38.[7] It would be of interest to learn the effect of deuterium substitution on the vapor pressure of acetaldehyde hydrate. Efforts to detect aldehyde hydrates in the vapor phase over concentrated aqueous solutions by Fourier transform infrared spectroscopy at atmospheric pressure were unavailing (P. Cullis and R. Wolfenden, unpublished experiments). Additivity considerations[3] suggest that its vapor pressure should be at least 1000-fold less than that of the free aldehyde.

Deuterium Isotope Effect on the Partitioning of *N*-Methylformamide between Water and Chloroform. The affinity of amides for water is so extreme[5] that, in order to determine distribution coefficients with the needed accuracy, it proved necessary to use a relatively polar solvent, water-saturated chloroform, as the reference phase in order to determine the influence of deuterium on the water-leaving character of *N*-methylformamide. In a typical experiment, *N*-methylformamide-*1-d* and *N*-methylformamide (0.15 mmol each) were dissolved in D_2O (2 mL, saturated with $CDCl_3$) and shaken vigorously with $CDCl_3$ (20 mL) that had been saturated previously with D_2O. The mixture was shaken repeatedly and allowed to remain in a water bath at 25 °C during the intervals. The $CDCl_3$ layer was then extracted with D_2O (2 mL), using the same procedure. Isotopic enrichment was determined by proton magnetic resonance spectroscopy, comparing the integrated intensity of the methyl protons (δ 2.6 ppm) and the formyl proton (δ 7.9 ppm) in the D_2O layers obtained from the first and second extractions. Pyrazine and *p*-dioxane were added as integration standards, in order to obtain the actual concentrations of the two *N*-methylformamides.

In double-labeling experiments identical with those just described except for the method of analysis, methylamines labeled with ^{14}C and ^{3}H were incorporated in *N*-methylformamide and *N*-methylformamide-*1-d*, respectively, and used as radioactive tracers. Concentrations were determined in each phase using a Packard scintillation spectrometer.

After the concentrations of *N*-methylformamide and *N*-methylformamide-*1-d* had been determined in each of the two D_2O layers, by nuclear magnetic resonance or scintillation spectrometry, distribution coefficients were determined using the formula:

$$K_d = \frac{(\text{concn})_{D_2O}}{(\text{concn})_{CDCl_3}} = \left(\frac{W}{C} - 1\right)\frac{M}{L}$$

where W = concentration in first D_2O layer, C = concentration in second D_2O layer, M = volume of second $CDCl_3$ layer, and L = volume of second D_2O layer. For *N*-methylformamide, analysis by the magnetic resonance procedure yielded $K_d(H) = 26.10 \pm 0.04$. For *N*-methylformamide-*1-d*, the same procedure yielded $K_d(D) = 26.87 \pm 0.04$. The ratio of distribution coefficients, obtained in three double-label radioisotope experiments, was $K_d(D)/K_d(H) = 1.0309 \pm 0.0015$. Isotope effects obtained by nuclear magnetic resonance and distribution of radioactive tracers were thus in reasonable agreement, indicating that deuterium enhances the affinity of *N*-methylformamide for solvent water.

Discussion

The present results would be understandable if deuterium, by enhancing the electron density at carbonyl oxygen were to enhance the strength of carbonyl hydrogen bonds to solvent water. The anharmonicities of C–H vibrations exceed those of C–D vibrations, with the result that deuterium can be expected to serve as an electron-donating substituent relative to hydrogen,[8] and, indeed, DCOOH is a weaker acid than HCOOH in water.[9,10] The isotope effect observed in the present experiments is in the wrong direction to be explained in terms of the reduced steric requirements of carbon-bound deuterium, a theoretical possibility[11] that appears to have been realized in the rotation of substituted biphenyls.[12,13]

For acetaldehyde, several orders of magnitude less strongly solvated than *N*-methylformamide, the effect of deuterium substitution at the carbonyl group does not exceed the level of experimental error estimated in these experiments as less than 0.5%. Aldehydes and ketones show relatively small shifts in infrared C=O stretching frequency upon removal from water, compared with carboxlic acid derivatives.[2,5] Thus it appears that the sensitivity of solvation to deuterium substitution may be related to the extent of bond rehybridization that accompanies introduction of the compound into solvent water.

Peptides and amides are extreme among neutral organic molecules in their affinity for solvent water.[5] The presently observed effect of deuterium in enhancing the affinity of *N*-methylformamide-*1-d* for water may accordingly be one of the largest carbon-bound deuterium isotope effects on solvation that is likely to be encountered in chemical practice. Evidently isotope effects on solvation can be significant in derivatives of formic acid, suggesting a need for caution in interpreting very small isotope effects that may be encountered in studying rates and equilibria of their reactions. These effects, which probably represent an upper limit on solvation effects that will be commonly encountered in organic reactions in water, are small enough to leave intact most mechanistic arguments that have been based on the tacit assumption that C–D isotope effects on solvation can be neglected.

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Registry No. Acetaldehyde-*1-d*, 4122-13-8; *N*-methylformamide-*1-d*, 26103-38-8; deuterium, 7782-39-0.

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(13) When deuterium substitution occurs β to a carbonyl group, interference between hyperconjugation and carbonyl solvation may result in preferential partitioning of the protio compound from the aqueous to the nonaqueous phase, relative to the deuterio compound. Such an effect has, in fact, been observed for acetone and *p*-nitroacetanilide and ascribed to this interference (Kovach, I. M.; Quinn, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 0000). We are grateful to these investigators for sending us their manuscript in advance of publication.