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Hydrolysis of Cholinergic Anabaseine and *N*-Methylanabaseine: Influence of Cosolvents on the Position of the Ring-Chain Equilibrium—Compensatory Changes

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The position of the ring-chain equilibrium established by the addition of water to the iminium ring (Im) in the title compounds and subsequent ring cleavage to give the acyclic conjugate acid amino ketones (AK) were investigated by proton NMR using D_2O, dimethyl sulfoxide (DMSO)-d_6, and CD_3OD solvents. A linear free energy relationship expressing the change in the logarithmic value of the equilibrium constant [AK]/([Im][D_2O]) for the mixed solvent with the mole fraction of water added to methanol is found for the substrates, giving the equilibrium constants for the pure solvents. Equilibrium constants for the pure solvents increase in the order water, methanol, DMSO. Although there is less water in the largely nonaqueous solvents, the increase in the magnitude of the equilibrium constants partially compensates for this decrease, thereby resulting in a moderated reduction of the value of the ratio [AK]/[Im]. Replacement of the deuteron bonded to the imine nitrogen atom by a methyl group causes much more acyclic keto-ammonium ion to form in D_2O due to steric compression between this methyl group and the adjacent pyridine ring causing an increase in energy of about 1.7 kcal/mol. © 1990 Academic Press, Inc.

INTRODUCTION

Anabaseine, **1** (3,4,5,6-tetrahydro-2,3′-bipyridine), naturally occurring in the neurotoxic venom of nemertine sea worms, is a potent reversible activator of nicotinic acetylcholine receptor sites in the nervous system (*1*). On contact with water the imino group of the tetrahydropyridine ring rapidly hydrolyzes to the open-chain amino ketone **2.** Our previous report establishes quantitatively for the first time the position of this equilibrium as a function of acidity. Briefly, at low pD in D_2O the acyclic form is favored as a dication, with the deuteron on both the pyridine and amine nitrogen atoms (the conjugate acid of **2**), whereas at high pD the conjugate base of cyclic iminium ion **1** is the major component. Under the approximately neutral conditions of physiological acidity the ratio of open-chain keto-ammonium ion **2** to cyclic iminium ion **1** is 1.2 (*2*). Suspected but not yet proven is the suggestion that only one of these two rapidly equilibrating forms, possibly the cyclic structure, binds at cholinergic receptor sites (*3*). If this is true,

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then the sea worm may store the other nontoxic form and generate the active one at the time of injection.

Because the nicotinic receptor for cholinergic agents may be hydrophobic (*4–6*), we present the results of studies designed to determine what influence a hydrophobic environment might have on the equilibrium ratio for **1,** its derivatives, and its analogs. The position of the ring-chain equilibrium of **1** with **2** and of its *N*-methyl derivative **3** (1-methyl-3,4,5,6-tetrahydro-2,3′-bipyridinium ion) interconverting with **4** was measured in aqueous and nonaqueous solvents and their mixtures. Analog **5** [1-methyl-6-phenyl-2,3,4,5-tetrahydropyridinium ion (*7*)] has a phenyl substituent in place of the 3-pyridyl ring and hydrolyzes to **6.** *N*-Methylated **5** was included to further substantiate the influence of a steric effect in the *N*-methyl compounds on the position of the ring-chain equilibrium.

Claisen-type syntheses of the known compounds **1** (*8*), **3** (*7*), and **5** (*7*) gave the desired substrates. The nonaqueous model solvents include two that are moderately polar, amphiprotic CD_3OD and the more basic dimethyl sulfoxide-d_6 (DMSO-d_6), a strong hydrogen bond acceptor (*9–11*).

RESULTS

Ring-Chain Equilibria: Nomenclature

A question arises as to how to name the solid compounds used as starting materials. Elemental analyses indicate they contain one equivalent of water in the solid state; the associated structures are compatible either with a ketone or with an imine containing a stoichiometric amount of free water in the lattice (water of hydration). Which structure is correct and therefore which name should be designated? This is a classical question that could be answered by, say, "magic angle" solid-state NMR but it was not addressed because we are interested in the solution chemistry. Further confusion arises about how to indicate the degree of protonation of *N*-methylated substrates in the solid state because, for example, the conjugate acid of ketone **4** is diprotic (a dihydrohalide) while that for iminium ion **3,** its equilibrium component, is monoprotic (a monohydrohalide). Equation [1] schematically illustrates the stoichiometry for the hydrolysis reaction, showing the equilibrium between the cyclic and acyclic forms where G is H or CH_3.

[1]

We have elected to name our starting materials using the common name for the cyclic imine as have others (*7*) and therefore designate its associated degree of protonation. The systematic name for the open-chain amino ketone is not employed in spite of the possibility that in the solid state the predominant form is acyclic. Thus, we prefer the common name *N*-methylanabaseine cation **(3)** over the cumbersome name for its hydrolysis product, **4,** 5-methylammonio-1-(3-pyridinyl)-1-

pentanone. Hence, salts of **3** will be referred to schematically as **3** · Cl for that cyclic chloride having an unprotonated pyridine ring while **3** · Cl · HCl denotes its conjugate acid, the pyridinium salt. Similarly, the dihydrobromide of **1** is designated **1** · 2HBr and the monohydrobromide **1** · HBr.

Survey of Solvent Effects

Preliminary studies first show the scope of solvent changes on the position of the equilibrium (*12*). Then a quantitative investigation with mixed water–methanol solvents along with a more limited study of water–dimethyl sulfoxide mixtures is described.

1. Water. *N*-Methylated **3** · Cl · HCl gave a solution with a pD of 3.0 where the material exists largely as the dication. The only form present was the conjugate acid of open-chain amino ketone **4.** A solution of **3** · Cl gave a pD of 6.0 while carbonate base added to **3** · Cl · HCl raised the pD to 9.2 or 9.6 in two separate experiments. In these three cases approximately 6–7% of the cyclic iminium ion **3** appeared (Table 1). The ratio of open-chain keto-ammonium ion to cyclic iminium cation under neutral conditions thus is about 14. The assignment of structures was made by comparison with the proton NMR shifts we previously observed for the nonmethylated derivatives **1** and **2** under similar conditions (*2*).

1 · 2HBr exists largely as the amino ketone while **1** · HBr has nearly equal amounts of the two forms, the ratios of the open-chain to cyclic structures being about 16 and 1.3, respectively. Phenyl derivative **5** exists largely as open-chain protonated amino ketone; there is 3.5 times more ketone than iminium ion (Table 1).

2. DMSO. NMR spectra of **1** and **3** either as their dihydrohalide or monohydrohalide salts were recorded using DMSO-d_6 over the concentration range 0.04 to 0.16 M. The added solids contain one equivalent of water as indicated by elemental analyses and so an equilibrium exchange of water is possible in the absence of any added water.

Results summarized in Table 1 show that (i) dications exist almost entirely as their open-chain keto-ammonium ions; (ii) monocations give more of the cyclic iminium ions, iminium ion being the major product from **1** and a minor product from **3.** In addition, the enamine **7** (1-methyl-1,4,5,6-tetrahydro-2,3′-bipyridine) is clearly detectable from the monohydrohalide of **3.** Formation of the enamine under such mild conditions is remarkable. By contrast, in aqueous solution the addition of base and a high pH are required for enamine formation (*13*).

An authentic sample of the enamine derivative **7** was generated by extracting with chloroform an aqueous solution of **3** made alkaline with sodium carbonate. Its NMR spectrum contains among other signals a triplet due to the alkene proton at 5.03 ppm as proof of its structure.

Varying the concentrations of **3** · Cl causes the product ratio to change. More dilute solutions give rise to lower concentrations of ketone and consequently more iminium ion and more of its conjugate base, the enamine **7.** The ratio of ketone to the total amount of iminium ion and enamine decreases from 3.5 to 2.6 to 1.5 on diluting substrate (Table 1). Both the presence of enamine and its increasing

TABLE 1

Composition of Ring-Chain Equilibrium Mixtures in Various Solvents at 22°C

Compound	Concentration (M)	Solvent	Ketone	Percentage imine	Other
1·2HBr		D_2O	94	6	0
	0.085	DMSO-d_6	100	0	0
	0.071	CD_3OD	72	19	9[a]
	0.071	CD_3OD	32	42	26[a,b]
1·HBr	0.080	D_2O	57	43	0
	0.079	CD_3OD	Trace	100	0
	0.075	CD_3OD/D_2O	27	73	0[c]
1·HCl	0.080	DMSO-d_6	10	90	0
	0.074	$DMSO/D_2O$	76	24	0[d]
	0.071	$DMSO/D_2O$	78	22	0[e]
3·Cl·HCl	0.17	D_2O	100	0	0
	0.053	D_2O/Na_2CO_3	94	6	0[f]
	0.039	D_2O/Na_2CO_3	93	7	0[g]
	0.036	DMSO-d_6	97	3	0
	0.068	CD_3OD	65	26	9[a]
	0.083	CD_3OD	86	5	9[a]
3·Cl	0.13	D_2O	93.5	6.5	0
	0.043	DMSO-d_6	60	21	19[h]
	0.082	DMSO-d_6	72	23	5[h]
	0.16	DMSO-d_6	78	16	6[h]
	0.078	$DMSO/D_2O$	100	Trace	Trace[e,h]
	0.087	CD_3OD	34	66	0
	0.080	CD_3OD/D_2O	77	23	0[e]
5·Br		D_2O	78	22	0

[a] Unknown structure.
[b] Same sample as above but after 10 days.
[c] 2.6 M D_2O.
[d] 4.1 M D_2O.
[e] 5.9 M D_2O.
[f] pD = 9.62, 70 mM Na_2CO_3, 0.5 M NaCl.
[g] pD = 9.20, 42 mM Na_2CO_3, 0.5 M NaCl.
[h] Enamine.

contribution with dilution are readily understandable. In an equilibrium such as that given by Eq. [1] where unequal numbers of particles are involved, dilution favors that side having the larger number of particles, in this case the iminium ion and water. Thus, on dilution the solution becomes less acidic as the keto-ammonium ion acid is converted to the weaker acid water. This decrease in medium acidity causes more of the *N*-methyl iminium ion to dissociate to its conjugate base the enamine **7.** The halide counterion, although substantially more basic in DMSO than in water, is not likely to serve as the active base for deprotonation. Instead it is the more abundant solvent. Adding 5 vol% D_2O to **3** · Cl shifts the equilibrium almost completley to ketone as expected from mass action considerations.

3. Methanol. Samples about 0.08 M in CD_3OD show the following characteristics

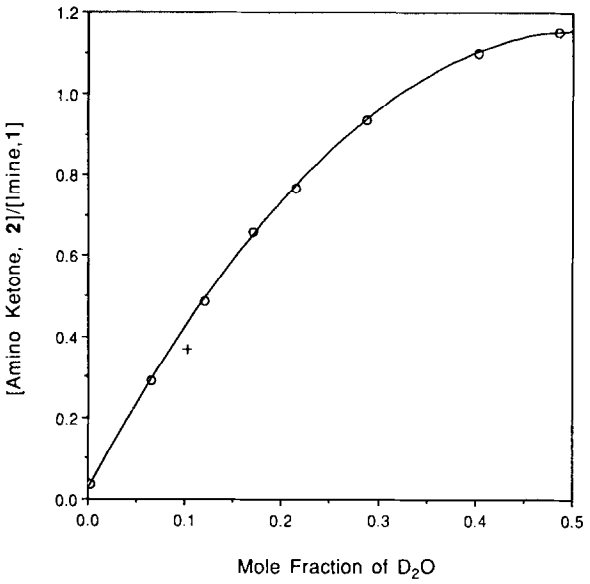


FIG. 1. Plot of the observed ratio of concentrations of keto-ammonium ion **2** to iminium ion **1** as a function of the mole fraction of D_2O in CD_3OD at 22°C uncorrected for "impurity" water in the methanol. The cross denotes the result of a separate determination.

(Table 1): (i) dications exist largely as open-chain ketones but substantial amounts of cyclic iminium ions are present as well, (ii) monocations exist mostly as the iminium ion, more being favored from **1** than from **3**, (iii) adding 5 vol% D_2O to the monocations causes more ketone to form, and (iv) both dications give <10% of an unknown material which may be a cyclic hemiaminal (*14*) or an acyclic ketal. The structure was not identifiable because some of the high field peaks were overlapped by the major components.

Quantitative Studies

1. Water–Methanol. Serial dilution experiments were performed with **1** · HCl and with **3** · Cl in the mixed solvent. To a methanolic solution of each substrate was added a measured amount of D_2O and the NMR spectrum of the mixture was recorded to provide the equilibrium composition. As expected from Table 1 the addition of water causes the amount of acyclic ketone to increase. This increase occurred rapidly at first with small additions of water and then more slowly. The smooth increase in the ratio of the concentrations of acyclic keto-ammonium ion **2** to cyclic iminium **1** is shown in Fig. 1 and similarly for the ratio of **4** to **3** in Fig. 2 as the mole fraction of water increases to about 0.45.

A linear free energy relationship (LFER) quantitatively describes the change in the ratio of the two forms of the two substrates as the composition of the solvent is varied and provides the two desired equilibrium constants for the pure solvents water and methanol:

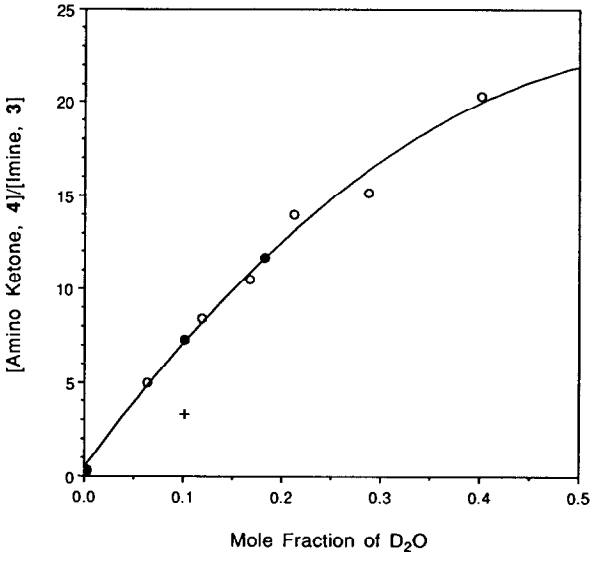


FIG. 2. Plot of the observed ratio of concentrations of keto-ammonium ion **4** to iminium ion **3** as a function of the mole fraction of D_2O in CD_3OD at 22°C uncorrected for "impurity" water in the methanol. Open and filled circles are due to two separate serial dilution experiments. The cross represents a separate determination.

$$\ln K_{mix} = F_D \ln K_D + (1 - F_D) \ln K_M. \quad [2]$$

In the LFER given by Eq. [2] K_{mix} represents the observed equilibrium constant for the mixed solvent, K_D denotes the equilibrium constant when pure D_2O is the medium, K_M is that for pure CD_3OD, F_D is the mole fraction of deuterated water, and $1 - F_D$ is the mole fraction of CD_3OD in the mixture. The equilibrium constant is defined as [ammonio ketone]/([iminium ion][D_2O]) or $K = [AK]/[Im][D_2O]_{tot}$, where $[D_2O]_{tot}$ denotes the total amount of water from all sources as explained below. The LFER actually was applied using the rearranged equation

$$\ln K_{mix} = F_D \ln (K_D/K_M) + \ln K_M \quad [3]$$

and the outcome is presented graphically in Figs. 3 and 4 for **1** and **3**, respectively. An equation similar to [2] has been reported, for example, for rates of reactions in mixed solvents but in these cases, unlike our own, one of the solvents was not a reactant (*15*).

Calculation of the mole fraction F_D and the concentration of water in a mixture requires some care because two minor corrections are needed. They are based on the following considerations: (i) a correction needs to be applied to reflect the release of water into the medium when the ketone is converted to iminium ion, (Eq. [1]) and (ii) the commercial sample of CD_3OD usually was not dried and the initial amount of water "impurity" in the methanol was not determined independently. Both corrections are made easily. In the first the observed equilibrium quantities of ketone and imine provide a measure of the amount of water liberated

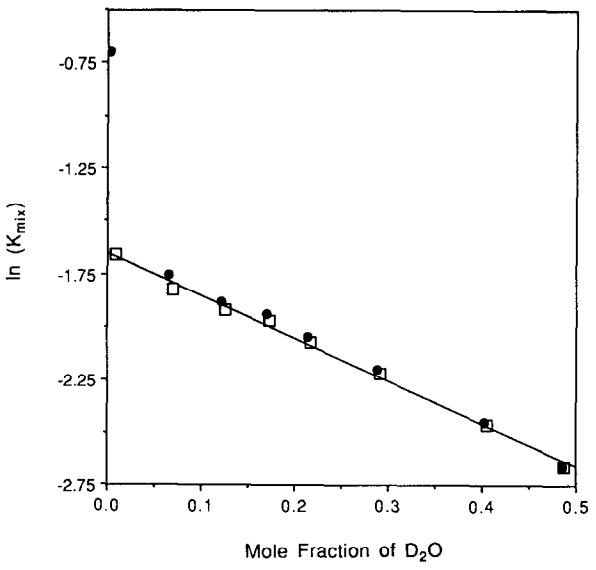


FIG. 3. Linear relationship between ln K_{mix} and the mole fraction of D_2O in CD_3OD at 22°C where K_{mix} = [2]/([1][D_2O]). The filled circles represent the actual data points uncorrected for the amount of "impurity" water present in the solvent; the open squares include this adventitious water calculated according to Eqs. (7) and (8). The slope is −2.02 and the intercept ln K_M at no water is −1.65.

when ketone cyclizes. In the second the unknown quantity of "impurity" water is treated as an adjustable parameter, thereby avoiding the need for tedious drying and determination of the actual water concentration. Determination of the amount of water impurity in the solvent is achieved by a computer fitting of all the experimental data based on Eq. [3] as described under Experimental. These are minor corrections and only materially affect K_{mix} when the measured amount of added water is small but they provide gratifyingly improved fits of the data. Figure 3 shows a typical set of data points with and without the correction for water impurity; there is only one value that changes substantially.

The LFER given by Eq. [3] first was rearranged and then the data were fit using a nonlinear regression microcomputer program that yielded the desired equilibrium constants as well as the amount of impurity water. The exact form of the equation is given under Experimental.

Having a measure of the total water concentration allows the construction of a plot of Eq. [3] to show clearly the influence of water on the apparent equilibrium constant. Accordingly, the intercept gives K_M while the product of the antilog of the slope and the antilog of the intercept provides K_D. The K_D and K_M values for **1** are 0.025 and 0.19 M^{-1}, respectively (correlation coefficient, r, for Eq. [3] is 0.997) and for **3** they are 0.49 and 3.3 M^{-1}, respectively (r = 0.984). For **1** the value of K_D determined in our earlier report (*2*) using purely aqueous solutions is 0.022 M^{-1} (K_H/55.3 M), which is in very good agreement with the present value. There is more scatter in the data for **3** (Fig. 3). The ratio of ketone to imine is so large

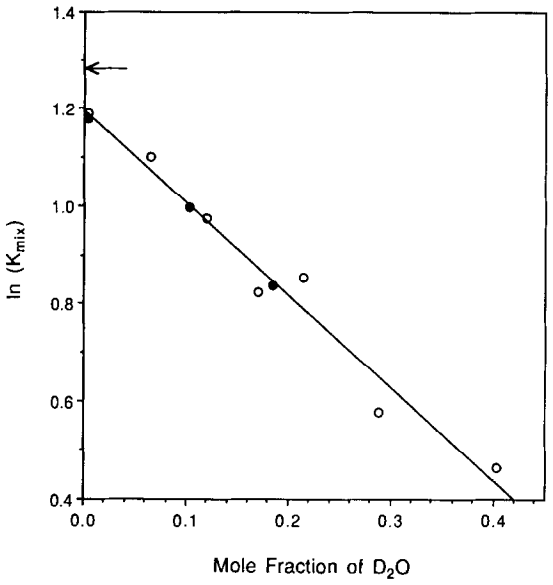


FIG. 4. The same coordinates as in Fig. 3 except that K_{mix} is given by **[4]**/([**3**][D_2O]); again the amount of "impurity" water calculated according to Eqs. (7) and (8) is included. The slope is −1.90 and the intercept ln K_M at no water is 1.20. Open and filled circles represent two separate serial dilution experiments; the arrow denotes the value of the equilibrium constant obtained using solvent dried with molecular sieves.

that the uncertainty in the measurement of the minor component is sizable. Thus, for example, the K_D value for **3** from the LFER is 0.49 M^{-1} while the experimental value measured directly is 0.26 M^{-1} (93.5/(6.5 × 55.3). However, in the former case this corresponds to a composition of 3.6% imine, in the latter to 6.5%, two values that are the same within the experimental uncertainty of our measurements. The computer fit indicates that the amounts of water initially present in the methanol are 0.13 and 0.036 M for **1** and **3**, respectively. Contributing to the quality of the fit is the expectation that the pK_a values for the nitrogen acids will be similar in the two solvents (*16, 17*) and that no significant change in the fraction of protonated material occurs in the mixtures. Only small variations in the chemical shifts of the substrates were observed in support of this suggestion.

The two linear relationships given in Figs. 3 and 4 may be interrelated through their common axis, mole fraction water. Thus, ln K^1_{mix} = 1.06 ln K^3_{mix} − 2.92, showing that both **1** and **3** respond in very nearly the same way to additions of water to methanol.

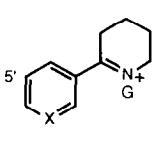
As a check on our computational approach to determine and to correct for the amount of adventitious water present in "dry" methanol two experiments were carried out following drying of the solvent with 3A sieves. Only when elaborate attempts were employed in the second of these to exclude moisture, including drying of the glassware, was it possible to obtain an equilibrium constant K_M having essentially the same value (3.6 M^{-1}) as that obtained from the LFER (3.3

M^{-1}). Moreover, in this case enamine **7** also was detected in about 6% yield. Clearly the additional effort required to dry the methanol is unnecessary; trace amounts of water may easily be established using our serial dilution technique and computer fitting of the data as given by Eqs. [7] and [8] under Experimental.

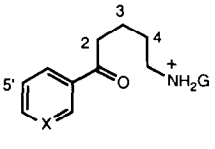
In addition some other new samples were prepared independently in the usual way without prior drying and the resultant data applied to the curves in the figures to check that the curves indeed are reproducible. Figures 1 and 2 show that the new points fit within the uncertainty of the measurements and therefore our results are verifiable. However, a generalization should be kept in mind: as the ratio of components changes from one in value, the uncertainty in the value of the equilibrium constant increases (*18*) and this accounts for the greater scatter in Fig. 4.

2. Water–DMSO. Serial dilution experiments were performed with **1** · HCl by adding D_2O to samples in DMSO-d_6. Analysis of the three data points (Table 1) produced an LFER based on the mole fraction of added water. The value of K_{DMSO} representing the equilibrium constant in pure DMSO is 1.5 M^{-1}. However, the derived value of K_D for pure water solvent is 0.078 M^{-1}, substantially higher than that observed directly for measurements made with pure water and also that obtained from methanol–water mixtures. Moreover, in contrast to the spectra obtained using methanol–water samples which did not show significant changes in chemical shift on addition of water, the NMR spectra of the DMSO–water samples contained large changes in shifts for the imine but not for the ketone. Pyridine signals usually moved upfield, suggesting that the mixture became less acidic. However, the methylene protons adjacent to the imino nitrogen moved in the opposite direction, consistent with increased protonation. As these shifts give inconsistent data about protonation, we made no attempt to interpret them and do not regard the LFER as particularly meaningful.

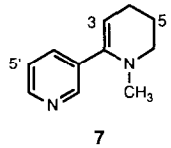
An estimate was made of the value of K_{DMSO} for **3** · Cl based on the assumption that all the water from the three entries in Table 1 comes from substrate when it forms imine and enamine. The derived equilibrium constant has the value 150 ± 13 M^{-1} and represents an upper limit because any water present as an impurity was not considered in the derivation.



1, X=N, G=H
3, X=N, G=CH_3
5, X=CH, G=CH_3



2, X=N, G=H
4, X=N, G=CH_3
6, X=CH, G=CH_3



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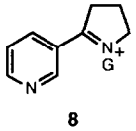

8

DISCUSSION

Solvent Effects

Three different solvents were chosen to study the influence of environment on the position of the ring-chain equilibrium involving the transfer of water to cyclic iminium ion to form acyclic keto-ammonium ion (Eq. [1]). These include water as the reference and hydroxylic but less polar methanol and polar, protophilic dimethyl sulfoxide. For the positively charged nitrogen acids examined, only small changes in their pK_a values are expected when dissolved in the chosen solvents. DMSO, being more basic than water, will bring about more dissociation than water (*9, 11, 19*). The pK_a values for the compounds in methanol and water are likely to be similar (*16, 17*).

No attempt was made to dry the commercial, perdeuterated solvents. Moreover, up to one equivalent of free water may be present in the nonaqueous solvents as provided by the heterocyclic salts themselves based on their composition. This stoichiometric water is bound in the amino ketone and is liberated as free water on conversion to imine (Eq. [1]). As our quantitative treatment of the data provided by methanol–water mixtures shows (Eq. [3]), drying is unnecessary for a systematic study because the amount of adventitious water can be determined on computer fitting of the data.

Dramatic shifts in the position of the ring-chain equilibrium do occur as a function of the composition of both the starting material and the solvent. While the dihydrobromide of **1** exists almost completely as the acyclic ketone in water and in DMSO, it is almost entirely in the cyclic iminium structure when present in methanol as the monohydrobromide. A similar but less extensive shift is found with **3** · Cl · HCl, which is virtually all ketone in both water and DMSO, whereas **3** · Cl is about 65% iminium ion in methanol (Table 1).

Major conclusions derived from the contents of Table 1 and the figures are as follows. (1) Ketone is favored in most of the trials listed in Table 1. That is, the equilibrium given by Eq. [1] need not be shifted largely to the left favoring iminium ion according to the law of mass action in spite of the concentration of water being low in "anhydrous" methanol and DMSO. (2) Generally, the least amount of cyclic iminium ion is present in pure water. (3) More iminium ion is present in methanol than in dimethyl sulfoxide. (4) More iminium ion is present in the less acidic solutions, especially when the starting compound is the monohydrohalide rather than the dihydrohalide salt. The latter conclusion is in keeping with our prior observations for purely aqueous solutions (*2*). Interestingly, although iminium ion and especially neutral imine are favored by basic conditions, the most basic solvent, DMSO, does not provide the most iminium ion from any salt, showing that the basicity of the medium is not the only relevant factor. Solvation, perhaps by means of H bonding, especially to stabilize the alkyl ammonium cation, which has a larger number of acidic protons than the iminium ion, should have an important influence on the position of the equilibrium and DMSO is an especially effective H-bond acceptor (*19*). (5) The values of K_D for **1** · HBr and for **3** · Cl are

0.025 and 0.49 M^{-1}, respectively, and for K_M they are 0.19 and 3.3 M^{-1}, respectively, as derived from Figs. 3 and 4.

The changes in the values of the equilibrium constants can be understood in terms of the activity coefficients for substrate transfer from one solvent to another. According to this, the concentration terms cancel in our ratio of equilibrium constants, leaving a ratio of solvent activity coefficients, (*20, 21*)

$$K_D/K_M = {}^D\gamma^M_{AK}/({}^D\gamma^M_{Im}\,{}^D\gamma^M_D), \qquad [4]$$

where ${}^D\gamma^M$ represents the solvent activity coefficient for transfer from D_2O (D) to CD_3OD (M) of the component designated by the subscript (*20*). Activity coefficients have been reported for H_2O in CH_3OH based on mole fraction, with unit mole fraction as the standard state (*22, 23*). These values indicate little deviation from ideality. Therefore, our water term appears to account for very little of the change in the observed equilibrium constant ratio, most of the change being associated with the two organic components. A similar activity coefficient ratio may be written for equilibria in DMSO where again data are available for water–DMSO mixtures. In this instance the value of the solvent activity coefficient for a mole fraction water of 0.1 is 0.28 (*24*). Again the major influence of the change in the solvent composition appears to be on the energy of the organic components. Perhaps the energy of the keto-ammonium ion is changed more than that of the iminium ion because H bonding of its ammonio protons to the DMSO solvent is so strong.

Steric Effects

The equilibrium composition of **3** · Cl in water is not that expected from a consideration of a model compound. Myosmine (**8,** G=H), an analog of **1** and a minor tobacco alkaloid binding to nicotinic cholinergic receptor sites, has a 5-membered 1-pyrroline ring rather than the 6-membered 1-piperideine ring in **1** and **3.** Replacing the hydron bonded to the imino nitrogen atom of **8** by a methyl group to give the 5-membered analog **8** (G=CH_3) of **3** causes a little less ketone ion to form on hydrolysis in D_2O. The ratio of the amounts of open-chain keto ammonium ion to cyclic iminium ion only decreases from 1.9 to 0.88 with this substitution on nitrogen because the electron-donating *N*-methyl group preferentially but slightly stabilizes the iminium ion at the expense of the ketone (*2, 13, 25*). Replacing the iminium hydron of **1** by a methyl group to give **3,** in marked contrast, has an effect opposite that just considered with **8.** The methyl group causes the iminium ion to become strongly disfavored in the equilibrium (Table 1). As given by the ratio of K_D and K_M values, the equilibrium constant for **3** is 20 and 17 times larger than that for **1** in D_2O and CD_3OD, respectively. Overriding the stabilizing electronic effect of the methyl group is a destabilizing steric effect for the larger 6-membered ring. As the iminium ring is made larger, the methyl group and the adjacent pyridine ring are forced closer together, raising the energy of the cyclic structure and thereby shifting the equilibrium to favor the acyclic

material where these two substituents now are separated and no longer can interact. The nominal angle defined by lines drawn from the substituents to the center of the ring marking the separation between the two annularly bonded groups on a 5-membered ring is 72°; for a 6-membered ring it is only 60°. The smaller angle supports the suggested steric compression between the two adjacent groups on the larger ring.

Making the approximation that the energies of the open-chain keto primary and secondary alkylammonium ions **2** and **4,** respectively, are similar allows the difference in energy between the two cyclic iminium ions to be estimated. Thus, from the ratio of the two equilibrium constants and their average for the two solvents there is about 1.7 kcal/mol (RT ln 18) of steric compression energy in **3** that is not present in **1,** providing a considerable driving force for ring opening.

The same kind of steric effect is also found with phenyl derivative **5** (*26*). Again, a consideration of model compounds is instructive, this time making changes at the carbon atom of the imine rather than at nitrogen. For cyclic 5-membered iminium **8** ($G{=}CH_3$) replacing the more electron-withdrawing 3-pyridyl ring by a phenyl substituent causes the ratio of acyclic to cyclic ions to decrease about 10-fold due to preferential electronic stabilization of the iminium group by conjugation with the more electron-donating phenyl ring (*13*). However, the equilibrium compositions of pyridyl **3** · Cl and phenyl **5** in water are similar (Table 1), contrary to the expectation raised by the model compounds. Steric compression between the *N*-methyl group and the benzene ring causes the iminium ring to be destabilized, negating the stabilizing electronic effect of the phenyl ring on the iminium ion.

Significance for Binding Studies

The results for **1** · HBr, **1** · HCl, and **3** · Cl provide the most useful information about the influence of solvent composition at a hydrophobic binding site on the position of the ring-chain equilibria for these substrates in biological experiments. The acidities of the aqueous solutions in our studies involving these substrates should reasonably approximate those of binding experiments while those for **1** · 2HBr and **3** · Cl · HCl are greater and therefore less relevant. The composition of **1** · HBr in D_2O (Table 1), is essentially the same (1.3) as that observed with aqueous phosphate buffers where the ratio (1.2) of the concentrations of the open-chain to cyclic forms is almost constant over the pD interval 4.5 to 7.5 (*2*).

Consideration of the results given in the figures is quite informative about the effect of the change in water content of methanol–water mixtures on the ring-chain ratio. But first it is necessary to understand what is meant by the definition of K_M in Eqs. [2] and [3] for an equilibrium involving the transfer of water between two substrates in a nonaqueous solvent. K_M reflects the value of the equilibrium constant under the conditions of a hypothetical state where water acts only as a reactant not as a solvent, i.e., as a hypothetically "infinitely" small amount of water in pure methanol solvent. As the amount of water is decreased, the value of K_{mix} increases (Eq. [3] and Figs. 3 and 4), and the two terms in the product $K_{mix}[D_2O]$ change in opposite directions. There are approximately compensating changes in the terms of this product and hence in the value of the ratio [AK]/

[Im]. The compensation is not exact because the concentration (activity) of water changes more than the value of K_{mix}. The range in the variation in K_{mix} is given by the ratio of the equilibrium constants for the pure solvents K_M/K_D, 7.6 for **1** and 6.7 for **3.** As the water concentration is reduced the net result is a decrease in the ratio of ketone to imine.

The variation in some of the results given for **3** in methanol–water in Table 1 now becomes understandable. Variable and small amounts of water in the commercial methanol account for the changes. Because the value of K_{mix} is so much larger for **3** than for **1** in the relationship $K_{mix}[D_2O]$ = [AK]/[Im], similar variations in the amount of water impurity will lead to a relatively larger change in the [AK]/[Im] ratio for the former.

Quantitative comparisons of the bioactivity of the solvent-sensitive and -labile **1** and **3** relative to those for other cholinergic substrates must be made with great care until it is established which one of the two forms involved in the hydrolytic equilibrium binds, if indeed it is only one form that is active. Thus, the data in Table 1 show that while the percentages of iminium ion from **1** · HBr in water and in methanol vary modestly, 43% vs 100% (a factor of 2.5), the changes in the amount of ketone are much larger, 57% vs a trace. Therefore, estimates of the concentrations of substrate undergoing binding based on the initial, *total* concentration uncorrected for the fractional amount of active material actually present may be grossly misleading. Similar errors may be made for **3** · Cl (Table 1).

The composition of **1** · HBr and **3** · Cl in methanol–water is not unlike that for pure water but is higher in iminium ion, the amount depending on the quantity of water present. If methanol–water rather than pure methanol or pure DMSO is a reasonable approximation of the environment at a hydrophobic receptor binding site and there are no special electrostatic effects between substrate and binding site to perturb the equilibrium, then application of our earlier data (*2*) about the composition of **1** in purely aqueous buffers should provide at least a semiquantitative measure of concentrations in the hydrophobic medium.

EXPERIMENTAL

NMR spectra were recorded on a Varian VXR-300. Tetramethylsilane (TMS) was used as an internal standard in $CDCl_3$ and sodium 2,2,3,3-d_4-trimethylsilylpropionate (TSP) was used in all other solvents. Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected.

Anabaseine (*8*), originally reported as the dipicrate, and *N*-methylanabaseine (*7*), originally reported as the monopicrate, are known compounds.

N-Methylanabaseine chloride hydrochloride (1-methyl-3,4,5,6-tetrahydro-2,3'-bipyridinium chloride hydrochloride)(***3*** · *Cl* · *HCl*). The chloride hydrochloride was isolated as a white precipitate from a solution of **3** in 12 M HCl following concentration of this solution and dilution with acetonitrile and enough ethanol to make the phases miscible. This compound melted at 173–177°C with decomposition. *Anal.* Calcd. for $C_{11}H_{16}N_2Cl_2 \cdot H_2O$: C, 49.82; H, 6.84; N, 10.56. Found: C, 49.83; H, 6.88; N, 10.51. ^{1}H NMR (0.5 M NaCl, D_2O), amino ketone: δ 9.34 (d, 1.6 Hz, 1 H,

H2'), 9.07 (dt, 8.1, 1.7 Hz, 1 H, H4'), 9.00 (d, 5.8 Hz, 1 H, H6'), 8.21 (dd, 8.2, 5.7 Hz, 1 H, H5'), 3.32 (t, 2 H, H2), 3.13 (unresolved t, H5), 2.75 (s, NCH_3), 1.84 (m, 2 H, H3 and H4). ^{13}C NMR (0.5 M NaCl, D_2O), amino ketone: δ 201.31 (carbonyl), 147.90, 147.71 (C2' and C6'), 145.15 (C4'), 137.52 (C3'), 130.40 (C5'), 51.69 (C5), 41.04, 35.67 (NCH_3 and C2), 27.61, 22.46 (C3 and C4).

1-Methyl-6-phenyl-2,3,4,5-tetrahydropyridinium bromide **(5).** This compound melted at 143–146°C. ^1H NMR (D_2O), ketone: δ 8.01 (d, phenyl, *ortho*), 7.63 (m, phenyl, overlaps imine), 3.19 (t, H2), 3.10 (t, H5), 2.74 (s, NCH_3), 1.78 (m, H3 and H4). Imine: δ 7.63 (m, phenyl, overlaps ketone), 3.96 (t, H2), 3.49 (s, NCH_3), 2.09, 1.97 (quintets, H4 and H3). The picrate has been prepared (*7*). ^1H NMR of the perchlorate in trifluoroacetic acid solution has been reported (*27*).

Anabaseine dihydrochloride (*3,4,5,6-tetrahydro-2,3'-bipyridinium dihydrochloride*) (***1*** · *2HCl*). The dihydrochloride was isolated as a white precipitate from a solution of **1** in 12 M HCl following dilution with isopropyl alcohol. This compound melted at 175–180°C with decomposition which was dependent on the rate of heating. *Anal.* Calcd. for $C_{10}H_{14}N_2Cl_2 \cdot H_2O$: C, 47.82; H, 6.42; N, 11.15. Found: C, 47.92; H, 6.55; N, 10.96. ^1H NMR (D_2O), ketone: δ 9.34 (s, H2'), 9.08 (dt, 8.3, 1.6 Hz, H4'), 8.99 (d, 5.9 Hz, H6'), 8.22 (dd, 8.1, 5.9 Hz, H5'), 3.30 (t, 6.6 Hz, H2), 3.07 (t, 6.6 Hz, H5), 1.81 (m, H3 and H4). Imine (7%): δ 9.04 (s, H2'), 8.92 (d, H6'), 8.45 (dt, H4'), 7.86 (dd, H5'), 3.91 (broad, H6), 2.04 (m, H4 and H5).

Anabaseine dihydrobromide (*3,4,5,6-tetrahydro-2,3'-bipyridinium dihydrobromide*) (***1*** · *2HBr*). The dihydrobromide was isolated as a light brown precipitate from a solution of **1** in 48% HBr following concentration of this solution and dilution with isopropyl alcohol. The compound melted at 168.5–172°C with decomposition. *Anal.* Calcd. for $C_{10}H_{14}N_2Br_2 \cdot H_2O$: C, 35.32; H, 4.74; N, 8.24. Found: C, 35.49; H, 4.76; N, 8.20.

N-Methylanabaseine chloride (***3*** · *Cl*). To a solution of 108 mg (0.41 mmol) of *N*-methylanabaseine dihydrochloride in 2 ml of methanol was added 175 mg of poly-4-vinylpyridine. After the suspension was stirred for 2 h at room temperature, the poly-4-vinylpyridine was removed by filtration. The methanol was evaporated to dryness to leave 88 mg (0.385 mmol, 94% yield) of white solid (mp 135.5–138°C, decomp). *Anal.* Calcd. for $C_{11}H_{15}N_2Cl \cdot H_2O$: C, 57.76; H, 7.49; N, 12.25. Found: C, 57.63; H, 7.40; N, 12.08. ^1H NMR (D_2O), amino ketone: δ 9.09 (d, 2.3 Hz, H2'), 8.74 (dd, 5.0, 1.7 Hz, H6'), 8.39 (dt, 8.1, 2.0 Hz, H4'), 7.61 (dd, 8.1, 5.0 Hz, H5'), 3.23 (t, 6.4 Hz, H2), 3.12 (t, 6.9 Hz, H5), 2.76 (s, NCH_3), 1.81 (m, H3 and H4). Imine: δ 8.81 (dd H6'), 8.72 (d, H2'), 8.06 (dt, H4'), 7.72 (dd, H5'), 4.03 (t, H6), 3.56 (s, NCH_3), 2.13, 2.00 (quintets, H4 and H5).

Anabaseine Hydrochloride (***3*** · *HCl*). This was prepared from the dihydrochloride as reported for **3** · Cl (mp 118.5–120°C, decomp). *Anal.* Calcd. for $C_{10}H_{13}N_2Cl \cdot H_2O$: C, 55.95; H, 7.04; N, 13.05. Found: C, 55.93; H, 7.02; N, 12.83. ^1H NMR (D_2O), ketone: δ 9.09 (d, 1.4 Hz, H2'), 8.75 (dd, 4.9, 1.4 Hz, H6'), 8.40 (dt, 8.0, 1.7 Hz, H4'), 7.63 (dd, 8.0, 4.9 Hz, H5'), 3.23 (t, residual H2), 3.08 (t, H5), 1.79 (m, H3 and H4). Imine: δ 8.95 (d, 1.9 Hz, H2'), 8.85 (dd, 5.0, 1.4 Hz, H6'), 8.29 (dt, 8.3, 1.7 Hz, H4'), 7.72 (dd 8.1, 5.0 Hz H5'), 3.91 (broad, H6), 2.04 (broad, H4 and H5).

TABLE 2

Chemical Shifts for Amino Ketones **2** and **4** (δ, ppm) in Nonaqueous Solvents

Solvent	Compound	H2′	H6′	H4′	H5′	H2	H3, H4	H5	*N*-Me
DMSO-d_6	**2** · 2HBr	9.38	9.05	8.79	8.02	3.25	1.70	2.88	—
	4 · Cl · HCl	9.31	8.97	8.65	7.90	3.23	1.72	2.94	2.53
	2 · HBr	9.17	8.82	8.35[b]	7.60	3.17	1.68	2.86	—
	4 · Cl	9.17	8.82	8.34	7.60	3.17	1.70	2.91	2.51
CD_3OD	**2** · 2HBr	9.43	9.06	9.16	8.26	[a]	1.84	3.01	—
	4 · Cl · HCl	9.42	9.06	9.15	8.26	[a]	1.88	3.07	2.71
	2 · HBr	9.11	8.73	8.40	7.58	[a]	1.79	2.98	—
	4 · Cl	9.12	8.73	8.41	7.58	3.17	1.80	3.05	2.71

[a] Overlapped or exchanged.
[b] Overlaps imine H4′.

Anabaseine hydrobromide (***1*** · *HBr*). This was prepared from the dihydrobromide by the method reported for **3** · Cl (mp 88–90°C). *Anal.* Calcd. for $C_{10}H_{13}N_2Br \cdot H_2O$: C, 46.35; H, 5.83; N, 10.81. Found: C, 46.72; H, 5.67; N, 10.60.

Measurements of equilibria by ^1H NMR. NMR samples were prepared by weighing the compound into an NMR tube and adding 500 μl of solvent. Concentrations ranged from 0.04 to 0.16 M. The spectra were recorded at probe temperature (21–23°C).

Spectra were recorded using a 37° pulse and no delay between transients. The fraction of a given component was obtained by averaging the values calculated using the intensity ratios for several sets of peaks. Spectra recorded using a 37° pulse and with no delay between transients gave intensity ratios similar to those recorded using a 90° pulse and a delay of five times the longest T_1. A ^1H NMR spectrum of **3** · Cl in CD_3OD gave values of 71.8% imine, 22.4% ketone, and 5.78% enamine when recorded, with no delay between transients and gave values of 72.0% imine, 22.2% ketone, and 5.80% enamine when recorded with a delay time of 35 s ($5 \times T_1$) between transients.

Chemical shifts for compounds **1** and **3** in nonaqueous solvents appear in Tables 2 and 3. Their chemical shifts and multiplicities are similar to those reported for the aqueous solutions in the preparations. Results are reported in Table 1.

Control experiments to establish that the hydrolysis reaction is at its equilibrium position. No change in the composition of **3** in D_2O was observed for a sample at pD 6.0 after 3 days at room temperature, showing that the intial spectrum obtained after a few minutes represented the sample at equilibrium, as expected.

All samples but **3** · Cl · HCl in DMSO were checked, usually after several days. No change was detected except in the case of **1** · HBr where a 40-min-old sample gave 35% ketone and 65% imine but 9 days later showed 15% ketone and 85% imine. The latter is taken to be the equilibrium composition (Table 1).

With the exception of **3** · Cl · HCl, which was not checked for time dependence in methanol, the data reported in Table 1 represent stable samples. When **1** · 2HBr

TABLE 3

Chemical Shifts for Cyclic Imines **1** and **3** (δ, ppm) in Nonaqueous Solvents

Solvent	Compound	H2′	H6′	H4′	H5′	H3	H4 and H5	H6	*N*-Me
DMSO-d_6	**3**·Cl·HCl	[a]	8.90	8.25	7.78	[a]	2.01, 1.87	3.94	3.46
	1·HBr	9.10	8.90	8.35[c]	7.70	3.28	1.91	3.81	—
	3·Cl	8.90	8.84	8.16	7.70	[a]	2.01, 1.89	3.93	3.44
CD_3OD	**1**·2HBr	9.32	[a]	8.79	8.08	[b]	2.07	3.95	—
	3·Cl·HCl	9.20	[a]	8.71	8.14	[b]	2.16, 2.02	4.05	3.56
	1·HBr	9.02	8.85	8.32	7.68	[b]	2.02	3.88	—
	3·HCl	8.8	8.8	8.12	7.68	[b]	2.13, 2.01	4.00	3.52
$CDCl_3$	**7**	8.63	8.48	7.69	7.26	5.03	2.16, 1.78	3.09	2.42

[a] Overlapped.
[b] Overlapped or exchanged.
[c] Overlaps ketone H4′.

was examined after standing 10 days at room temperature a substantial decrease in the amount of ketone and an increase in the amount of imine and unknown were noted. The initial reading was taken to be that for the equilibrium mixture.

Titration of methanolic solutions of ***1*** *and* ***3*** *with* D_2O. Initial samples were prepared by weighing the compounds into NMR tubes and adding 500 μl of CD_3OD. ^1H NMR spectra were recorded a few minutes after preparation. Both samples then were given several hours to come to equilibrium before titration. After 21 h, the 0.080 M solution of **1** went from 92 to 96% imine, and after 8 h, the 0.079 M solution of **3** went from 74 to 76% imine. The latter values were taken as the equilibrium values. Seven to eight aliquots of D_2O ranging in size from 15 to 60 μl were added successively to the samples 10 min before recording the spectra. After 3 days, the solution of **1** containing 150 μl of D_2O and 500 μl of CD_3OD had not changed significantly (47.6 to 47.4% imine). A final aliquot of 60 μl of D_2O was added to this solution.

Prior to fitting the titration data by computer, Eq. [3] was transformed into Eq. [6] to calculate the water impurity in the CD_3OD, considering this amount as an adjustable parameter. The ratio K_D/K_M and K_M were also fit as adjustable parameters.

$$\ln K_{mix} = \ln\left(\frac{[AK]}{[Im][D_2O]_{tot}}\right) = \ln\left[\left(\frac{K_D}{K_M}\right)^{F_D} \cdot K_M\right]. \quad [5]$$

$$\ln\frac{[AK]}{[Im]} = \ln\left[\left(\frac{K_D}{K_M}\right)^{F_D} \cdot K_M \cdot [D_2O]_{tot}\right]. \quad [6]$$

[AK] = concentration of amino ketone and [Im] = concentration of imine. The mole fraction of water, F_D, and the concentration of water were expressed in terms of moles of water:

$$F_D = \frac{M_{sub} + M_{solv} + M_o}{M_{sub} + M_{solv} + M_o + 0.0246(0.500 - 18.13M_o)}. \quad [7]$$

$$[D_2O]_{tot} = \frac{M_{sub} + M_{solv} + M_o}{(0.01813)(M_{sub} + M_{solv}) + 0.000500}. \quad [8]$$

M_{sub} = moles of water liberated from substrate, M_{solv} = moles of water from added D_2O, and M_o = moles of water impurity in CD_3OD. In Eq. [7], the volume of methanol (0.500 ml) is corrected for the volume of water impurity which is given by the term $18.13M_o$, where 18.13 is the ratio of the molecular weight (20.03 g/mol) to the density (1.105 g/ml) of D_2O (*28*). The corrected volume of methanol is then converted to moles of CD_3OD by multiplying by 0.0246 which is the ratio of the density (0.888 g/ml) to the molecular weight (36.07 g/mol) (*29*). In Eq. [8], the term 0.01813 is a conversion factor (the ratio of molecular weight, 20.03 g/mol, to density in grams per liter, 1105 g/liter) for converting the moles of D_2O to liters and the term 0.000500 is the volume of methanol in liters. The moles of water liberated from cyclization of amino ketone, M_{sub}, was calculated for each ratio of amino ketone to cyclic imine measured. The fraction of imine ([Im]/([Im] + [AK])) present is equal to the fraction of water liberated. The moles of water liberated then is the product of the fraction of water and the total moles of substrate present. The value of the moles of water impurity in the methanol which was calculated with the computer fit was added to the observed mole fraction of water (Eq. [7]) and to the observed concentration of water to correct the experimental values of K_{mix}. The values for K_{mix} were plotted against the mole fractions of water in the LFER. Results appear in Figs. 1–4.

Control experiments to measure the equilibrium constant for **3** *in dry methanol.* Measurements were made on two separate solutions of **3** · Cl in methanol which was dried over 3A sieves prior to making the NMR solutions.

The 3A sieves were rinsed with D_2O and dried by heating them under vacuum for 10 h. For the first NMR solution, a sample of perdeuterated methanol was dried over these sieves for 22 h in an oven-dried volumetric flask. Solid **3** · Cl was dried in a drying pistol, weighed into an NMR tube containing dry TSP, and dissolved in 500 μl of the dried methanol to make a 0.078 M solution of **3** · Cl. Three and a half hours after making the NMR sample, 72.9% imine, 22.9% amino ketone, and 4.2% enamine were present. After standing 32 h, 71.7% imine, 25.7% amino ketone, and 2.5% enamine were present and after 3 days, 70.8% imine, 29.2% amino ketone, and a trace of enamine were present. It is not clear whether the increase in the fraction of amino ketone and decrease in the fraction of enamine represent a slow approach to equilibrium or contamination of the sample with water on standing. The values calculated for K_M (K_M = [ketone]/([imine][D_2O]$_{tot}$)) are 5.2 M^{-1} after 3.5 h, 6.2 M^{-1} after 32 h, and 7.5 M^{-1} after 3 days.

The 3A molecular sieves were dried an additional 9 h for the second NMR sample. A fresh sample of perdeuterated methanol was dried over the sieves for 3 days in an oven-dried volumetric flask in a desiccator. Solid **3** · Cl was dried in a drying pistol and weighed into an NMR tube with dry TSP. The NMR tube containing the solids was dried in the drying pistol before adding 500 μl of the

dried CD_3OD with a dry syringe to make a 0.111 M solution of **3** · Cl. This NMR sample was stored in a desiccator. After standing 22 h, the sample contained 72.0% imine, 22.2% ketone, and 5.8% enamine. After standing 46 h, the sample contained 72.0% imine, 22.1% ketone, and 5.9% enamine. The fractions of each component in the sample represent equilibrium values and yield a calculated value for K_M of 3.6 M^{-1} which is in agreement with the value obtained from the LFER fit (K_M = 3.3 M^{-1}) of **3** · Cl in methanol which was not dried.

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