bar and connected to a mineral oil bubbler was cooled to room temperature under argon. The flask was immersed in a water bath at 25 °C. Then 3.35 mL of THF, 1.6 mL (2 mmol) of a 1.25 M solution in THF of lithium tri-*tert*-butoxyaluminohydride, and 1.0 mL (2 mmol) of a 2.0 M solution of *n*-dodecane in THF (to serve as the internal standard) were introduced in the order indicated. Finally, 2 mL (2 mmol) of a 1.0 M solution of phenyl disulfide in THF was added to this well-stirred mixture. The reaction mixture was now 0.25 M in both LTBA and disulfide. Vigorous hydrogen evolution was observed during the initial phases of the reaction. After 2 min, 0.1 mL of the reaction mixture was withdrawn by a syringe, quenched with dilute HCl, extracted with ether, and dried ($MgSO_4$). The dry ether extract was analyzed by capillary GLC. The analysis revealed the presence of 16% benzenethiol. Similarly, the reaction was monitored at 5, 10, 15, 30, 60, and 120 min. At 30 min, 53% of the reaction was complete. At the end of 120 min, reaction had proceeded only to the extent of 58%.

**Procedure for Rate Studies and Product Analysis.** The following procedure for the reduction of *p*-tolyl disulfide is representative. The experimental setup was the same as in previous experiments. THF (1.3 mL) was injected into the reaction flask followed by 3.8 mL (4.8 mmol) of a 1.28 M solution of LTBA in THF and 0.9 mL of *n*-dodecane (internal standard). To this well-stirred solution maintained at 25 °C, 2 mL (2 mmol) of a 1.0 M solution of *p*-tolyl disulfide in THF was injected. Hydrogen evolution was observed. At appropriate intervals of time, 0.1 mL of the reaction mixture was withdrawn and monitored by capillary GLC as in the previous experiment.

**General Preparative Procedure for the Selective Reduction of Organic Disulfides with Lithium Tri-*tert*-butoxyaluminohydride.** The following procedure for the selective reduction of 4-cyanophenyl disulfide is representative. An oven-dried 250-mL flask equipped with a side-arm, a magnetic stirring bar, and a pressure-equalizing graduated addition funnel connected to a mineral oil bubbler was cooled to room temperature under a stream of argon. The flask was charged with 9 g (33.5 mmol) of 4-cyanophenyl disulfide and 17 mL of freshly distilled THF. To this well-stirred slurry, maintained at ca. 25 °C (water bath) was added 56.3 mL (70 mmol) of a 1.25 M solution of lithium tri-*tert*-butoxyaluminohydride in THF (previously transferred to the addition funnel through a double-ended stainless steel needle) over a 15-min period. Vigorous hydrogen evolution was observed in the initial phases of the addition, which then subsided toward the end of addition. The resulting clear mixture was stirred for an additional period of 1 h. Water (3 mL) was added dropwise to destroy the excess hydride. The mixture was acidified by the addition of 6.0 N hydrochloric acid to attain a pH of <3. The organic phase was separated, and the aqueous phase was extracted with 3 × 50-mL portions of ether. The combined extracts were washed with 2 × 150-mL portions of saturated brine and dried ($MgSO_4$). Removal of volatile solvents on a rotary evaporator (ca. 35 °C) followed by vacuum drying yielded 9.1 g (100%) of 4-cyanobenzenethiol: mp 47–49 °C (lit.[18] mp 50–51 °C), >97.5% pure by HPLC; $^1H$ NMR ($CDCl_3$, 300 MHz, ppm) 3.70 (s, 1 H, SH), 7.34 (d, 2 H, aromatic), 7.52 (d, 2 H, aromatic).

# Quantitative Determination of the Ring–Chain Hydrolysis Equilibrium Constant for Anabaseine and Related Tobacco Alkaloids

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Anabaseine (1) (3,4,5,6-tetrahydro-2,3′-bipyridine), a neurotoxin, rapidly hydrolyzes in aqueous solution to its open-chain amino ketone. The constituents over the acidity range pD 2–10 include 1, its conjugate iminium ion acid 2, open-chain keto alkylammonium ion 3, and its conjugate acid 4, a pyridinium–ammonium dication. Three equilibrium constants are required to describe quantitatively the titration curve over this acidity range: the two dissociation constants for acids 2 and 4 as well as the hydrolysis constant $K_H$ for [3]/[2]. Values were established by using proton NMR ($D_2O$) at 22 °C and 0.6 M ionic strength. They are based on computer fitting of chemical shifts and areas. The major forms under physiological conditions and the interval pD 4.5–7.5 are 2 and 3, the latter slightly predominating ($K_H$ 1.2). Similar equilibrium constants were derived from literature data on the alkaloid myosmine (6) (3-(1-pyrrolin-2-yl)pyridine) and its *N*-methyl analogue 7 as well as for 2-phenyl-1-pyrroline 12 and its *N*-methyl analogue 13. Comparison of the p$K_a$ values for the 5- and 6-membered iminium ions shows that the smaller ring is more acidic by at least 1.5 ($\Delta pK_a$), a most surprising conclusion considering that the saturated ammonium ion counterparts have very similar p$K_a$ values.

Anabaseine (1) is a naturally occurring neurotoxin.[1-4] Hoplonemertines, a class of ubiquitous predatory marine worms, produce it to paralyze their prey and to repel potential predators. The toxin is more active than nicotine on cholinergic receptors.[5]

Two rings are present in 1, an aromatic 3-pyridine and a tetrahydropyridine (a 1-piperideine) attached to the former by its 2-position in such a way that the aromatic ring and the imine are in conjunction. The imino group in the tetrahydropyridine ring is water labile,[6] as in many other imines,[7,8] easily hydrolyzing to its acyclic amino ketone 3 via the conjugate acid of 2 of 1, Scheme I.

Interesting questions arise concerning the biological roles of the ring-opened versus the closed forms of 1 and its

† Department of Chemistry.
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(3) Kem, W. R.; Abbott, B. C. *Toxicon* **1971**, *9*, 15.
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(6) For a review of ring-chain tautomerism, see: Valters, R. E.; Flitsch, W. *Ring-Chain Tautomerism*; Plenum: New York, 1985.
(7) Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 832; **1963**, *85*, 2843.
(8) Koehler, K.; Sandstrom, W.; Cordes, E. H. *J. Am. Chem. Soc.* **1964**, *86*, 2413.

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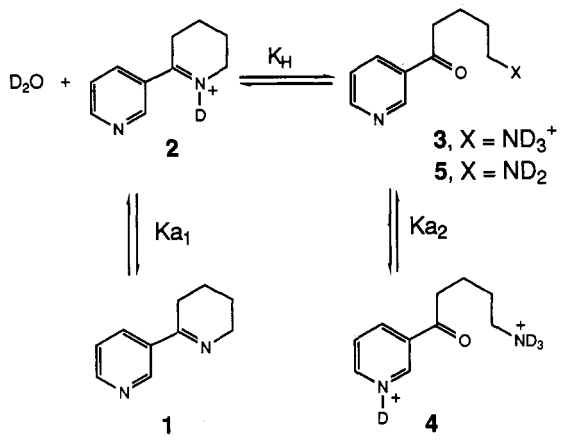
**Scheme I**




related substances. Which of the two forms is neurotoxic? Why is the substance not toxic to the worm? There has been speculation that the worm stores an inactive form and then generates the second active component at the time of injection into its prey.[5]

Prior to attempting to answer such fascinating questions we have investigated the hydrolysis reaction. For the first time we are able to describe quantitatively the various equilibria associated with the hydrolysis and now present our observations and conclusions.

## Results

The hydrolytic equilibrium for anabaseine in $D_2O$ was examined with the aid of proton NMR at 22 ± 1 °C at an ionic strength of 0.6 ± 0.08 M. The hydrolysis reaction was complete in the 5–10 min it took to prepare the sample and record the spectrum. Found were the cyclic imine 1, its conjugate acid 2, and the hydrolysis product, the open-chain amino ketone 3, as well as its conjugate acid 4. The samples were stable; after standing in a refrigerator for 9 months there was no evidence of change in the NMR spectrum. Some yellowing did occur, however. While the tetrahydropyridine ring in 1 may undergo dimerization and cyclic trimerization,[9,10] our solutions evidently were too dilute (0.008–0.018 M) for this to be an important side reaction.

**Assignment of Structures.** Both the high- and low-field portions of the spectra were used to assign structures. The high-field NMR signals were attributed to cyclic and open-chain compounds using the chemical shifts of the methylene protons next to the nitrogen atom. Assignments rest on (a) the relative chemical shifts of these signals and (b) the influence of pD on their positions. For equivalent states of protonation, an $sp^2$-hybridized nitrogen atom is expected to be more deshielding than one that is $sp^3$ hybridized. Moreover, the amino group of the open-chain ketone of 3 is likely to remain largely as its conjugate acid because of its basicity and hence be invariant in shift over the pD range of our studies (2–10). But the position of the signal due to the less basic imine 1 will change as the pD of the medium is varied and the ratio of base and its conjugate acid changes. Therefore, the signal at δ 3.07 ± 0.01, which remained constant, was assigned to the open-chain amino ketone and that which varied from δ 3.72 to 3.89 to the cyclic imine. The ratio of the two forms was easily obtained by integration.

Shifts due to the $CH_2CH_2$ unit of the open and ring-closed forms overlapped and could not be used to determine structures. The protons next to the carbonyl group and adjacent to the imine carbon were replaced by deuterium. For example, at high pD little of these signals remained by the time the first spectrum was obtained. Even an acidic sample (pD 3.64), where isotope exchange was much slower, demonstrated deuterium incorporation over a period of a day at room temperature.

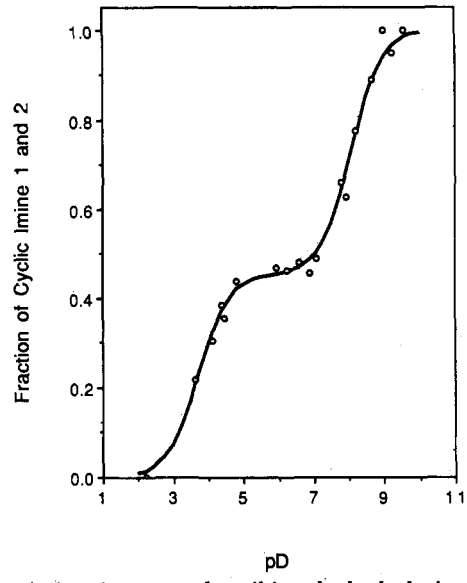



**Figure 1.** A titration curve describing the hydrolysis of anabaseine (1) in $D_2O$ at 22 °C at 0.6 M ionic strength as a function of acidity. The open circles are experimental values expressing the fractional amount of the total reaction mixture existing as 1 and 2. The solid line was calculated with the aid of eq 2 and the values in Table I.

These structural assignments also are supported by a consideration of the chemical shifts at low field associated with the pyridine ring. For example, the signal for H-2 of the pyridine ring of one (imine) component moves to lower field (δ 8.75–8.95) over the pD range 9.57–6.59 and then remains constant. However, H-2 for the other (ketone) component was essentially constant at δ 9.10 from pD 9.57–6.24 and then gradually moved to lower field as the solution was made more acidic, reaching a final value of δ 9.34 at pD 2.20.

This variation in shift of the protons associated with the pyridine ring at high pD confidently can be assigned to the cyclic imine while movement of the signal at low pD can be associated with acyclic ketone. The imine is more basic than the pyridine ring, and its protonation will influence the chemical shifts of the attached pyridine ring. Since the nitrogen atom in the pyridine ring of the ketone is less basic than the imine, it will only undergo protonation and associated deshielding in more acidic media.

Figure 1 graphically reports our findings concerning the influence of solution acidity on the equilibrium composition of anabaseine during hydrolysis. The data are given in terms of the fraction of the total amount of substrate present as cyclic imine rather than as a ratio of two forms. This fraction varies from 0 to 1 and therefore is more easily understood. At high acidity (pD 2) nearly all the substrate is present in the open-chain form as its dication 4 where both the pyridine and the amino groups exist as their conjugate acids. As the solution is made more basic, monocationic ketone 3 is produced along with cyclic iminium ion 2. From about pD 5–7 the composition of the mixture is approximately constant. Then there is a sharp increase in the amount of cyclic imine as the solution is made still more alkaline until finally only cyclic, free imine 1 is present at about pD 9.5.

(9) Blaha, K.; Cervinka, O. *Adv. Heterocycl. Chem.* **1966**, *6*, 147.
(10) Ferles, M.; Pliml, J. *Adv. Heterocycl. Chem.* **1970**, *12*, 43.

Table I. Equilibrium Constants and Chemical Shifts for the Compounds in Scheme I in $D_2O$ at 22 ± 1 °C and 0.6 M Ionic Strength[a]

| $10^8K_{a_1}$ | $10^4K_{a_2}$ | $K_H$ | $\delta_N$ | $\delta_{NH}$ |
|---|---|---|---|---|
| 1.29 (0.51) | | | 3.71 (0.02) | 3.90 (0.01), imine[b] |
| | 1.23 (0.44) | | 9.12 (0.02) | 9.34 (0.01), H-2[b] |
| | 1.64 (0.15) | | 8.50 (0.01) | 9.14 (0.006), H-4[b] |
| | 1.23 (0.21) | | 7.67 (0.03) | 8.26 (0.01), H-5[b] |
| | 1.37 | | | |
| 1.82 (0.25)[c] | 1.03 (0.21)[c] | 1.21 (0.06)[c] | | |
| 15–16[d] | 4.8–5.0[d] | 0.9–1.1[d] | | |

[a] Standard deviation in parentheses. [b] Using eq 1. [c] Using eq 5. [d] Using absorbance changes at 238 mm, 25.0 °C, and 0.15 M ionic strength in $H_2O$. Based on eq 3.

**Calculation of p$K_a$'s from pD-Dependent Chemical Shifts.** Those proton signals that show chemical shift changes with pD provide information about p$K_a$ values for the basic nitrogen sites. The observed chemical shift ($\delta_{obs}$) at any pD then is the population weighted average of the chemical shifts of the unprotonated ($\delta_N$) and protonated ($\delta_{NH}$) forms.

Values of the dissociation constant ($K_a$) for the conjugate acid **2** of the imine nitrogen of **1** ($K_{a_1}$) and the chemical shifts $\delta_N$ and $\delta_{NH}$ were obtained by a nonlinear, iterative regression analysis using eq 1 to fit the observed chemical shifts associated with the signals of the methylene protons $\alpha$ to the nitrogen atom. Values so obtained are recorded in Table I. The fractional amount of free base is given by the expression $K_a/(K_a + [D^+])$ and the fraction of protonated substrate by $[D^+]/(K_a + [D^+])$.

$$\delta_{obs} = \delta_N K_a/(K_a + [D^+]) + \delta_{NH}[D^+]/(K_a + [D^+]) \quad (1)$$

Similarly, the observed chemical shifts of each of the pyridine protons in the open-chain amino ketone is a weighted average of the chemical shifts of the protonated and unprotonated forms. From a consideration of the p$K_a$ values of model compounds such as 2,3′-bipyridine (p$K_a$'s 1.5, 4.4[11]) we assume that protonation of the pyridine ring conjugated with the positively charged iminium ion will not be important over the pD range where the pyridine ring of the ketone is protonated. Therefore, the dissociation constant of the pyridinium ion in the cyclic imine was neglected, and the changes in the chemical shifts of the pyridine protons at low pD were assigned to the open-chain ketone **4**. Its $K_{a_2}$ was calculated by the nonlinear regression technique using the data for the H2, H4, and H5 protons separately and eq 1. The overlapping values are given in Table I and are within the usual agreement found for an NMR method.[12]

While our assumption concerning the absence of protonation of the pyridine ring of the cyclic imine is not strictly correct, little error should be introduced into our value of $K_a$ for the pyridine ring of ketone because the degree of such protonation of the less basic ring is likely to be small. Moreover, our data at very low pD are sparse, and attempts to include the additional $K_a$ value into the fit by our computer only gave absurd values for this additional $K_a$ and the same values for the other constants. Our p$K_a$ value of 3.36 (3.86 − 0.50) corrected for the solvent isotope effect (0.50) is not unlike that for 3-acetylpyridine (p$K_a$ 3.2, $H_2O$[13]), a reasonable model.

**The pD–Hydrolysis Profile and the Hydrolysis Equilibrium Constant $K_H$.** That the system is rapidly reversible and hence at equilibrium was demonstrated by taking a reaction mixture, changing its pD, and redetermining the substrate ratio. Thus, following observation of the hydrolysis ratio of a sample having pD 4.37, the pD was increased to 9.22 by the addition of carbonate ion, and the new ratio of open-chain to cyclic substrate was determined 2 h later. All the substrate now existed as cyclic imine as was found for a fresh sample of similar pD made directly from anabaseine. Similarly, a change was made immediately in the opposite direction; an alkaline sample was acidified from pD 8.20 to 4.82. Again the ratio was the same as that obtained on a fresh sample of similar pD.

The hydrolysis equilibrium is defined by the reaction $2 + D_2O = 3$ where $K_H = [3]/[2]$. However, NMR area measurements provide the total amounts of each of the two components, i.e., (3 + 4) and (1 + 2), and not just **3** and **2** alone. Therefore, the sum must be corrected for the amount of the unwanted component to obtain the value for $K_H$. This is accomplished easily by eq 2 with the aid of the appropriate $K_a$ values along with the concentration of acid obtained from pD measurements.

$$K_H = \frac{[\text{open-chain}]_{tot}}{[\text{ring-closed}]_{tot}} \cdot \frac{K_{a_2}/([D^+] + K_{a_2})}{[D^+]/([D^+] + K_{a_1})} \quad (2)$$

Equation 2 was solved with the aid of a microcomputer program using nonlinear regression analysis. Prior to computer fitting the equation was rewritten to express the fractional amount of total substrate present as the cyclic imine (free base **1** and its conjugate acid **2**) rather than as the ratio of [3]/[2]. The former fraction ranges in value from 0 to 1 whereas the other tends to a very large value at low pD owing to the small amount of **2**, thereby causing computational problems. This new equation, eq 5, is given in the Experimental Section. From the data in Figure 1 it was possible to obtain the three relevant equilibrium constants $K_{a_1}$, $K_{a_2}$, and $K_H$ to describe quantitatively the changes in the concentrations of the four species **1**–**4** as a function of pD. Table I lists the values.

Worthwhile is a comparison of the $K_a$ values obtained by using the variation in chemical shifts, eq 1, and by the overall fitting technique based on eqs 2 and 5. There is satisfactory agreement, Table I. The values for $K_{a_1}$ overlap within one standard deviation for the two approaches while the value for $K_{a_2}$ derived from a consideration of all the data in Figure 1 is 25% smaller than the average found by the chemical-shift method, reasonable agreement for NMR analysis. Our results are self-consistent.

The value of 1.21 for $K_H$ shows that almost equal amounts of the two mono cationic species **2** and **3** are present with the open-chain ketone being favored slightly.

**Ultraviolet Spectra.** Another approach using ultraviolet absorption spectra was taken to examine the hydrolysis reaction. Spectral changes were used to follow the reaction, this time in $H_2O$ at 25 °C and at a lower salt concentration, 0.15 M. Again spectra were taken as a function of solution acidity, and 238 nm was selected as the analytical wavelength because of the large and complex absorbance changes. Figure 2 shows how the absorption varies with acidity over the pH interval 1–12.

Attempts to obtain equilibrium constants were more difficult and unsatisfactory because unlike the NMR method individual species could not be observed. Our nonlinear regression program was employed to estimate constants from eq 3 where [1–4] denotes the total amount

(11) Krumholz, P. *J. Am. Chem. Soc.* **1951**, *73*, 3487.
(12) Sergeyev, N. M. *Org. Magn. Reson.* **1978**, *11*, 127.
(13) Hall, N. F.; Sprinkle, M. R. *J. Am. Chem. Soc.* **1932**, *54*, 3469.

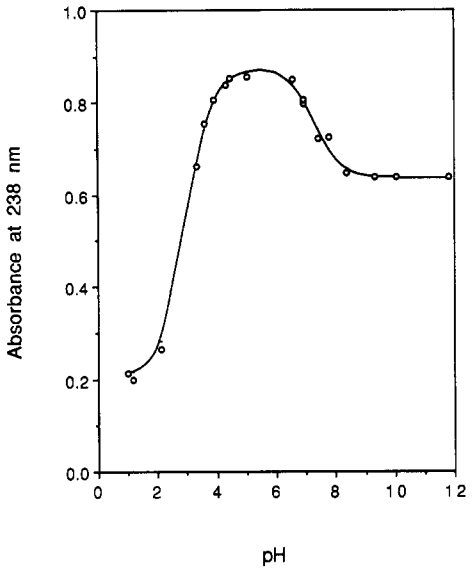



**Figure 2.** A titration curve for 1 according to Scheme I based on ultraviolet absorption data collected at 238 nm and 25 °C using $H_2O$ at 0.15 M ionic strength. The open circles are experimental absorbances and the line was calculated using eq 3 and 4 and the constants in Table I.

of substrate, Scheme I, $\epsilon$ represents the molar absorptivity, and $F$ indicates the fraction of the total amount of substrate present in solution as one of the four forms. Equation 4 serves as an example of how this fraction may be expressed in terms of the appropriate equilibrium constants and the $[H^+]$. The fractional amount of the

$$ABS_{obs} = (\epsilon_1 F_1 + \epsilon_2 F_2 + \epsilon_3 F_3 + \epsilon_4 F_4)\cdot[1\text{–}4] \quad (3)$$

$$F_1 = \frac{K_{a_1}K_{a_2}}{K_{a_1}K_{a_2} + K_{a_2}[H^+] + K_H K_{a_2}[H^+] + K_H[H^+]^2} \quad (4)$$

total substrate present as 1 is indicated. The other fractions may be written by taking the same denominator and replacing the numerator with one of the three remaining terms in the denominator. Thus, the second term when placed in the numerator gives $F_2$, the third $F_3$, and the fourth $F_4$. Additional unknowns, the molar absorptivities or $\epsilon$ values, are not present in the NMR equation but are found in eq 3. Estimates of these were made as follows. The $\epsilon$ value at high pH was assumed to be due to free imine 1, that at low pH (1.1) dication 4. The NMR ratios near neutrality where both 2 and 3 exist almost exclusively were considered in an attempt to dissect the observed absorbance and obtain estimated $\epsilon$ values for 2 and 3. A unique fit to eq 4 was not found because of the uncertainty in these values. For example, when the initial estimates for 2 and 3 were varied by about a factor of two from those reported in the Experimental Section, the value of $K_H$ had a very large uncertainty and so these estimates were rejected. The final values seem to be reasonable. The points in Figure 2 are the experimental values and the line is that calculated by the equilibrium constants given in Table I, the molar absorptivities in the Experimental Section, and eq 3, showing a satisfactory fit.

The $pK_a$ values derived from samples in $D_2O$ and in $H_2O$, Table I, differ by 0.5–1. Neglecting the small variation in temperature between our two studies, $pK_a$ values for pyridinium ions in heavy water are expected to be some 0.4–0.5 higher,[14] reflecting the greater acidity of $D_3O^+$ over $H_3O^+$. The difference in the ionic strength (0.6 vs 0.15 M)

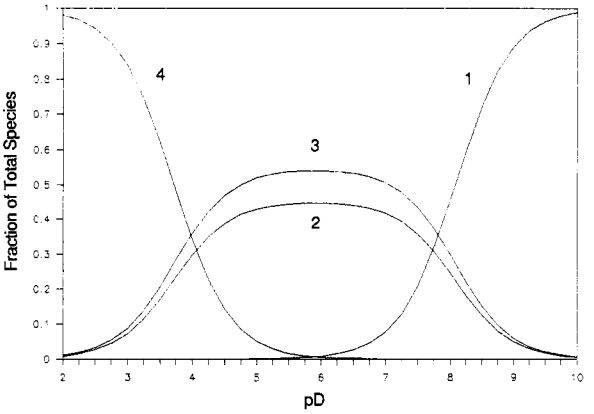



**Figure 3.** A plot showing how each of the four components of the anabaseine equilibrium given in Scheme I varies with pD.

**Table II. Equilibrium Constants Obtained from Analysis of Literature Data for the Hydrolysis of Cyclic Imines**[a]

| compd | $K_{a_1}$ | $K_{a_2}$ | $K_{a_3}$ | $K_H$ | ref |
|---|---|---|---|---|---|
| 6 | $1.20 \times 10^{-6}$ | $2.66 \times 10^{-4}$ | | 1.89 | 20 |
| 7 | | $2.42 \times 10^{-4}$ | $1.00 \times 10^{-10}$ | 0.88 | 21 |
| 12 | | | | 0.18 | 20 |
| 13 | | | $4.95 \times 10^{-11}$ | 0.085 | 21 |

[a] $K_{a_1}$ and $K_{a_2}$ as defined in the Schemes. $K_{a_3}$ refers to the dissociation constant for acyclic, protonated amine.

also leads to a small reduction in $pK_a$ value. The main result of the spectrophotometric analysis is confirmation of the value of $K_H$. A large displacement in the position of the titration curve along the axis expressing solution acidity for a related substance, myosmine[15] (6), has also been reported when the solvent was changed from heavy to light water and is considered below.

## Discussion

**Anabaseine.** The rapidly equilibrating set of four components, Scheme I, resulting from the hydrolysis of anabaseine is described quantitatively for the first time. The titration curve, Figure 1, obtained for this complex mixture may be expressed in another way, Figure 3, that shows how the concentrations of each one of the four components varies with solution acidity. Figure 3 was constructed using $[D^+]$ and fractions defined by eq 4 and its counterparts where the numerator for each fraction changes as explained in the Results. Open-chain dication 4 predominates at low pD, and cyclic free base 1 is the major component at high pD. Over a wide acidity range near neutrality and under physiological conditions the two mono cations 2 and 3 are essentially the only two materials present in solution, acyclic ketone slightly predominating. At pD 7 for example, there is 8% of 1, 42% of 2, and 50% of 3. This composition will be different in $H_2O$. Making the reasonable assumptions that $K_H$ will not change significantly but that the two relevant $pK_a$ values will decrease by 0.5 due to the solvent isotope effect gives rise to a new equilibrium distribution: 1, 21%, 2, 36%, and 3, 43%. There is a major increase in the amount of 1 on going to $H_2O$.

The $K_H$ term describing the hydrolysis equilibrium in terms of the two mono cationic constituents may be written in an alternate form, as an equilibrium between the cyclic free base 1 and the open-chain free base 5, where 5 is the conjugate base of acid 3. This new constant is given by the expression $K_h = (K_H K_{a_3})/K_{a_1}$ where $K_{a_3}$ equals

(14) Bellobono, I. R.; Beltrame, P. *J. Chem. Soc. B* **1969**, 620. Bellobono, I. R.; Diani, E. *Ibid.* **1972**, 1707.

(15) Myosmine is also known as 3-(3,4-dihydro-2*H*-pyrrol-5-yl)-pyridine, 3-(1-pyrrolin-2-yl)pyridine, and as 2-(3-pyridyl)-1-pyrroline.

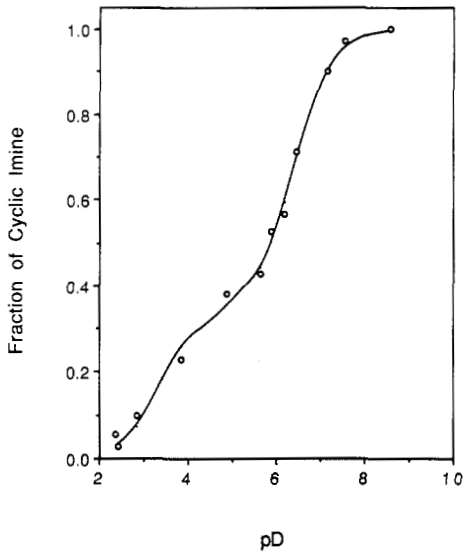



**Figure 4.** A titration curve for the hydrolysis of mysomine (**6**) in $D_2O$ as described in Scheme II. The open circles represent the fraction of imine and its conjugate acid taken from ref 20; the line was calculated as given in Figure 1.

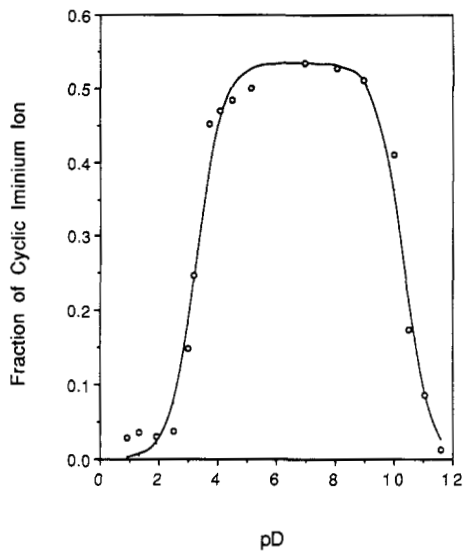



**Figure 5.** A titration curve for *N*-methylmyosmine **7**, R = $CH_3$. The values given by the open circles were taken from ref 21, and the line was calculated as indicated by the approach in Figure 1.

$[D^+][5]/[3]$.[16] Using 10.5 as an estimate of $pK_{a_3}$, Table II, the value for the alkylammonium ion, provides a value of $3 \times 10^{-3}$ for $K_h$, indicating that only 0.3% of **5** exists in the presence of **1** at high pD. Although $K_h$ expresses the hydrolytic equilibrium in terms of neutral rather than charged substrates, we prefer $K_H$. As Figure 3 shows, $K_H$ pertains to the predominant constituents of the equilibrium under most acidity conditions whereas $K_h$ does not.

A $pK_a$ value of 6.7 already has been reported for anabaseine based on a titration with aqueous HCl in $H_2O$ containing 5% methylcellosolve at 25 °C.[17] Our work shows that this value cannot be for the pure substance but rather for the mixture given in Scheme I. That is, during the titration hydrolysis of **1** occurs and this produces the more basic open-chain, aliphatic amine **5**. And so the $K_a$ value is skewed to include the more basic substance that will be present as its conjugate acid **3**. The reported dissociation constant is $K_{app} = [H^+][1]/([2] + [3])$ and not $K_{a_1}$ as desired.[16] This apparent dissociation constant is related to the true dissociation constant $K_{a_1}$ as follows: $K_{app} = K_{a_1}/(1 + K_H)$ which on a logarithmic scale is $pK_{app} = pK_{a_1} + 0.34$, including the logarithmic value for $(1 + K_H)$. In this case the correction for the presence of the amine component is not very large. Moreover, this analysis is supported by our titration curve, Figure 1, where careful examination of the inflection point at high pD shows that this point does not have the value for $pK_{a_1}$ but rather for $pK_{app}$ as expected. Correcting our $pK_a$ value of 7.8 for solvent isotope and salt effects by as much as 0.7 gives a value of 7.1 for $H_2O$. This is only in fair agreement with the corrected value of 6.4 obtained by titration.

**Myosmine and Analogues.** Encouraged by our success with anabaseine we elected to examine the titration curves carefully constructed with the aid of NMR data for the hydrolysis of substances structurally quite close: the tobacco alkaloid myosmine[18-20] **6** and its cationic N-methylated derivative **7**,[21] substrates having the 5-membered 1-pyrroline rather than the 6-membered 1-piperideine ring of **1**, Scheme II. Figures 4 and 5 show how the composition of the hydrolysis reaction mixtures for these substances changes with pD. The published data for **6**[20] and **7**[21] had not been subjected to a computer analysis, and so equilibrium constants describing the titration curve had not been defined. Our analysis allows these values to be extracted from the data for the first time. Again the points in the figures are experimental values, estimated from the published figures, and the solid line is that given by the equilibrium constants in Table II. The fit again is satisfactory.

Because of the expected interest in the heretofore undescribed variation in the composition of reaction mixtures of **6** by those engaged in pharmacological studies, a plot is given in Figure 6 showing how the fractional amounts of its four components change with pH. These curves were constructed using the equilibrium constants in Table II after making a reduction of 0.5 in $pK_a$ values to reflect the change from $D_2O$ to $H_2O$. Equations such as that given by 4 describe how the fractional amount of a given component varies with pH.

The titration curve for **7** is different in shape from those in **1** and **6** in that at high pD values the uncharged open-chain amino ketone predominates rather than neutral, cyclic compound as in the cases of **1** and **6**. However, with **7** the cyclic form is constrained to be a cation due to quaternization instead of protonation of the nitrogen atom and so the cyclic iminium ion hydrolyzes to the amino ketone at high pD. Because of the presence of large amounts of this open-chain material at high pD, Scheme II does not describe correctly the hydrolysis of **7**. $K_{a_1}$ is not relevant and $K_{a_3}$ must be added to reflect conversion of the open-chain alkylammonium ion to its conjugate base. Hence, eq 2 must be modified; the concentration ratio of open-chain to cyclic substrate must now be multiplied by

(16) Careful consideration of the relevant equilibria lead to a more complex equation. At high pD, $K_{app} = [D^+]([1] + [5])/([2] + [3])$ that may be transformed to $K_{app} = K_{a_1}(1/(1 + K_H)) + K_{a_3}(K_H/(1 + K_H))$. The latter equation has the same form as that of NMR eq 1; in both cases the observed value is a population weighted average of two constants, in one case the limiting chemical shifts and in the other the $K_a$ values. Because the amount of **5** is so small under our conditions, the second term of the $K_{app}$ equation may confidently be neglected.

(17) Yamamoto, I.; Kamimura, H.; Yamamoto, R.; Sakai, S.; Goda, M. *Agr. Biol. Chem.* **1962**, *26*, 709.

(18) The open-chain hydrolysis product of myosmine is named poikiline.[19]

(19) Haines, P. G.; Eisner, A.; Woodward, C. F. *J. Am. Chem. Soc.* **1945**, *67*, 1258.

(20) Brandänge, S.; Rodriguez, B. *Acta Chem. Scand. B* **1983**, *37*, 643.

(21) Brandänge, S.; Lindblom, L.; Pilotti, A.; Rodriguez, B. *Acta Chem. Scand. B* **1983**, *37*, 617.

the fraction $[D^+]K_{a_2}/([D^+]^2 + [D^+]K_{a_2} + K_{a_2}K_{a_3})$ instead of the fraction given therein to reflect the amount of open-chain material present as the monocation. This consideration produces a satisfactory fit.

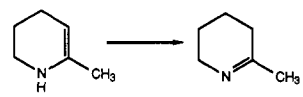
The hydrolysis profile for **7** could be made even more complex because the cyclic iminium ion is the conjugate acid of an enamine and could be deprotonated to the enamine at high pD values.[22] However, after a careful search the enamine was not detected[21] and so it was not included in our scheme. Redefining $K_{a_3}$ as a dissociation constant to reflect the formation of enamine conjugate base with the same value for $K_{a_3}$ rather than to yield acyclic amine base would have no influence on the shape of the titration curve.

In the most acidic solutions for **7** there are some deviating points (Figure 5). It is not clear whether these points reflect the incursion of a new conjugate acid of substrate or simply represent NMR errors in estimating small amounts of minor component.

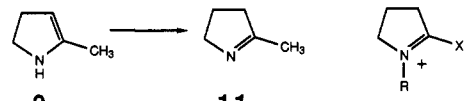
The titration curve for **6** is similar in shape to that of **1** and their $K_H$ values also are alike, 1.9 vs 1.2, respectively. However, comparison of the $K_{a_1}$ values for the two substances produces a major surprise. The $pK_a$ value of 5.9 for the smaller ring is very much less than the value of 7.7 for the larger ring. This lower value serves to change markedly the populations of the various components. For example at pD 7 there is 80% of **6**, 7% of its conjugate acid, and 13% of monoprotonated, ring-opened amino ketone. In $H_2O$ these values are likely to be 94%, 2%, and 4%, respectively (Figure 6). Much more of the cyclic free base is present compared with **1** under the same conditions.

**Iminium Ion Acidities.** The remarkably large difference in $pK_a$ values for the 5- and 6-membered iminium ions stands in marked contrast to the insignificant difference in p*Ka* values for the corresponding saturated, secondary amines, pyrrolidine[23] and piperidine.[24] This large variation certainly is not due to the different ionic strengths used in the two studies, the former not being reported but is likely to be less than our own.

Two other reports support our claim that the $pK_a$ values of 5- and 6-membered iminium ion acids are very different. (1) A large difference in $pK_a$ values ($H_2O$) had been reported much earlier for anabaseine (6.7) and myosmine (5.5) from classical titrations.[17] (2) Another old study on similar, simpler structures also has gone largely unrecognized, possibly because the initial structures were assigned their incorrect tautomeric forms. We know today that enamines of secondary amines really exist as imines so that the corrected forms of **8** and **9** are the tautomers[25,26] **10** and **11**, respectively. The $pK_a$ value for the conjugate acid of **10** is 9.55[27] and for **11** it is 7.91.[28] Again there is a large difference and again the acid with the 5-membered ring is stronger. The early workers recognized but denied the possibility of hydrolysis during their titrations of **10** and **11**. Considering the effect of the 2-methyl group on the value of $K_H$ given below, this assumption may be largely correct. Even if the reported $pK_a$ values are not for a pure substance but are for an equilibrium mixture that is somewhat skewed by the presence of more basic amine,



**8** **10**



**9** **11**

**12**, R=H, X=$C_6H_5$
**13**, R=$CH_3$, X=$C_6H_5$

the difference is real and highly significant. Therefore, the amount by which the reported $pK_a$ ($pK_{app}$) differs from the true value must be about the same for both the conjugate acids of **10** and **11**, since $pK_{app} = pK_a + \log(1 + K_H)$.

We suggest that the greater acidity of the iminium ion with the 5-membered ring over that of the 6-membered ring is largely due to the difference in energy between the free bases. The greater s-orbital character of the lone pair associated with the smaller interior bond angle of the 5-membered ring provides a larger stabilization, thereby making the nitrogen atom less basic. While the direction of the stabilization is expected, the large magnitude is a surprise and raises the question of whether other cyclic imines of varying ring size show similar hybridization effects.

Two other reports concerning the equilibrium hydrolysis of 1-pyrrolines are of interest because they provide additional insight into the influence of structure on the value of $K_H$. Compounds **12** and **13** have a phenyl substituent at position 2, the latter also an *N*-methyl group. The titration curve for **13** resembles that for **7** at high pD in that open-chain material is favored while acidic solutions show no variation in composition because of the lack of a second basic group, the pyridine ring.[21] Again, we were able to fit the reported titration curve for **13** (not shown) with our computer, equilibrium constants being listed in Table II. Although an enamine equilibrium could be incorporated into the hydrolysis model, we find this to be unnecessary. The observed inflection point ($pK_{app}$) of 11.4 in the reported titration curve is nicely reproduced by considering only the $pK_a$ of the open-chain amine and $K_H$, i.e., $pK_{app} = pK_{a_3} - \log(K_H/(K_H + 1)) = 11.4$ where $pK_{a_3} = 10.3$ and $K_H = 0.085$. Moreover, even if the situation is made more complex by the inclusion of a $pK_a$ value for an enamine (12.7), both $pK_{a_3}$ and $K_H$ are essentially unchanged, now being 10.2 and 0.083, respectively.

The titration curve was not presented for **12** but from the reported equilibrium composition[20] we were able to estimate a value for $K_H$, Table II.

Consideration of all the $K_H$ values, Tables I and II, for the cyclic imines as a function of substituents suggests a trend. As the group at the imino carbon atom is made less electron-withdrawing in the order 3-pyridyl and phenyl there is less hydrolysis. That is, cyclic imine is favored more as the substituent is made more electron-donating, the same kind of electronic effect as found earlier.[21] Similarly, addition of a methyl substituent to the imine nitrogen atom causes the cyclic form to increase in abundance. These limited data should be considered with caution, but they suggest that in the absence of steric effects electron-donating groups preferentially stabilize the protonated iminium ion more than the open-chain ketone. But the combined effect of both changes is modest, being only a factor of 22. This conclusion also suggests that the old $pK_a$ values reported for the conjugate acids of **10**[27] and **11**[28] may be skewed only a little by the presence of open-

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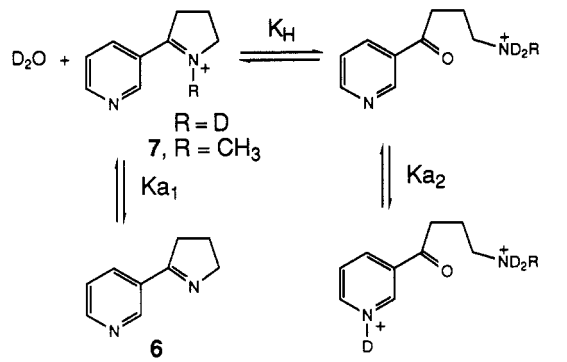
(28) Starr, D. F.; Bulbrook, H.; Hixon, R. M. *J. Am. Chem. Soc.* **1932**, *54*, 3971.

**Scheme II**



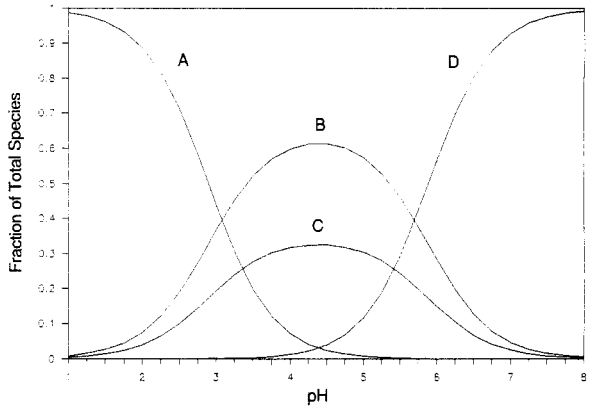




**Figure 6.** Calculated changes in the composition of hydrolysis reaction mixtures of **6**, Scheme II, as a function of pH. Prior to using the data in Table II, p$K_a$ values were decreased by 0.5 to correct for the solvent isotope effect. Regions A and B refer to open-chain hydrolysis products, di- and monocations, respectively, while C is **7** (R = H) and D is **6**.

chain hydrolysis product because only a little may be present.

## Conclusions

The composition of aqueous solutions of **1** and **6** may be described quantitatively for the first time. The state of protonation as well as the degree of conversion to their open-chain constituents is presented in Figures 3 and 6 as a function of acidity. These findings make it possible to develop a strategy to ascertain the active form responsible for the pharmacological action of **1** and **6**.

By varying the pH of physiological experiments from about 6 to 8 the following predictions may be made with the important assumptions that the receptor site is largely aqueous, that pH changes in solution are mirrored at the receptor site, and that variations in receptor properties due to pH may be corrected by comparison with, say, carbamylcholine. The concentration of anabaseine free base over this interval will increase by a large factor of about 30 while the concentrations of its monocationic cyclic and acyclic ions will decrease by a modest factor of approximately 3. For myosmine over this same pH interval the cyclic free base concentration will increase only by about 2-fold and the concentrations of the cations will fall by about a large factor of 50. Fortunately, the observations for these two substances reinforce each other because there is a major change in composition of different species as the pH is varied, the neutral form of anabaseine and the monoprotonated entities for myosmine. It therefore should be possible from the increase or decrease in bioactivity to state whether it is the neutral or cationic form that is responsible for promoting activity at the receptor site. However, because both the amounts and pH dependence of the monocations are so similar, it will be necessary to employ noninterconverting model compounds to distinguish between them.[29,30] In this way it should be possible for the first time to identify both the structure and the state of protonation of these interesting, old neurotoxins when bound to the active site.[31,32]

## Experimental Section

Anabaseine·2HClO$_4$·$^1/_2$H$_2$O had mp 106–109 °C dec. Anal. Calcd for $C_{10}H_{15}N_2Cl_2O_{85}$: C, 32.45; H, 4.08; N, 7.57. Found: C, 32.67; H, 4.29; N, 7.45. The material was isolated as the picrate as originally reported[33] and then converted to the perchlorate.

**Buffers.** Solutions for NMR measurements were prepared by dissolving the buffer salts in a solution of 0.5 M NaCl and 0.002 M TSP (sodium 3-(trimethylsilyl)propionate) in 99.8% $D_2O$. Phosphate and carbonate buffers ranged in ionic strength from 0.52 to 0.68 M. Measurements of pH were made using Bates' buffers for standardization.[34] The pH meter readings were converted to pD by adding 0.40.[35]

Solutions for UV measurements were prepared by making 0.01 M solutions of the acidic buffer component and adding KCl to make an ionic strength of 0.15 M. Acidic buffer was mixed with an appropriate volume of 0.01 M NaOH and 0.14 M KCl solution to bring the buffer to the desired pH. Glass-distilled water was used. Buffers include formate, acetate, MES, HEPES, bicine, and carbonate ion.

**$^1$H NMR Measurements.** Spectra were recorded using a Varian VXR 300 instrument at ambient temperature (usually 21–23 °C). Samples were prepared by dissolving 2–4 mg of anabaseine in 0.4–0.6 mL of the appropriate buffer; concentrations ranged from 0.008 to 0.018 M. A pD value was obtained by measuring the pH and adding 0.40 for each sample after recording the spectrum. The ratios of the areas of the methylene protons $\alpha$ to the nitrogen atom in the open chain form and the methylene protons $\alpha$ to the imine nitrogen in the cyclic form were obtained by integration. A delay time of 5 s between transients was used. This is 2.9 and 3.3 times the two $T_1$'s of interest. Use of a delay of 10 s which is 5.7 and 6.5 times the two $T_1$'s of interest did not have a significant effect (±2%) on the integral ratios so the shorter delay was used. Each spectrum was the result of 64 transients. The methyl signal of TSP served as an internal shift standard. Equation 2 was transformed to eq 5 prior to fitting the data by the computer. The fraction of cyclic imine is given by ([1] + [2])/([1] + [2] + [3] + [4]).

fraction of cyclic imine =

$$\frac{K_2(K_1 + [D^+])}{K_2(K_1 + [D^+]) + K_H[D^+](K_2 + [D^+])} \quad (5)$$

**UV Measurements.** A Perkin-Elmer 330 spectrophotometer was used. A 3-mL aliquot of each buffer was equilibrated in the spectrophotometer at 25 °C for 15 min before adding 100 $\mu$L of 3.57 mM anabaseine in 0.15 M KCl. A wavelength scan from 220 to 320 nm was recorded for each sample. The following molar absorbtivities were used with eq 3: **1**, 5570; **2**, 10 000–10 500; **3**, 5000–5200; **4**, 1790.

**Computer Program.** The program written by Dr. D. Whitman does a nonlinear least-squares analysis to fit parameters to observed data and the equations given in the text. It is based on the program of Wentworth.[36]

**Acknowledgment.** This work was kindly supported in part by a grant from Taiho Pharmaceutical Company, Ltd., Tokyo, Japan.

(29) Previous analyses of nicotine action upon the neuromuscular acetylcholine receptor as a function of the external pH have provided strong evidence that the monocationic species is much more active than the neutral form.[30]

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