BIOORGANIC CHEMISTRY 18, 85–90 (1990)

The Apparent pK_a of the Oxidation Product of Spermine or Spermidine: A pK_a Masked by a Ring-Chain Tautomeric Equilibrium

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Received October 5, 1989

The previously reported acid titration curve (S. Brandänge, L.-H. Eriksson, and B. Rodriguez, *Acta Chem. Scand.*, 1984, **B38,** 526) for the aminal oxidation product of the title compounds in D_2O is computer simulated based on a model consisting of a ring-chain tautomeric equilibrium (K_T) for the monodeuteronated bicyclic aminal followed by a second deuteronation of the resultant monocyclic primary amine (K_{a1}) to give a dication. The fractional amount of dicationic acid product is given by the term $[D^+]/([D^+] + K_{app})$ where K_{app}, the apparent dissociation constant, equals 5.47×10^{-6}, the product of K_T and K_{a1}.

Spermine[2] and spermidine[3] are two of several aliphatic polyamines important in the control of proliferative processes (*1*). They may be oxidatively degraded either chemically or enzymatically (*2*). The structure of the product from the enzymatic reaction depends on both the nature of the polyamine and the enzyme (*3*), in some instances being the same as that from the nonenzymatic reaction (*2*). One of these products has a structure that has been controversial. It formally arises from the cyclization of a diamino aldehyde. The early assignment of a monocyclic 2-pyrroline or enamine structure **1** (see Scheme 1) provides a compound with properties that are not entirely consistent with some of those reported for the oxidation product and so **2** was proposed (*4*). The latter is a racemic, bicyclic aminal having been formed by the spontaneous cyclization of the same diamino aldehyde precursor. More recently, a family of rapidly interconverting structures including **2**,[4] **3**, and **4** has been suggested for the oxidized material based on a careful consideration of ^1H and ^{13}C NMR spectra of a synthetic sample in a series of aqueous solutions of varying acidity (*5*). Bicyclic ion **3** consists of two rapidly interconverting conjugate acids of secondary **3a** and tertiary **3b** amines. Its monocyclic conjugate acid **4** is a 3-ammoniopropyl-1-pyrrolinium dication.

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[2] *N,N'*-Bis(3-aminopropyl)-1,4-butanediamine.

[3] *N*-(3-Aminopropyl)-1,4-butanediamine.

[4] Octahydropyrrolo[1,2-*a*]pyrimidine or 1,5-diazabicyclo[4.3.0]nonane.

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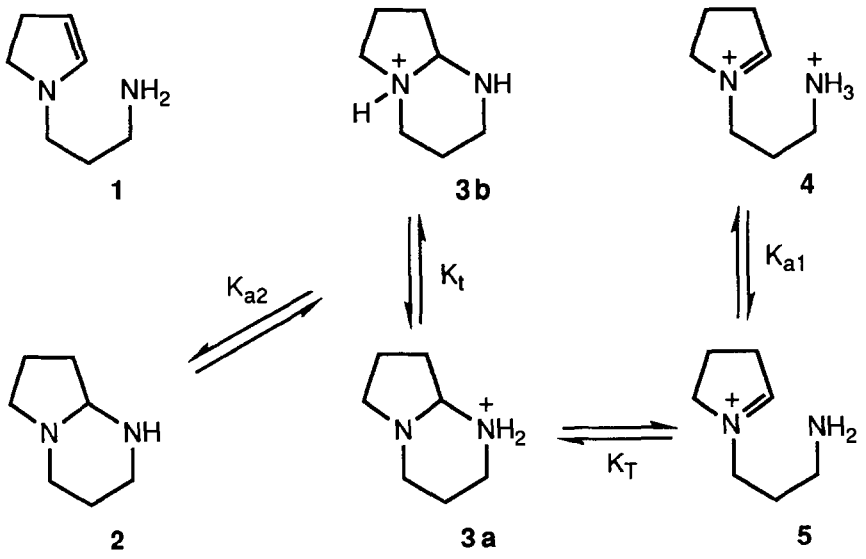

$$F = \frac{[4]+[5]}{[3]+[4]+[5]} = \frac{[D^+]+K_{a1}}{[D^+]+K_{a1}+K_{a1}K_T(1+K_t)} \qquad [1]$$

$$F \sim \frac{[D^+]}{[D^+]+K_{app}} \qquad [2]$$

$$F = \frac{[D^+]K_{a1}+[D^+]^2}{[D^+]K_{a1}+[D^+]^2+[D^+]K_{a1}K_T(1+K_t)+K_{a1}K_{a2}K_T(1+K_t)} \qquad [3]$$

$$F \sim \frac{[D^+]^2}{[D^+]^2+[D^+]K_{1app}+K_{1app}K_{a2}} \qquad [4]$$

SCHEME 1

Enamine **1** must be only one of several rapidly interconverting structures, its presence being inferred by the observation of hydrogen–deuterium exchange at the β position of the enamine (*5*). The minor contribution of **1** also is consistent with the high basicity of enamines (*6*); it is expected to be an unimportant contributing structure except in highly alkaline solutions.

A titration curve based on proton chemical shifts of the oxidation product dissolved in D_2O was reported and the data points are reproduced in Fig. 1.

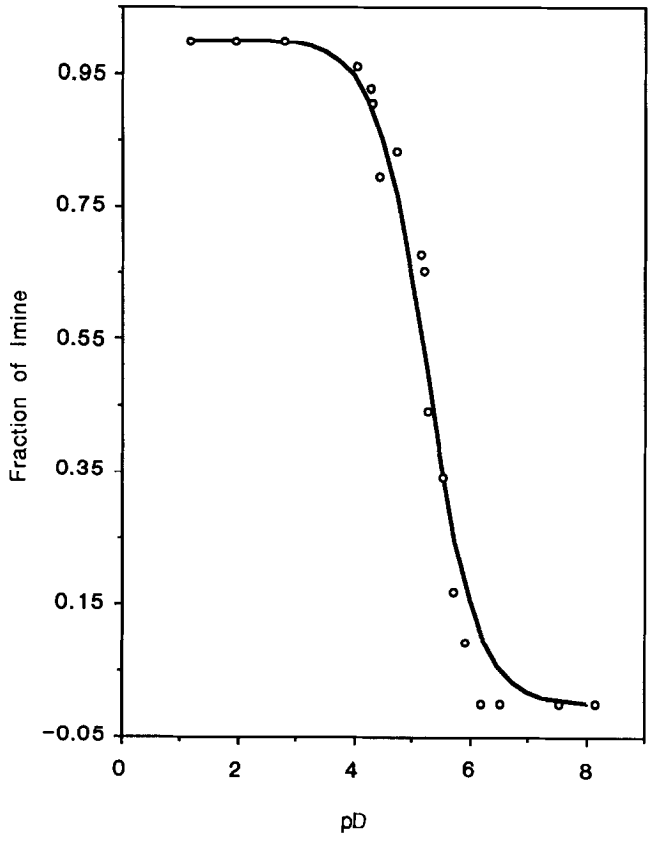

FIG. 1. Titration curve for the oxidation product of spermine and spermidine (D_2O). The circles are taken from Ref. (*5*) while the solid line is calculated using Eq. [2] and a K_{app} value of 5.47×10^{-6} (pK_a 5.26).

According to this report bicyclic **2** converts to monocyclic **4** on acidification (*5*).[5] Taking the pD value at the midpoint of this curve as being equal to a pK_a value provides an apparent pK_a (pK_{app}) of 5.2. Is this value consistent with the structural assignments or does it invalidate them?

On first consideration this would seem to be a pK_a value for the mixture of secondary and tertiary amino groups in the conjugate acid of **3**, a dication. Comparison of this pK_a value with that of a model compound initially seems to be consistent.

Aminals are hydrolytically labile (*7*) and so we make comparison not with a desired gem-diamine as in **3** but rather with a vic-diamine, as its dicationic acid, e.g., the diprotonated form of 1,2-diaminoethane which has as its first pK_a (H_2O) a value of 7.4 (*8*). The diprotonated form of animal **3** would be expected to have a

[5] In D_2O solvent all the NH bonds in the structures are replaced by ND bonds.

pK_a value much lower than this owing to the larger acidifying effect of the more closely situated ammonio group (*7, 9*) in apparent agreement with the assignment.

This superficial analysis is not correct, however. The reported titration curve not only applies to a deuteronation[6] step but also to a ring cleavage reaction, likely to be a ring-chain tautomeric equilibrium between aminal **3a** deuteronated at its secondary amino group and its monocyclic aminoalkyl iminium ion **5.** This equilibrium provides a major perturbation on the true pK_a value.

Our first analysis starts with the pair of equilibrating bicyclic monodeuteronated cations **3a** and **3b,** said to be the major forms in neutral water (*5*). They are in equilibrium with monocyclic, tautomeric monocation **5.** This amine, expected to be more basic than the aminal because it resembles a simple aliphatic amine with a remotely situated electron-withdrawing group, accepts the deuteron, trapping the substrate in its monocyclic form to give the observed product **4.** According to this model the fraction (F) of the total amount of substrate present as imine is given by Eq. [1] which assumes that in the acidity interval in question there are only three significant forms present, **3, 4,** and **5** (*7*). K_{a1} is the dissociation constant for **4** going to **5** while K_T denotes the ratio of ring-chain tautomers (*11*) [**3a**]/[**5**] and K_t gives the ratio of acids [**3b**]/[**3a**]. Because K_{a1} is small relative to the concentration of acid [D^+] in Eq. [1] it may be neglected as indicated in Eq. [2]. Moreover, this reduced fraction has another form where the term $K_{a1}K_T(1 + K_t)$ is a constant equal to K_{app}, Eq. [2], the apparent K_a value as given by the half-titration point.

We have been able to fit the experimental data as taken from the reported titration curve (*5*) using a nonlinear regression microcomputer program and Eq. [2]. The fit shown by the solid line in Fig. 1 is satisfactory except that our calculated values for the fraction of imine appear to be slightly too large at a pD of about 6. If this analysis is accepted, then the K_{app} value of 5.47×10^{-6} (pK_a 5.26) with a standard deviation of 0.56×10^{-6} may be converted to its two associated constants using an estimate of 9.68 for pK_{a1} (*12*) from a model, the dideuteronated form of 1,3-diaminopropane.[7] The value for $K_T(1 + K_t)$ becomes 2.6×10^4. Since tertiary alkylammonium ions are likely to be slightly stronger acids than their corresponding secondary relatives the term $(1 + K_t)$ is expected to be about 1 in value and so K_T is approximately 2.6×10^4 showing that only a very small amount of the open-chain amino tautomer is present along with the deuteronated cyclic aminal. K_{app} then is the product of an equilibrium constant for a tautomeric step (K_T) and an equilibrium constant (K_{a1}) for a weak acid in which the conjugate base of this acid is disfavored by the prior ring-chain equilibrium. The large K_T term makes the apparent acidity constant of the ammonium ion large. The apparent acidity constant is not a value for dissociation alone as a simple consideration of the data might first suggest.

We can also simulate the titration curve using a more complex expression starting with aminal **2** as its free base. In this simulation two hydrons are involved in the overall conversion to monocyclic dication **4.** As shown in Fig. 2 the fit in the

[6] Nomenclature follows the IUPAC recommendation (*10*).

[7] The reported pK_a of 8.88 at 25°C (H_2O) is statistically corrected for two equivalent acidic sites (0.30) and a solvent isotope effect of 0.50 (*13*) to give the value of 9.68.

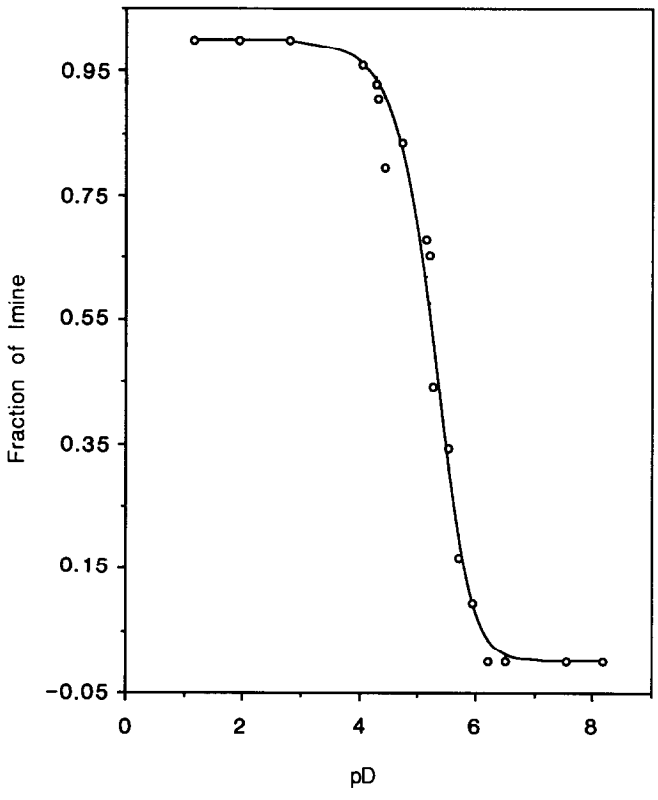

FIG. 2. The same titration data as in Fig. 1 except that the data are computer fitted with the aid of Eq. [4] and $K_{1app} = 3.33 \times 10^{-6}$ and $K_{a2} = 2.67 \times 10^{-6}$.

pD 6 region is much better. According to this model the fraction (F) of total substrate present as the monocyclic imine **4** and **5** is given by Eq. [3] where the previously employed K values are as represented earlier and K_{a2} stands for the combined dissociation constant for the mixture of **3a** and **3b** going to **2.** Again, since K_{a1} is small relative to $[D^+]$ over the pD range of the titration, the term $[D^+]K_{a1}$ can be neglected and the constants combined into two where K_{1app} equals $K_{a1}(K_T(1 + K_t))$, Eq. [4]. Reduced Eq. [4] has the same form as that for the titration of a diprotic acid in which the fraction of the total amount of acid in the final diprotonated state is expressed. On computer fitting both values turn out to be about the same 5.48 and 5.57, for pK_{1app} and pK_{a2}, respectively.

Unfortunately this second analysis is flawed. (i) At the start of the titration the substrate was said to exist as a mixture of aminal monocations (*5*). (ii) Our estimate from the computer fit for the pK_a value (5.57) of the conjugate acid of **2** is not reasonable for K_{a2}. The calculated pK_a value for K_{a2} is much too low. The pK_a (H_2O) of the conjugate acid of the model aminal 2-isopropyl-1,3-diethylimidazolidine is 8.42 at 35°C (*9*). (iii) The standard deviation of K_{a2} is an unsatisfactory 57%

of the equilibrium value. We favor, therefore, the first scheme based on a single degree of deuteronation.[8]

The identity of another of the oxidation products from spermine and spermidine now has been firmly established. Under physiological conditions the major form is **3**. The titration data are consistent with the proposed structures.

EXPERIMENTAL

The titration curve was simulated on an IBM microcomputer using a nonlinear regression program based on the method of Wentworth (*14*).

ACKNOWLEDGMENT

We thank Dr. Svante Brandänge for helpful comments.

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[8] Dideuteronated aminal is not likely to be present in significant amounts (*7, 9*). Our superficial analysis based on this dication can be made to fit the experimental data when a step for the direct conversion of this ion to **4** is included. Both ring cleavage and proton transfer then are required, perhaps by the participation of a water bridge.