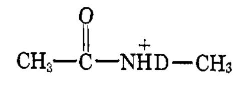
Table I. Kinetic Parameters for Hydrogen–Deuterium Exchange in Amides

Amide	Temp, °C	pD_{min}	Obsd rate constant, k', min^{-1}	Acid catalytic constant, k_{D+}, min^{-1} M^{-1}	Base catalytic constant, k_{OD^-}, min^{-1} M^{-1}	Activation energy, E_A*, kcal/mole[a]	Entropy of activation, ΔS_A*, cal/mole deg[a]
N-Methylacetamide	25.5	5.42	0.480	5.5×10^4	5.4×10^8	15.7	−15.6
	15.0	5.53	0.137	2.1×10^4	2.7×10^8		
N-Ethylacetamide	26.0	5.60	0.310	6.6×10^4	2.9×10^8	...	...
2-Pyrrolidone	19.5	5.20	1.30	13.6×10^4	55.0×10^8	26.0	20.6
	11.0	5.21	0.520	3.5×10^4	28.4×10^8		

[a] Calculated from acid catalytic constants.

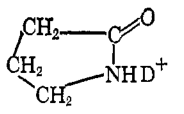
the acid catalytic constants we have computed by standard procedures[11] E_A*, the activation energy for exchange, and thereafter, ΔS_A*, the entropy of activation. These parameters are also listed in Table I.

As has been remarked earlier,[4] the activation energy for H–D exchange in the straight-chain amide, N-methylacetamide (I), is surprisingly high. The even higher E_A* for 2-pyrrolidone is, therefore, even more striking. The mechanism of the acid-catalyzed exchange presumably involves the protonated intermediates IV and V. The activation energy may be ascribed, therefore, to the energy required to rearrange the orbitals of I into IV, and of II into V, so that the D^+ may be placed on the amide in an orbital comparable to that of the H already on. On this basis one could rationalize the greater activation energy for the pyrrolidone as being due to the greater stability of the ground state of the amide bond in the five-membered ring.



$$CH_3-\overset{\overset{O}{\|}}{C}-\overset{+}{N}HD-CH_3$$

IV



V

Molecular models also indicate that access to the amide N is more open in the cyclic amide II than in the straight-chain amide. This difference in structure is in accord with the higher ΔS_A* for the cyclic amide.

In any event, the experimental observations demonstrate unequivocably that the cyclic *cis* amide has a higher rate of H–D exchange than do comparable *trans* amides. Although it seems somewhat far-fetched, one is inclined to wonder whether various bulk reagents, such as urea or dioxane, which speed up exchange in proteins, may not be operating by facilitating a change in configuration of the amide bond.

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Thermodynamics of Proton Dissociation in Dilute Aqueous Solution. VI. p*K*, $\Delta H°$, and $\Delta S°$ Values for Proton Ionization from 9-β-D-Xylofuranosyladenine at 25°[1a]

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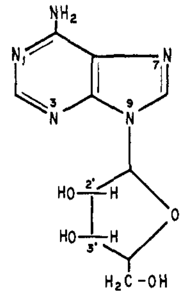
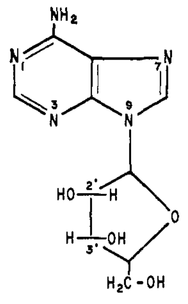
Abstract: Thermodynamic p*K* (12.34 ± 0.04), $\Delta H°$ (8.4 ± 0.2 kcal/mole), and $\Delta S°$ (−28.3 ± 0.8 eu) values are reported for proton ionization from the xylose group of 9-β-D-xylofuranosyladenine. The values were obtained by thermometric titration calorimetry and are valid at 25° and zero ionic strength. Comparisons are made between these values and those reported earlier for adenosine and its derivatives.

In previous publications[2,3] we have discussed the site of acidity and reported the p*K*, $\Delta H°$, and $\Delta S°$ values valid at 25° and zero ionic strength, μ, for proton ionization from adenosine (I). An extensive entropy titration study of derivatives of I including 2′-deoxyadenosine, 3′-deoxyadenosine, 2′-O-methyladenosine,



I



II

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[1] (a) Supported by National Institutes of Health Grant No. RG 9430-05. (b) To whom inquiries should be directed.

[2] R. M. Izatt, L. D. Hansen, J. H. Rytting, and J. J. Christensen, *J. Am. Chem. Soc.*, 87, 2760 (1965).

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and adenosine 5′-monophosphate showed that both the 2′- and 3′-hydroxyl groups are necessary for adenosine to be acidic in the pH region studied (11.5–13.5). It was felt that it would be of interest to make a similar study of 9-β-D-xylofuranosyladenine (II) in which the 2′- and 3′-hydroxyl groups are *trans*, whereas in I they are *cis*. Thus, one could learn whether the acidity of I depends upon the 2′- and 3′-hydroxyl groups being *cis* to each other. It was suggested earlier[3] that the acidity of I might result from stabilization of the anion by hydrogen bonding involving the 2′ and 3′ positions. The concept of hydrogen bonding affecting the acidity of molecules (*e.g.*, cytidine) similar to those studied here has been suggested by Fox, Cavalieri, and Chang.[4] Molecular models (Leybold, A. S. La Pine and Co.) of I and II indicate that hydrogen bonding is favorable between the 2′ and 3′ positions in I, but not in II. Thus one would expect that, if stabilization due to hydrogen bonding contributes significantly to the observed acidity of I, then II should show reduced acidity. We have extended our previous study to include II and report here thermometric titration data from which p$K°$, $\Delta H°$, and $\Delta S°$ values for proton ionization from II are calculated.

Experimental Section

Materials. The 9-β-D-xylofuranosyladenine used in this study was obtained from the Cancer Chemotherapy National Service Center, Bethesda, Md., and the NaOH titrant was prepared from Baker and Adamson Reagent Grade 50% NaOH. All solutions were prepared, stored, and used under a pure nitrogen atmosphere.

Procedure. A 9-β-D-xylofuranosyladenine solution was titrated with a NaOH solution using a precision thermometric titration calorimeter which together with its operation and calibration has been described.[5]

Calculations. The entropy titration procedure used to determine the pK and $\Delta H°$ values in this study and the method of data analysis have been described.[6] The heat of dilution data for NaOH used in making the calculations were determined by Sturtevant.[7] Values for the heat of ionization (13.34 kcal/mole) and ion product (1.004×10^{-14}) of water are taken from Hale, *et al.*,[8] and Harned and Owen,[9] respectively. The calculations were aided by an IBM 7040 computer.

Results

In Table I the corrected heat of reaction values are given as a function of millimoles of NaOH titrant added.

The limited amount (200 mg) of II available permitted only one run to be made. The data were analyzed assuming the reaction $\mathrm{HII} = \mathrm{H^+} + \mathrm{II^-}$ to obtain the pK, $\Delta H°$, and $\Delta S°$ values valid at 25° and $\mu = 0$ which are given in Table II, together with previously determined values for I.

Discussion

No previous pK, $\Delta H°$, or $\Delta S°$ values have been reported for proton ionization from II. The pK values for I and II (Table II) are, within experimental uncertainty, identical, but the $\Delta H°$ and $\Delta S°$ values are quite different for the two compounds. Thus, the acidity is independent of whether the hydroxyl groups are *cis* or *trans*.

Table I. Corrected Heat of Reaction, Q, for Interaction of 0.5853 F NaOH with II[a]

Titrant delivered, moles × 10^3	Q,[b] cal	Titrant delivered, moles × 10^3	Q,[b] cal
0.3001	−0.3783	2.1006	−1.6456
0.6002	−0.6769	2.4007	−1.7741
0.9003	−0.9241	2.7008	−1.8834
1.2003	−1.1408	3.0008	−1.9866
1.5004	−1.3307	3.3009	−2.0800
1.8005	−1.4968		

[a] Initial molar concentration of II, 0.007183; initial volume in calorimeter, 97.62 ml. [b] Corrected for heat of stirring and heat losses from the calorimeter.

Table II. pK, $\Delta H°$, and $\Delta S°$ Values Valid at 25° and $\mu = 0$ for Proton Ionization from I and II

Compound	pK	$\Delta H°$, kcal/mole	$\Delta S°$, eu
II[a]	12.34 ± 0.04	8.4 ± 0.2	−28.3 ± 0.8
I[b]	12.35 ± 0.03	9.7 ± 0.2	−24.0 ± 0.7

[a] Values are reported as the average of the results obtained from 20 different combinations of data points in one run with the uncertainties expressed as standard deviations between these combinations. [b] Reference 2; values are the average of 16 runs with the uncertainties expressed as standard deviations between runs.

The fact that the pK values are the same for I and II suggests, but does not prove, that stabilization of the anion by hydrogen bonding is not the reason that two adjacent hydroxyl groups are necessary for the acidity of adenosine since greater hydration in II could also account for the identical pK values. The difference between the $\Delta S°$ values for I and II is 4.3 eu, which is in the range of values (2–6 eu) which can be estimated from entropy of hydration data,[10] structural considerations,[11–13] and reactions in solution[14] for the addition of a water molecule to an ion with no resulting change in ion charge. Thus, greater hydration in II could account for the difference in the $\Delta S°$ and $\Delta H°$ values between I and II with a compensating effect resulting in identical pK values. Lower hydration of I could result from steric hindrance due to the vicinal OH groups or hydrogen bonding in the anion.

Further studies involving derivatives of II similar to the study previously carried out with I[1,2] should lead to a better understanding of acidity in both I and II, including a knowledge of the proton ionization site in II, and whether both hydroxyl groups must be present in II for acidity.

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