The limits of the electron-impact estimate, *i.e.*, 96–115 kcal mole^{-1}, bracket the value of 107 ± 3 kcal mole^{-1} estimated from thermochemical data. The latter value is thus used in all discussions.

Acknowledgments. This work was made possible, in large part, by grant-in-aid support from the American Chemical Society Petroleum Research Fund. J. B. F. acknowledges the award of a NASA traineeship during the tenure of this study. The contributions of B. Hardman in performing experimental rate measurements and F. Dampier in computer analysis of data are acknowledged with pleasure.

Deuterium–Hydrogen Exchange in a *cis*-Lactam Amide Group

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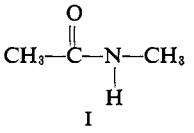
Contribution from the Biochemistry Division,[1] *Department of Chemistry, Northwestern University, Evanston, Illinois.* *Received May 18, 1966*

Abstract: Rates of deuterium–hydrogen exchange have been measured for 2-pyrrolidone (II) at 11.0 and 19.5°, and compared with values for two *trans* amides, N-methyl- and N-ethylacetamide. The *cis* amide shows markedly faster exchange rates, a higher activation energy, and a larger entropy of activation. The amide nitrogen seems more accessible in the *cis* amide but the energy for placing a D^+ on it is greater.

There are numerous factors that may influence the rates of hydrogen–deuterium exchange in biological macromolecules. Before the observed kinetics can be interpreted in terms of structure and conformation of a macromolecule, it will be necessary to examine the behavior of simple model systems under a variety of environmental conditions. N-Methylacetamide (I) has

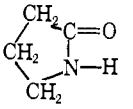


I

been used as one such model in several laboratories.[2-4] Its rate of N–H → N–D exchange has been found sensitive to D^+ or OD^- in several different solvent systems, and also subject to catalysis by a number of acids (*e.g.*, formic, acetic) and bases (imidazole, amines).

It seems almost certain that the amide group in N-methylacetamide is in the *trans* form, as depicted in I, in view of the large dielectric constant of this substance as well as its infrared and nmr properties.[5-7] The *trans* state is also that of amide groups in either the α helix or β form of polypeptides or proteins. On the other hand, it is possible that in some environments, a *cis* form of the amide might exist, even if only as a transient state. It seemed of interest, therefore, to compare the rate of deuterium–hydrogen exchange in a *cis* amide with that in a *trans* state.

Large cyclic lactams can fold in various ways, but the five-membered ring compound 2-pyrrolidone (II)



II

must have the amide group locked in a *cis* configuration. The kinetics of its exchange reaction with D_2O were examined, therefore, under a variety of conditions.

Experimental Section

Materials. 2-Pyrrolidone was obtained from the Dyestuff and Chemical Division of General Aniline and Film Corp., New York, N. Y. It was vacuum distilled at 3 mm pressure, and the fraction boiling at 88–90° was collected. N-Methylacetamide was obtained from Eastman Organic Chemicals and distilled at atmospheric pressure. The fraction boiling at 205–206° was collected. N-Ethylacetamide was provided by Lachat Chemicals, Chicago, Ill. The fraction boiling at 68–69° at a pressure of 3 mm was collected. The purity of this material was checked by nmr spectroscopy and vapor phase chromatography and found to be greater than 99%.

Heavy water containing a minimum of 99.8% D_2O was purchased from Bio-Rad Laboratories, Richmond, Calif. A solution of 38% DCl in D_2O was also obtained from Bio-Rad and was diluted as necessary.

Sodium acetate was obtained from Eastman Organic Chemicals and used directly.

Preparation of Reaction Mixtures. A solution of 0.02 *M* sodium acetate in D_2O was prepared as the buffer medium for H–D exchange. The necessary pD was reached by addition of small quantities of 1 *M* DCl to the buffer.

The amide to be studied, contained in a 250-μl Hamilton syringe, was injected into a 1-cm quartz cell containing 2.5 cc of buffer. The cell was manually shaken, centrifuged to remove air bubbles, and then placed in the spectrophotometer. An optical reference cell contained the same buffer solution but no amide.

Optical Measurements. Rates of exchange were followed by the increase in absorption at 1.43 μ for both N-methylacetamide and N-ethylacetamide and 1.41 μ for 2-pyrrolidone as measured in a Cary Model 14R recording spectrophotometer. The quartz cells were the Infrasil type sold by Precision Cells Inc., New York, N. Y.

pH Measurements. All pH values were measured with a Beckman Model G pH meter at 25°. The meter was standardized with H_2O buffer solutions and the meter reading converted to pD values

(1) This investigation was supported in part by Grant GM-09280 from the National Institute of General Medical Sciences, Public Health Service. It was also assisted by support made available by Public Health Service Training Grant No. 5T1-GM-626 from the National Institute of General Medical Sciences.

(2) A. Berger, A. Loewenstein, and S. Meiboom *J. Am. Chem. Soc.*, **81**, 62 (1959).

(3) S. O. Nielsen, *Biochim. Biophys. Acta*, **37**, 146 (1960).

(4) I. M. Klotz and B. H. Frank, *J. Am. Chem. Soc.*, **87**, 2721 (1965).

(5) G. R. Leader and J. F. Gormley, *ibid.*, **73**, 5731 (1951).

(6) S. Mizushima, T. Simanouti, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba, and O. Fujioka, *ibid.*, **72**, 3490 (1950)

(7) L. A. LaPlanche and M. T. Rogers, *ibid.*, **86**, 337 (1964).

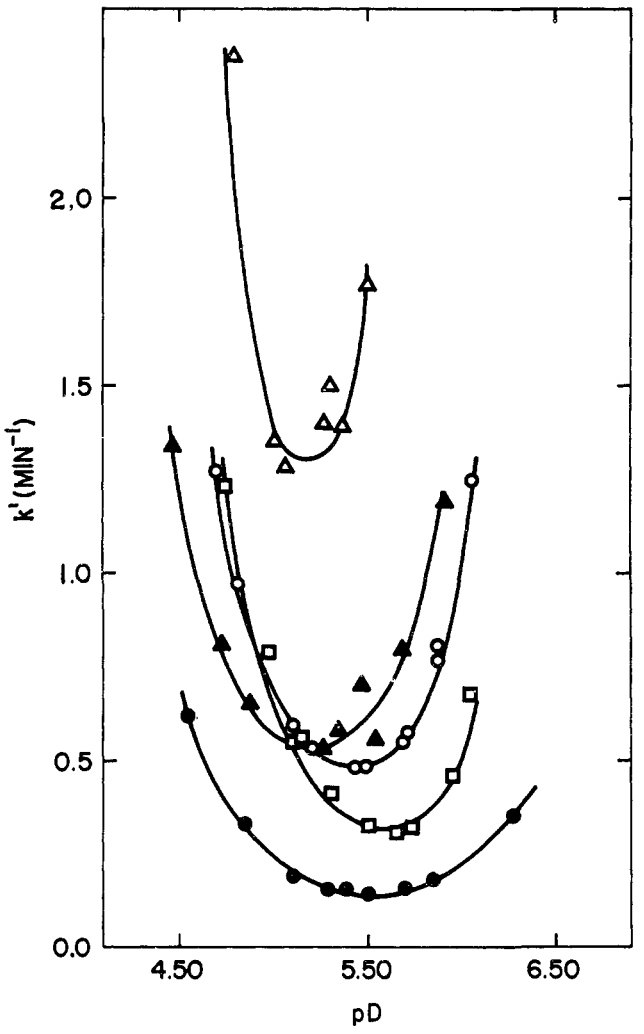


Figure 1. Rates of hydrogen-deuterium exchange for various amides at 1 M concentration in D_2O: △, 2-pyrrolidone at 19.5°; ▲, 2-pyrrolidone at 11.0°; ○, N-methylacetamide at 25.5°; □, N-ethylacetamide at 26°; ●, N-methylacetamide at 15°.

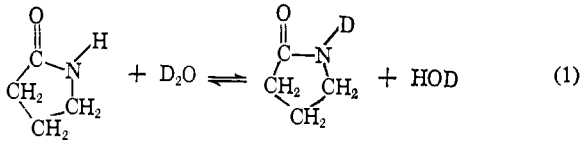
following the equation of Glasoe and Long[8]

$$pD = pH(\text{meter reading}) + 0.40$$

Temperature. Temperature regulation was maintained by thermostating the cell holder and the cell compartment with a Haake and a Sargent circulating water bath, respectively. The actual temperature was measured with a Model 425C thermoprobe from the Yellow Springs Instrument Co.

Results and Discussion

The reaction of 2-pyrrolidone with D_2O may be represented as



(1)

Since the concentration of D_2O is 55 M and that of 2-pyrrolidone is 1 M, this reaction may be analyzed according to first-order kinetics. Therefore we write

$$\frac{\partial[\text{DOH}]}{\partial t} = k[\text{2-P}][D_2O] = k'[\text{2-P}] \qquad (2)$$

(8) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

where t represents the time, k the rate constant, and [2-P] the concentration of 2-pyrrolidone.

We may use the half-life method to solve for k'.

$$k' = \frac{\ln 2}{t_{1/2}} \qquad (3)$$

Alternatively we may apply the Guggenheim analysis.[9] In this case we write

$$A_t = A_0 e^{-k't}; \; A_{t+\Delta} = A_0 e^{-k'(t+\Delta)} \qquad (4)$$

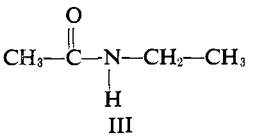
from which one can obtain[9]

$$k' = \frac{\ln (A_{t+\Delta} - A_t)}{t} + \frac{C}{t} \qquad (5)$$

where A_t is the absorbancy at time t, $A_{t+\Delta}$ is the absorbancy at time $t + \Delta$, k' is the observed rate constant, Δ is a constant interval of two or three half-lives, and C is a constant. A program was written for the CDC 3400 computer to calculate k' in eq 5 by a least-squares analysis of the data.

The observed rate constants for H–D exchange of 2-pyrrolidone are summarized in Figure 1. Comparing minimum exchange rates with those for N-methylacetamide,[4] we see that the *cis*-lactam undergoes exchange considerably faster (Table I). For example, the former amide at 15° has a k' of 0.14 min^{-1}, whereas that of the pyrrolidone at 11°, a slightly lower temperature, is 0.52 min^{-1}, almost four times greater. At 25.5°, N-methylacetamide shows a minimum rate constant of 0.48 min^{-1}, substantially smaller than that (1.3 min^{-1}) of 2-pyrrolidone at a slightly lower temperature, 19.5°.

Actually a more appropriate comparison would be between a *trans* amide and a *cis* amide containing the same numbers of carbon atoms. For this purpose rates of H–D exchange were measured with the straight-chain amide N-ethylacetamide (III). At 26° this substance shows a minimum rate constant of 0.31 min^{-1}, a factor of 4 smaller than that of 2-pyrrolidone (1.3 min^{-1}) at the slightly lower temperature of 19.5°.



III

As with the straight-chain *trans* amides, both acid and base catalysis of H–D exchange has been found for the *cis* amide 2-pyrrolidone. We may write, therefore[10]

$$k' = k_0 + k_{D^+}[D^+] + k_{OD^-}[OD^-] \qquad (6)$$

where k_0 is the specific rate constant in the absence of catalysts, k_{D^+} is the acid catalytic constant, and k_{OD^-} is the base catalytic constant. Catalytic constants, calculated from the k'–pD profile of exchangeable amide from (Figure 1) and from K_w, the ionization constant of D_2O, are listed in Table I. Again it is apparent that the catalytic effects of D^+ and of OD^- are much greater for the *cis* lactam than for either straight-chain amide.

The temperature coefficients of the rate constants also provide some interesting comparisons. Using

(9) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926).

(10) Although 0.02 M acetate buffer was also present, this concentration is much too small for acetic acid–acetate to contribute significantly by general acid–base catalysis; see ref 4.

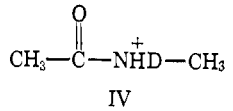
Table I. Kinetic Parameters for Hydrogen–Deuterium Exchange in Amides

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.



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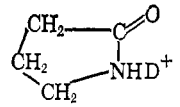

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Molecular models also indicate that access to the amide N is more open in the cyclic amide II than in the straight-chain amide. This difference in structure is in accord with the higher ΔS_A* for the cyclic amide.

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 farfetched, 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.

(11) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, pp 22–24, 100–101.

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

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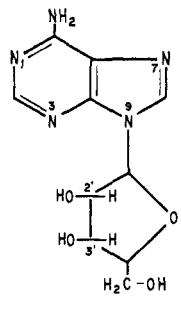
Contribution from the Departments of Chemistry and Chemical Engineering, Brigham Young University, Provo, Utah. *Received August 22, 1966*

Abstract: Thermodynamic pK (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 pK, $\Delta H°$, 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,



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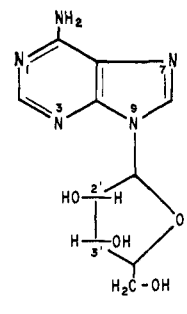

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(1) (a) Supported by National Institutes of Health Grant No. RG 9430-05. (b) To whom inquiries should be directed.

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

(3) R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, *ibid.*, **88**, 2641 (1966).