Kinetic Studies of Hydrogen Bonding. 1-Cyclohexyluracil and 9-Ethyladenine[1]

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Abstract: Rate constants for the association and dissociation of the hydrogen-bond stabilized dimers of 1-cyclohexyluracil and of 1-cyclohexyluracil-9-ethyladenine in chloroform have been determined from ultrasonic attenuation measurements over the frequency range 15–175 MHz. Standard enthalpies of formation of the dimers have also been calculated from the ultrasonic data. Lower bounds for the rate constants associated with the dimerization of 9-ethyladenine have been obtained. Although the thermodynamic stabilities of the three dimers are quite different, the association rate constants are similar: $1.5 \times 10^9\ M^{-1}\ \text{sec}^{-1}$ for 1-cyclohexyluracil at 25°, $\geq 2 \times 10^9\ M^{-1}\ \text{sec}^{-1}$ for 9-ethyladenine at 25°, and $4.0 \times 10^9\ M^{-1}\ \text{sec}^{-1}$ for 1-cyclohexyluracil-9-ethyladenine at 20°. The magnitudes of these rate constants indicate that in all three cases the association is essentially diffusion controlled. The corresponding dissociation rate constants, $25 \times 10^7\ \text{sec}^{-1}$, $\geq 60 \times 10^7\ \text{sec}^{-1}$, and $3.2 \times 10^7\ \text{sec}^{-1}$, respectively, differ considerably and are direct measures of the strength of the hydrogen bonds. The implications of these results for the mechanism of hydrogen bonding in general are discussed.

The importance of hydrogen bonds in the structures of proteins and nucleic acids is well known, and many studies of the equilibrium properties of hydrogen bonds have been published.[2] However, relatively little is known about the dynamics of hydrogen bonding, primarily because of the extreme rapidity of hydrogen-bonding processes. Kinetic investigations of the formation of the hydrogen-bond-stabilized dimers of benzoic acid,[3,4] ε-caprolactam,[5] and 2-pyridone[6] and the interaction of water with a number of compounds, including amines,[7] dioxane,[8] diglycine,[9] polyethylene glycol,[10–12] and polyglutamic acid[13] have been carried out using dielectric and ultrasonic techniques. Of particular importance to biology are the hydrogen-bond interactions between the bases in nucleic acids. A number of equilibrium studies of the hydrogen-bonding interactions between these bases in relatively inert solvents have been reported.[14–19]

The self-association of 9-ethyladenine and of 1-cyclohexyluracil and the association of these two compounds in chloroform have been studied by nuclear magnetic resonance[20] and infrared techniques.[19,21] Hamlin, Lord, and Rich[14] convincingly demonstrate in their infrared studies that 1:1 complexes are found in all three cases. In the work reported here, the kinetics of these three reactions in chloroform have been studied using an ultrasonic absorption technique. The rate constants for the dimerization of 1-cyclohexyluracil and for the formation of the mixed dimer have been determined over a limited temperature range, and lower bounds have been estimated for the rate constants associated with the formation of the 9-ethyladenine dimer. In addition, standard enthalpies of formation of the former two dimers have been calculated from ultrasonic data. The relationship of these measurements to the mechanism of hydrogen bonding in general is discussed.

Experimental Section

Chloroform (Mallinckrodt AR), which contains about 0.75% ethanol, was distilled and passed slowly through an aluminum oxide (Woelm, basic, activity 1) column 30 cm long and 4 cm in diameter. The fraction between 150 and 500 ml, which had a maximum alcohol and water content of 0.005%,[22,23] was collected and stored in a brown glass container. Fresh samples were prepared every 2 weeks; during this period the ultrasonic properties of the chloroform showed no detectable changes. The 9-ethyladenine and 1-cyclohexyluracil were obtained from the Cyclo Chemical Corp. (Los Angeles, Calif.). 9-Ethyladenine was recrystallized from the purified chloroform. Both compounds were vacuum dried and solutions of the desired concentrations were prepared just before ultrasonic measurements were made. After each set of measurements, the solutions were flash evaporated and the bases were again vacuum dried.

The apparatus and procedure for making ultrasonic measurements have been previously described.[13,24] The only change was the use of Teflon gaskets throughout the apparatus. The pressure amplitude absorption coefficient, α, and the ultrasonic velocity, v, were determined over the frequency range 15–175 MHz.

Results and Treatment of Data

The ultrasonic absorption coefficient divided by the square of the frequency of the ultrasonic wave, α/f^2,

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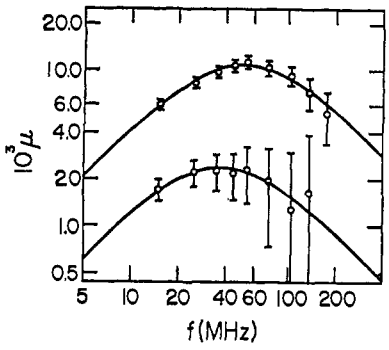

Figure 1. Typical plots of absorption per wavelength, μ, as a function of frequency, f. Bottom: 0.065 M 1-cyclohexyluracil in chloroform at 10°. Top: equimolar mixture (0.234 M in each constituent) of 1-cyclohexyluracil and 9-ethyladenine in chloroform at 35°. The lines are calculated by use of eq 2 and the parameters in Tables II and III. The standard deviations shown correspond to ±2% error in the absorption coefficient, α.

and the ultrasonic velocity, v, are constant over the frequency range 15–175 MHz for pure $CHCl_3$ and for saturated solutions of 9-ethyladenine in $CHCl_3$ at 10 and 25° (~0.05 and ~0.1 M, respectively). The values obtained are given in Table I. The experimental error in α/f^2 is ±2%, while that in v is less than 1%.

Table I. Ultrasonic Parameters for $CHCl_3$ and Saturated Solutions of 9-Ethyladenine

Solution	Temp, °C	$10^{17}\alpha/f^2$, sec^2/cm	$10^{-5}v$, cm/sec
$CHCl_3$	10	449	1.014
	25	501	0.962
9-Ethyladenine in $CHCl_3$ (~0.05 M)	10	415	1.016
9-Ethyladenine in $CHCl_3$ (~0.1 M)	25	410	0.969

In solutions of 1-cyclohexyluracil, the data at a given concentration and temperature are consistent with the assumption of a single relaxation process and therefore can be described by the equation[25]

$$\alpha/f^2 = \frac{A\tau}{1 + \omega^2\tau^2} + B \qquad (1)$$

where A and B are constants, ω ($=2\pi f$) is the angular frequency, and τ is the relaxation time. Alternatively, eq 1 can be written in terms of the chemical absorption per wavelength,[26] μ_{ch}

$$\mu_{ch} = 2v/(\alpha/f^2 - B) = 2\mu_m\frac{\omega\tau}{1 + \omega^2\tau^2} \qquad (2)$$

where μ_m is equal to $Av/2\pi$. A plot of μ_{ch} *vs.* log ω goes through a maximum when $\omega = 1/\tau$. The ultrasonic parameters were obtained using the template technique of Piercy and Subrahmanyam.[27] A typical plot of the data according to eq 2 is given in Figure 1 and a summary of the ultrasonic parameters obtained is given in Table II. The estimated error in τ is ±10% at 10° and ±15% at 25° while that in μ_m is ±15% at 10° and ±30% at 25°. Data were obtained over as wide a concentration and temperature range as possible. At higher temperatures than those employed, the relaxation times became too short to measure, and the amplitude of the relaxation process decreased considerably. At lower concentrations the amplitude of the relaxation process was too small to be observed.

Table II. Ultrasonic Parameters for 1-Cyclohexyluracil in Chloroform

$(U)_0$, M	$10^9\tau$, sec	$10^3\times\mu_m$	$10^{-5}v$, cm/sec	$10^{17}B$, sec^2/cm	$10^{-8}k_1$, M^{-1} sec^{-1}	$10^{-7}k_{-1}$, sec^{-1}	$-\Delta H°$, kcal/mol
				At 10°			
0.245	2.3	7.5	1.031	298	8.9	10.2	3.9
0.123	3.2	4.7	1.025	348	8.8	10.2	4.1
0.065	4.3	2.4	1.021	387	8.6	9.9	4.0
				Av	8.8	10.1	4.0
				At 25°			
0.065	1.9	3.7	0.969	422	16	26	5.4
0.030	2.8	1.5	0.964	458	14	23	5.4
				Av	15	25	5.4

If the relaxation process is attributed to the reaction

$$U + U \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} U_2 \qquad (3)$$

where U designates 1-cyclohexyluracil, the reciprocal relaxation time is[26]

$$1/\tau = k_{-1} + 4k_1\bar{c}_U \qquad (4)$$

where $\bar{c}_U$ is the equilibrium concentration of the monomer. Equilibrium constants, $K_1 = k_1/k_{-1}$, determined by Kyogoku and coworkers[21] were used to evaluate $\bar{c}_U$ at the concentrations and temperatures studied. Also by use of K_1, k_{-1} can be eliminated from eq 4 and k_1 can be calculated at each concentration. The constants obtained are given in Table II. Although reliable activation energies cannot be obtained over such a restricted temperature range, Arrhenius activation energies for the forward and reverse rate constants in eq 3 can be estimated as 6 and 10 kcal/mol, respectively. The amplitude parameter, μ_m, is a function of thermodynamic variables only, and for the reaction of eq 3 under the experimental conditions employed it is given to a good approximation by[26]

$$\mu_m = (\rho v^2\pi\Gamma/10^3RT)[\Delta V° - \beta\Delta H°/\rho c_P]^2 \qquad (5)$$

where ρ is the density of the solution, R is the gas constant, $\Delta V°$ and $\Delta H°$ are the standard volume and enthalpy changes for the reaction, β is the coefficient of thermal expansion of the solvent, and c_P is the constant pressure specific heat of the solvent. For a self-association reaction of this type

$$\Gamma = \frac{1}{8K_1}\left[\frac{1 + 4K_1c_{0U}}{(1 + 8K_1c_{0U})^{1/2}} - 1\right]$$

where c_{0U} is the total concentration of 1-cyclohexyluracil. In nonaqueous solvents, $\Delta V° << \beta\Delta H°/\rho c_P$ so that $\Delta H°$ can be calculated from μ_m. Although the sign of $\Delta H°$ cannot be ascertained because it is obtained as a square root, it is assumed to be negative;

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the calculated values of $\Delta H°$ are included in Table II. The corresponding standard entropy changes at 10 and 25° are −9.8 and −14.5 eu, respectively. Because the amplitudes at 25° are small, the data at 10° are considered more reliable. The estimated uncertainty in the average rate constants at 10° is ±20% and that in the enthalpy change is ±0.5 kcal/mol.

Equimolar mixtures of 9-ethyladenine and 1-cyclohexyluracil displayed a relaxation process having more than ten times the amplitude (*i.e.*, a ten times greater value of $A\tau$; see eq 1) of the effect seen with 1-cyclohexyluracil at corresponding concentrations. The data were again consistent with the assumption of a single relaxation process; and a typical plot of the data according to eq 2 is shown in Figure 1. The relaxation process associated with the dimerization of 1-cyclohexyluracil could not be detected, presumably because of its relatively small amplitude. The maximum concentration of the 1-cyclohexyluracil dimer relative to that of the mixed dimer was about 8% if the equilibrium constants of Kyogoku and coworkers[21] were assumed. The ultrasonic parameters for the equimolar mixtures at 20 and 35° are summarized in Table III. The temperature range studied was restricted to one where the relaxation times were within the experimentally accessible frequency range at the concentrations which could be employed.

Table III. Ultrasonic Parameters for Equimolar Mixtures of 9-Ethyladenine and 1-Cyclohexyluracil

c_0,[a] M	$10^9\tau$, sec	$10^3\mu_m$	$10^{-5}v$, cm/sec	$10^{17}B$, sec²/cm
		At 20°		
0.234	4.1	10.0	1.006	254
0.162	5.2	8.4	0.996	316
0.102	6.1	7.4	0.988	348
0.050	9.4	4.4	0.989	417
		At 35°		
0.234	3.1	11.0	0.961	290
0.162	3.7	8.5	0.946	346
0.102	4.7	6.7	0.943	394
0.050	6.4	4.6	0.933	452

[a] Concentration of each base.

As a first approximation, we neglect the self-dimerization of the bases and assume that the only reaction of importance can be represented as

$$A + U \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} AU \qquad (6)$$

where A designates 9-ethyladenine. The reciprocal relaxation time for this reaction is[26]

$$1/\tau = k_{-2} + k_2(\bar{c}_A + \bar{c}_U) \qquad (7)$$

where $\bar{c}_A$ and $\bar{c}_U$ represent the equilibrium concentrations of the monomers. For equimolar mixtures of the bases, eq 7 can be squared and rearranged to give

$$1/\tau^2 = k_{-2}^2 + 4k_2k_{-2}c_0 \qquad (8)$$

where c_0 is the total concentration of either of the bases. Plots of $1/\tau^2$ *vs.* c_0 are shown in Figure 2 and the rate and equilibrium constants obtained from a least-squares analysis of these plots are given in Table IV. The equilibrium constants are in reasonable

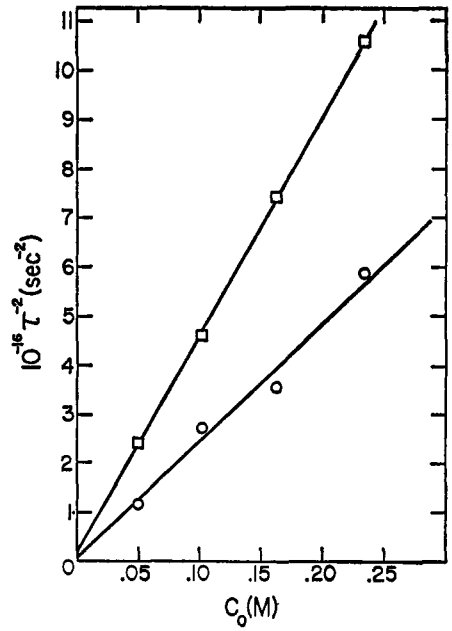

Figure 2. Square of the reciprocal relaxation time, τ^{-2}, as a function of the concentration, c_0, of 1-cyclohexyluracil (or 9-ethyladenine) for equimolar mixtures of the bases in chloroform at 35° (□) and 20° (○) plotted according to eq 8. The lines were obtained from a least-squares analysis of the data.

agreement with the average of the values reported by Kyogoku and coworkers[21] although the values obtained in this work contain considerable experimental error due to the smallness of the intercept of the plots. Standard enthalpy changes can be calculated using eq 5, in which, for this case

$$\Gamma = \frac{1}{2K_2}\left[\frac{1 + 2K_2c_0}{(1 + 4K_2c_0)^{1/2}} - 1\right] \qquad (9)$$

The average calculated values of $\Delta H°$ at the two temperatures are included in Table IV. (Average values of the equilibrium constants of Kyogoku and coworkers[21] were used in carrying out these calculations.)

Table IV. Rate and Thermodynamic Constants for $A + U \rightleftharpoons AU$

Temp, °C	$10^{-9}k_2$, M^{-1} sec^{-1}	$10^{-7}k_{-2}$, sec^{-1}	K_2, M^{-1}	$-\Delta H°_{AU}$, kcal/mol
20[a]	2.8	2.2	124	5.0
35[a]	3.3	3.4	99	4.9
20[b]	4.0	3.2	(125)[21]	6.1
35[b]	4.0	5.3	(75)[21]	5.9

[a] Neglecting the formation of U_2 and A_2. [b] Neglecting the formation of A_2, but including the formation of U_2.

A more exact treatment of this problem requires that the self-association of the monomers be included in the analysis. However, since a relaxation process is not observed in 9-ethyladenine solutions, self-association of this species will be neglected. (Assumption of the equilibrium constants of Kyogoku and coworkers[21] for dimerization of 9-ethyladenine indicates that the concentration of the dimer A_2 is always less than 3% of the AU concentration in the solutions studied.) This point will be considered further in the discussion. The relaxation times found for 1-cyclohexyluracil

dimerization and those found in solutions containing equimolar mixtures of the bases are sufficiently similar to indicate that some kinetic coupling must occur. Thus the reaction mechanism can be written as

$$\begin{array}{ccc} A + 2U & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & AU + U \\ {\scriptstyle k_1}\downarrow\uparrow{\scriptstyle k_{-1}} & & \\ U_2 & & \end{array} \qquad (10)$$

The relaxation times for such a mechanism are[26]

$$1/\tau_{1,2} = -{}^1/_2(a_{11} + a_{22})\{1 \pm [1 - 4(a_{11}a_{22} - a_{12}a_{21})/(a_{11} + a_{22})^2]^{1/2}\} \qquad (11)$$

where the plus sign corresponds to one relaxation time and the negative sign to the other; $a_{11} = -4k_1\bar{c}_U - k_{-1}$, $a_{12} = -2k_1\bar{c}_U$, $a_{21} = -2k_2\bar{c}_A$, and $a_{22} = -k_2(\bar{c}_A + \bar{c}_U) - k_{-2}$. The observed relaxation times are almost certainly the negative roots of eq 11 since these relaxation times are longer than those found for 1-cyclohexyluracil solutions of similar concentrations. If the equilibrium constants of Kyogoku and coworkers[21] are assumed, eq 11 can be written as a function of the rate constants k_1 and k_2 of eq 10. These rate constants were varied at each temperature until the minimum deviation was found between the relaxation times calculated from eq 11 and the experimentally determined relaxation times at each concentration. The best value of k_1 was found to be 1.9×10^9 M^{-1} sec^{-1} at both temperatures and the best values of k_2 and k_{-2} are included in Table IV. These rate constants reproduced the experimental relaxation times within $\pm 15\%$ or better. Because of the limited data available and the uncertainties in the equilibrium constants, the rate constants cannot be considered more reliable than ± 25–30%.

The amplitude of the coupled relaxation process is again given by eq 5 except that

$$\Gamma = \Gamma_2 = \left\{\frac{(1/\tau_1 - 1/\tau_2)}{a_{11}^2}\right\}\left\{\left[\frac{1}{(AU)(1/\tau_1 + a_{11})} + \frac{1}{(A)(1/\tau_1 + a_{11})} + \frac{(1/\tau_1 + a_{11})}{(U_2)a_{21}^2} + \frac{(1/\tau_1 + a_{11} + a_{21})\left(\frac{a_{21}}{1/\tau_1 + a_{11}} + 2\right)}{(U)a_{21}^2}\right]^{-1} + \left[\frac{1}{(AU)(1/\tau_2 + a_{11})} + \frac{1}{(A)(1/\tau_2 + a_{11})} + \frac{(1/\tau_1 + a_{11})^2}{(U_2)a_{21}^2(1/\tau_2 + a_{11})} + \frac{(1/\tau_1 + a_{11} + a_{21})(a_{21} + 2/\tau_1 + 2a_{11})}{(U)a_{21}^2(1/\tau_2 + a_{11})}\right]^{-1}\right\} \qquad (12)$$

and

$$\Delta H^\circ = \Delta H_2 = \frac{-\Delta H^\circ_{AU} - \left(\frac{(1/\tau_1 + a_{11})}{a_{21}}\right)\Delta H^\circ_{UU}}{(1/\tau_2 - 1/\tau_1)/a_{11}} \qquad (13)$$

where ΔH°_{AU} and ΔH°_{UU} are the standard enthalpy changes for formation of the AU and U_2 dimers, respectively. Equations 12 and 13 are derived in the Appendix. The average values of ΔH°_{AU} obtained from the measured values of μ_m and eq 5, 12, and 13 are given in Table IV. The assumption was made that ΔH°_{AU} is negative in carrying out the calculations and ΔH°_{UU} was assumed to be -4.3 kcal/mol, as reported by Kyogoku and coworkers.[21] The average deviation in the calculated values of ΔH°_{AU} is only ± 0.1 kcal/mol, and the average value is probably reliable to ± 0.5 kcal/mol.

Discussion

We first consider the ultrasonic absorption in pure chloroform. The values of α/f^2 given in Table I for the pure liquid are considerably higher than those previously reported by Sette,[28] Heasell and Lamb,[29] and Willard,[30] who obtained values of 420×10^{-17}, 365×10^{-17}, and 380×10^{-17} sec^2/cm, respectively, at 25°. This is undoubtedly due to the fact that previous workers did not adequately purify their chloroform. The chloroform employed in this work contained about 0.75% ethanol as a preservative and had a value of α/f^2 of 412×10^{-17} sec^2/cm before purification. Sette[28] has shown for a number of liquids with high ultrasonic absorptions that the presence of a small amount of impurity having a low absorption can greatly reduce the value of α/f^2 for these liquids. The strong absorption of ultrasonic radiation by unassociated liquids is attributed to the slow intermolecular exchange of internal energy. The relaxation frequency for this process in chloroform is still at considerably higher frequencies than are employed in this investigation. Impurities markedly decrease this relaxation time for energy transfer, and since the amplitude of a relaxation process is equal to $A\tau$ when $\omega\tau \ll 1$ (eq 1), α/f^2 is decreased correspondingly.[31] The fact that the parameters B (Tables II and III) for solutions of 1-cyclohexyluracil and 1-cyclohexyluracil-9-ethyladenine are less than α/f^2 for the pure liquid and decrease monotonically with increasing concentration can be explained in a similar manner.

Although α/f^2 is decreased in 9-ethyladenine solutions, a relaxation process due to dimerization is not observed. However, infrared spectra indicate that dimerization does occur although the equilibrium constant is quite small.[21] From the equilibrium data, the amplitude parameter A (eq 1, 2, and 5) expected for the dimerization process can be calculated. The calculated value of A for a saturated solution at 10° (0.05 M) is 9.7×10^{-8} sec/cm, while the corresponding value at 25° (0.1 M) is 2.0×10^{-7} sec/cm. The amplitude of the experimentally observed relaxation process is determined by the product $A\tau$ (see eq 1). The minimum detectable value of $A\tau$ is about 16×10^{-17} cm^2/sec (twice the estimated experimental precision of α/f^2); therefore for the equilibrium data to be consistent with the absence of a relaxation effect, the relaxation times at 10 and 25° must be less than 2×10^{-9} and 8×10^{-10} sec, respectively. (These

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Table V. Rate Constants for Hydrogen Bond Dimerization

Reactants	Solvent	$10^{-9}k_f$, M^{-1} sec^{-1}	$10^{-7}k_r$, sec^{-1}	Temp, °C	Ref
Benzoic acid	CCl_4	5	0.073	25	3
	Toluene	1.6	0.37	25	3
	Hexane	8.1	0.022	20	4
	C_6H_5Cl	5.1	0.21	20	4
	$CHCl_3$	4.7	0.75	20	4
ε-Caprolactam	CCl_4	5.5	4.6	22	5
	Benzene	6.5	26	22	5
2-Pyridone	Dioxane	2.1	13	25	6
1-Cyclohexyluracil	$CHCl_3$	1.5	25	25	This work
9-Ethyladenine	$CHCl_3$	≥ 2	≥ 60	25	This work
1-Cyclohexyluracil-9-ethyladenine	$CHCl_3$	4.0	3.2	20	This work

correspond to relaxation frequencies of about 100 and 200 MHz, respectively.) If these are assumed to be the maximum values of the relaxation times, then the minimum values of the rate constants can be calculated by use of the known equilibrium dimerization constants and eq 4. The calculated minimum values of the association and dissociation rate constants at 10 and 25° are 2×10^9 M^{-1} sec^{-1} and 4×10^8 sec^{-1}, while at 25° the corresponding values are 2×10^9 M^{-1} sec^{-1} and 6×10^8 sec^{-1}. These rate constants are similar in magnitude to those obtained for the dimerization of 1-cyclohexyluracil and are not greater than the theoretical limits for such rate constants.[32] Thus it can be concluded that the failure to detect the dimerization of 9-ethyladenine with ultrasonics is not inconsistent with available equilibrium data.

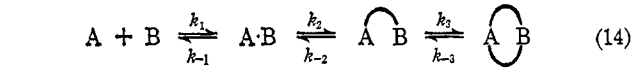
Therefore, in solutions containing both 9-ethyladenine and 1-cyclohexyluracil, three coupled reactions occur, two self-associations and mixed dimer formation. However, if the equilibrium constants of Kyogoku, *et al.*,[21] are assumed, the concentration of the 1-cyclohexyluracil dimer is never greater than 8% of the mixed dimer while that of the 9-ethyladenine dimer is always less than 3% of the mixed dimer concentration. This being the case, a good first approximation is to neglect the self-association reactions entirely. The rate constants obtained by this simple analysis (Table IV) are consistent with the equilibrium data. As a second approximation, the dimerization of 1-cyclohexyluracil can be taken into account since the kinetics of its formation could be studied independently. The rate constants characteristic of the self-association of 1-cyclohexyluracil obtained from this analysis are consistent with the values obtained in independent experiments while the rate constants for mixed dimer formation (Table IV) are slightly larger than those obtained with the simple analysis. This increase is primarily due to the reduction in the concentration of free 1-cyclohexyluracil, rather than to the kinetic coupling; that is, use of the lower equilibrium concentrations in eq 7 gives only slightly smaller rate constants than the more exact analysis. Although the effect on the rate constants is not very pronounced, the magnitude of the standard enthalpy change is significantly increased (see Table IV). An exact analysis of the kinetic problem that includes all three dimerizations requires the solution of a cubic secular equation to obtain the three coupled relaxation times. Each relaxation time would be a function of all six rate constants or three rate constants if the equilibrium constants are assumed known. In view of the complexity of such an analysis and the relatively small amount of data which could be obtained—only one of the three relaxation times over a limited concentration range—this approach was not explored further. Moreover, the effect of the kinetic coupling of the 9-ethyladenine self-association on the calculated rate constants for the mixed dimer formation will almost certainly be negligible based on the effect of the 1-cyclohexyluracil. If the 9-ethyladenine self-association is neglected, an estimate of the amplitude of the unobserved relaxation process associated with τ_1 (eq 11), $A_1\tau_1$, can be made using eq 5 and 11 and eq A-7 (Γ_1) and A-6 (ΔH_1) given in the Appendix. At the highest concentrations, $A_1\tau_1$ is about 25% of $A_2\tau_2$ but τ_1 is sufficiently short so that only about half of the associated relaxation process occurs in the accessible frequency range. Construction of theoretical plots of α/f^2 which include both relaxation processes indicates that the expected deviations from a single relaxation process are of the same order of magnitude as the experimental error. At the lowest concentration, $A_1\tau_1$ is less than 10% of $A_2\tau_2$, which is not detectable experimentally. Thus the failure to observe more than one relaxation time is to be expected.

The standard enthalpy changes for formation of the 1-cyclohexyluracil dimer at 10° and the mixed dimer at 20 and 35° (for the analysis including kinetic coupling) are virtually identical with the values reported by Kyogoku and coworkers.[21] The somewhat high values obtained for the enthalpy change associated with the 1-cyclohexyluracil dimerization at 25° are not very precise because of the small amplitude of the relaxation effect.

A summary of the rate constants associated with the formation of hydrogen-bond-stabilized dimers in systems studied thus far is given in Table V. Regardless of the type of hydrogen bond or solvent, all of the association rate constants are about 10^9 M^{-1} sec^{-1}, which is the approximate value expected for a diffusion-controlled process.[32] The quantitative differences between the rate constants can be attributed to experimental error, and to differences between the diffusion coefficients of the reactants, the effective reaction radii, and the steric factors. Note that all other things being equal, the maximum value of the rate constant for identical reactants is one-half that for nonidentical reactants.[32] Such rate constants would be expected to have activation energies of only a few kilocalories/mole.

(32) I. Amdur and G. G. Hammes, "Chemical Kinetics: Principles and Selected Topics," McGraw-Hill Book Co., New York, N. Y., 1963, p 59 ff.

The association rate constants reported in this work showed little or no temperature dependence, over the very restricted temperature range accessible, which is consistent with a small energy of activation. The mechanism of dimer formation can be depicted as diffusion together of the reactants followed by formation of the first hydrogen bond and finally formation of the second hydrogen bond. Schematically this can be written as



(14)

where k_1 and k_{-1} are the rate constants for diffusion-controlled association and dissociation. Since only one relaxation time is observed, the intermediates can be assumed to be present in very small concentrations, and the steady-state approximation can be employed to calculate the experimentally determined rate constant as a function of the rate constants in eq 14. The association rate constant is

$$k_f = \frac{k_1}{1 + \frac{k_{-1}}{k_2}\left(1 + \frac{k_{-2}}{k_3}\right)}$$

Since it is observed experimentally that $k_f \approx k_1$, this implies $k_2 \gg k_{-1}$, or once the reactants have diffused together, the rate of formation of the first hydrogen bond is rapid compared to the rate of diffusion apart of the monomers. Since k_{-1} can be estimated to be about 10^{10} sec^{-1}, the rate constant for intermolecular hydrogen-bond formation, k_2, is probably about 10^{11}–10^{12} sec^{-1}. For the limiting case where $k_f \approx k_1$, the reverse rate constant is

$$k_r = k_{-1}\frac{k_{-2}k_{-3}}{k_2k_3}$$

The rate of diffusion apart of the monomers characterized by the rate constant k_{-1} is about the same for all reactants and solvents so that the relative stabilities of the dimers are determined by the equilibrium constants for the last two steps of the mechanism, or in other words by the equilibrium competition between solute–solute hydrogen bonds and solute–solvent interactions.

The details of the mechanism of hydrogen-bond formation in water remain an open issue. It might be expected that some of the intermediate states in eq 14 would be stabilized. From a biological standpoint, stable hydrogen bonds are usually found in a nonaqueous-like environment well shielded from water so that the results obtained in nonaqueous solvents might be quite relevant. Certainly in the case of the free nucleoside bases in water, association *via* vertical stacking of rings rather than *via* hydrogen bonding occurs,[33] whereas hydrogen bonding is of crucial importance in the structure of deoxyribonucleic acid in water. In any event, the available kinetic data confirm the fact that hydrogen-bond reactions occur rapidly enough to accommodate the most rapid transformations found to occur in nucleic acids and proteins.

Appendix

General methods for calculating coupled relaxation times and normal concentration and thermodynamic variables are available,[26] so that only an outline of the application of these methods to the problem of interest here is presented. Rate equations for the coupled reaction mechanism of eq 10 can be linearized in the neighborhood of equilibrium to give

$$\begin{aligned} dx_2/dt &= a_{11}x_2 + a_{12}x_3 \\ dx_3/dt &= a_{21}x_2 + a_{22}x_3 \end{aligned} \qquad \text{(A-1)}$$

where the a_{ij}'s have been previously defined and the x's are the deviations from equilibrium of the concentrations of the reacting species. More precisely, $x_1 = \delta U$, $x_2 = \delta U_2$, $x_3 = \delta AU$, and $x_4 = \delta A$ where δ designates the deviation from equilibrium. The rate equations (eq A-1) can be written as

$$d\mathbf{X}/dt = \mathbf{AX} \qquad \text{(A-2)}$$

where **X** is the column matrix of x's and **A** is the 2×2 matrix made up of the a_{ij}'s. By the usual normal coordinate transformation, eq A-2 can be transformed to

$$d\mathbf{y}/dt = \mathbf{By} \qquad \text{(A-3)}$$

where **y** is the column matrix of the eigenvectors or normal concentration variables and **B** is a diagonal matrix of the negative reciprocal relaxation times. The transformation matrix M can be defined such that

$$\mathbf{y} = \mathbf{MX}$$

$$\mathbf{B} = \mathbf{MAM}^{-1}$$

Direct computation gives[8]

$$\mathbf{M} = \begin{bmatrix} 1 & -\left[\dfrac{(1/\tau_1) + a_{11}}{a_{21}}\right] \\ \dfrac{a_{21}}{a_{11}} & -\left[\dfrac{(1/\tau_2) + a_{11}}{a_{11}}\right] \end{bmatrix}$$

The relaxation times are given by eq 11 and the normal concentration variables are

$$\begin{aligned} y_1 &= x_2 - [(\tau_1^{-1} + a_{11})/a_{21}]x_3 \\ y_2 &= (a_{21}/a_{11})x_2 - [(\tau_2^{-1} + a_{11})/a_{11}]x_3 \end{aligned} \qquad \text{(A-4)}$$

The over-all enthalpy change for the reaction is

$$\Delta H = y_1\Delta H_1 + y_2\Delta H_2 = \bar{H}_U x_1 + \bar{H}_{U_2}x_2 + \bar{H}_{AU}x_3 + H_A x_4 \qquad \text{(A-5)}$$

where the ΔH_i's ($i = 1$ or 2) are the normal enthalpy changes for reactions 1 and 2 of eq 10 and the H_i's are the partial molar enthalpies of the species shown as subscripts. Combination of eq A-4 and A-5 with mass conservation relationships gives ΔH_2 according to eq 13 and

$$\Delta H_1 = \frac{\Delta H^\circ_{AU} + \left(\dfrac{1/\tau_1 + a_{11}}{a_{21}}\right)\Delta H^\circ_{UU}}{(1/\tau_2 - 1/\tau_1)/a_{21}} \qquad \text{(A-6)}$$

where $\Delta H^\circ_{AU} = \bar{H}_{AU} - \bar{H}_A - \bar{H}_U$ and $\Delta\bar{H}_{UU} = \bar{H}_{U_2} - 2\bar{H}_U$. The quantity Γ_2 in eq 12 is equal to $-RT(\partial y_2/\partial A_2)_{T,P,y_1}$ and was evaluated by use of the eigenvectors and normal affinity which is defined analogous to the

(33) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *J. Am. Chem. Soc.*, 86, 4182 (1964); M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *ibid.*, 87, 5241 (1965).

normal enthalpy (eq 13). The corresponding expression for Γ_1 in this case is

$$\Gamma_1 = -RT\left(\frac{\partial y_1}{\partial A_1}\right)_{T,P,y_2} = \left\{\frac{1/\tau_2 - 1/\tau_1}{a_{21}^2}\right\}\times \left\{\left[\frac{1}{(AU)(1/\tau_2 + a_{11})} + \frac{1}{(A)(1/\tau_2 + a_{11})} + \frac{(1/\tau_2 + a_{11} + a_{21})(2/\tau_2 + 2a_{11} + a_{21})}{(U)a_{21}^2(1/\tau_2 + a_{11})} + \frac{(1/\tau_2 + a_{11})}{(U_2)a_{21}^2}\right]^{-1} - \left[\frac{1}{(AU)(1/\tau_1 + a_{11})} + \frac{1}{(A)(1/\tau_1 + a_{11})} + \frac{(1/\tau_2 + a_{11} + a_{21})(2/\tau_2 + 2a_{11} + a_{21})}{(U)a_{21}^2(1/\tau_1 + a_{11})} + \frac{(1/\tau_2 + a_{11})^2}{(U_2)a_{21}^2(1/\tau_1 + a_{11})}\right]^{-1}\right\} \quad \text{(A-7)}$$

where A_1 is the normal chemical affinity associated with y_1.

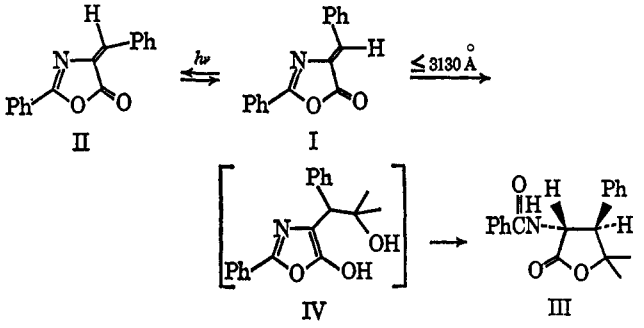
Communications to the Editor

Wavelength-Dependent Photochemical Reactions of Lactones

Sir:

During a study of potential photochemical ring atom transposition reactions in heterocycles, the photochemical behavior of 3-benzal-5-phenylazlactone (I) was found to display a marked wavelength dependence. As there are relatively few authentic examples of wavelength-dependent reactions in solution involving excitation of only one species,[1] the photochemistry of this compound and simpler related esters has been examined.

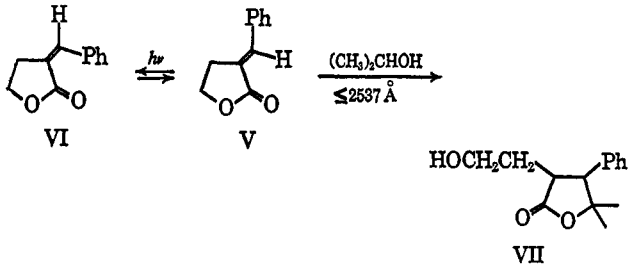
Irradiation of I [$\lambda_{max}^{i\text{-PrOH}}$ 259 (ϵ 26,500), 346 (32,000), 361 (42,000), and 381 mμ (30,000)] in degassed isopropyl alcohol with 3650-Å light led to diminution of the long-wavelength maximum with the appearance of isosbestic points at λ 345, 355, 364, and 377 mμ. After the rapid initial spectral changes no further changes were observed on prolonged irradiation. The only components of the reaction mixture were I and a previously known geometrical isomer II.[2] Irradiation of II with 3650-Å light gave the same photostationary mixture.



Irradiation of the azlactone I in isopropyl alcohol with 2537-Å light led to spectroscopic changes characteristic of the above geometric isomerization plus a gradual disappearance of absorption throughout the ultraviolet region. A similar result was observed with 3130-Å light although the rate of azlactone disappearance was reduced relative to the isomerization reaction. The major product, isolated in 17% yield, was the adduct III: ν_{NH} 3430, ν_{COO} 1757, and ν_{CON} 1667 cm^{-1}; nmr ($CDCl_3$) τ 8.48 and 8.92 ($2CH_3$), 6.2 (PhCH, d, J = 13 Hz), 4.5 (O=CCH, 2d, J = 13 Hz, J' = 8 Hz), and 1.06 (NH, d, J' = 8 Hz); *m/e* 309 (M), 265 (M − CO_2), and 223 (M − CO − $(CH_3)_2CO$).

A plausible pathway for the formation of III is through hydrogen abstraction from solvent by the excited azlactone I (or II) followed by combination of the resulting radical pair to give IV which subsequently lactonizes. This interpretation is supported by the finding that the rate of disappearance of the azlactones using 2537-Å light was related to the availability of solvent hydrogen, *i.e.*, isopropyl alcohol > ether > cyclohexane ≫ acetonitrile. Moreover, the reaction was inhibited by trace amounts of oxygen and yielded numerous side products and polymeric products characteristic of a free-radical mechanism.

The photochemistry of α-benzal-γ-butyrolactone (V) [$\lambda_{max}^{i\text{-PrOH}}$ 218 (ϵ 12,400), 224 (10,700), and 281 mμ (24,000)] was analogous to that of the azlactone I. On irradiation of V in isopropyl alcohol with 3130-Å light, only geometrical isomerization was observed (isosbestic points at 226, 244, and 300 mμ). Thermal (160°) re-formation of V from the isomer VI and deshielding of the olefinic hydrogen of V (τ 2.45) relative to VI (τ 3.01)[3] confirm the assigned stereochemistry.



(1) Reports of reactions yielding qualitatively different products with different wavelengths include: (a) N. C. Yang, N. Nussim, M. J. Jorgenson, and S. Murov, *Tetrahedron Letters*, 3657 (1964); (b) R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, **88**, 509 (1966); (c) E. F. Ullman and B. Singh, *ibid.*, **88**, 1844 (1966); **89**, 6911 (1967); (d) J. Streith and J. Cassal, *Comp. Rend. Ser. C*, **264**, 1307 (1967).

(2) Speculative stereochemical assignments for I and II based on melting point comparisons (R. E. Buckles, R. Filler, and L. Hilfram, *J. Org. Chem.*, 17, 233 (1952)) are contrary to the present tentative assignments based on near-quantitative thermal conversion (150°) of II to I.

(3) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p 124.