Kinetic and Thermodynamic Studies of Hydrogen Bonding[1]

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Abstract: Rate constants and standard enthalpy and entropy changes for the formation of dimers stabilized by hydrogen bonds of the type N–H···O, N–H···S, and N–H···N have been determined in chloroform from ultrasonic attenuation measurements over the frequency range 15–175 MHz. The self-association of 2-pyridone, 2-thiopyridine, mephobarbital, and thiopental and the association of 9-ethyladenine with mephobarbital and thiopental have been studied. The 2-pyridone dimer is considerably more stable and has a more negative heat of formation than the 2-thiopyridine dimer; this reflects the relative stability of N–H···O *vs.* N–H···S hydrogen bonds. The enthalpy changes associated with the self-association of mephobarbital and thiopental are significantly less negative than those associated with mixed-dimer formation. An unusually large standard enthalpy change for formation of the thiopental–9-ethyladenine dimer has been interpreted in terms of a structure involving three hydrogen bonds. All of the association rate constants have values characteristic of a diffusion-controlled process ($\sim 10^9\ M^{-1}\ \text{sec}^{-1}$), so that the dissociation rate constant is a direct measure of the stability of the hydrogen bonds. The estimated rate constant for the formation of the first hydrogen bond after the molecules have diffused together, 10^{11}–10^{12} sec^{-1}, is similar to the rate constant for intramolecular hydrogen bond formation in poly-L-ornithine in water–methanol solutions.

A wide variety of hydrogen bonds occur in biological systems; for example, hydrogen bonding is of crucial importance in the structure of DNA and double-stranded RNA and in the maintenance of protein structures. Numerous sulfur compounds, especially the thiobarbiturates, have found widespread application as medicinal agents. Recently Kyogoku and coworkers[2] have shown that of the four bases involved in the double-stranded helix of DNA or RNA (adenine, uracil (thymine), guanine, and cytosine), only adenine associates to any significant extent with a large number of barbiturates. This preferential binding of adenine to the barbiturates is considered to be due to the fact that the structures of the barbiturates closely resemble the structure of uracil (or thymine) which is the complementary base of adenine in the nucleic acids. In order to characterize and understand the nature of these hydrogen-bonding interactions, detailed thermodynamic and kinetic data are needed for reactions involving many different types of hydrogen bonds. Although a large accumulation of thermodynamic data is available,[3] very few investigations of the kinetics of hydrogen bonding have been made. A summary of the available kinetic data has been given in an earlier paper.[4]

In this work, thermodynamic parameters and rate constants have been determined from ultrasonic studies in chloroform for the self-association of 2-pyridone, 2-thiopyridine, mephobarbital, and thiopental, and for the formation of the mixed dimers mephobarbital–9-ethyladenine and thiopental–9-ethyladenine. Many of the types of hydrogen bonds of importance in biological systems are involved in the dimers studied. The relationship of these results to more complex biological processes is considered.

Experimental Section

Materials. Chloroform (Mallinckrodt AR) and 9-ethyladenine (Cyclo Chemical Co.) were purified as previously described.[4] All solutions were made up by weight, with freshly prepared chloroform, just prior to use.

Mephobarbital obtained from Winthrop Laboratories (New York, N. Y.) was recrystallized from the pure chloroform, mp 176.5–177.5° (lit.[5] 176°). Thiopental was obtained as the sodium salt (pentothal) from Abbott Laboratories (Chicago, Ill.). The free acid precipitated when an aqueous solution of the salt was neutralized with hydrochloric acid. The precipitate was filtered, washed several times with water, and three times recrystallized from chloroform. The pure thiopental melted over the temperature range 156.5–157.5° (lit.[6] 156–157°). Practical grade 2-pyridone (J. T. Baker Chemical Co.) and 2-thiopyridine (K & K Laboratories) were purified by several recrystallizations from benzene. The 2-thiopyridine crystallized in the form of yellow plates, mp 132.5–133.5° (lit.[7] 128°). The initial benzene solution of 2-pyridone was brown in color and contained some insoluble material. This was treated with decolorizing charcoal and filtered, and the 2-pyridone was precipitated. The crystals obtained were dissolved in fresh benzene and further treatment with decolorizing charcoal produced a clear solution from which 2-pyridone precipitated as colorless needles, mp 110.5–111.5° (lit.[8] 106–107°).

Procedure. The apparatus and procedure for making ultrasonic measurements have been previously described.[9] For the purpose of obtaining the association constant for the dimerization of 2-pyridone, spectra of 2-pyridone solutions were determined using a Cary 14 and a Zeiss PMQ II spectrophotometer. Absorbancy readings were taken over as wide a concentration range as possible using cells which varied from 1 mm to 10 cm in path length. All spectral experiments were conducted at 25 ± 0.1° with the slit width maintained at 0.1 mm.

(1) This work was supported by a grant from the National Institutes of Health (GM 13292).

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Results and Treatment of Data

In all of the solutions studied the ultrasonic data were consistent with the assumption of a single relaxation process and therefore could be described by the equation[10]

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

where α is the ultrasonic absorption coefficient, f is the frequency, A and B are constants, ω $(=2\pi f)$ is the angular frequency, and τ is the relaxation time. The estimated experimental error in α/f^2 is approximately $\pm 2\%$ in the frequency range covered, 15–175 MHz. Alternatively, eq 1 can be written in terms of the chemical absorption per wavelength,[11] μ_{ch}

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

where v is the ultrasonic velocity and μ_m is equal to $Av/2\pi$. A plot of μ_{ch} *vs.* ω goes through a maximum when $\omega = 1/\tau$. Where possible, the ultrasonic parameters were obtained using the template technique of Piercy and Subrahmanyam.[12] In order to use this technique, a reliable estimate of the parameter B (the infinite frequency value of α/f^2) must be made. For very short relaxation times, B is quite difficult to obtain directly since the measured values of α/f^2 do not reach a plateau at high frequencies. In this case, eq 1 can be written as

$$(\alpha/f^2 - B)^{-1} = 1/A\tau + (\tau/A)\omega^2 \qquad (3)$$

and a weighted least-squares analysis of the data using $(\alpha/f^2 - B)^{-1}$ and ω^2 as variables can be carried out. Using a program developed by Hammes and Spivey,[13] computer calculations were cycled through specified increments in B about the approximate value. The value of B producing the minimum standard deviation in τ was taken as giving the best fit of the data. Initial estimates of B were obtained by extrapolation of a plot of B *vs.* concentration since B usually can be precisely obtained in sufficiently dilute solutions. As a check on this analysis, data for some solutions for which B could be obtained accurately by inspection were analyzed by the weighted least-squares method and the template method; the same ultrasonic parameters were found within 1%. Some typical plots of the data according to eq 2 are given in Figure 1, and a summary of the ultrasonic parameters obtained for mephobarbital, mephobarbital-9-ethyladenine, thiopental, thiopental-9-ethyladenine, 2-pyridone, and 2-thiopyridine are given in Table I. The estimated error is $\pm 10\%$ in τ, $\pm 15\%$ in μ_m, $\pm 3\%$ in B, and $\pm 0.5\%$ in v. In all cases the temperature and concentration ranges investigated were picked so as to make a major portion of the relaxation curve lie in the accessible frequency range.

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Table I. Ultrasonic Parameters

C_0, M	$10^9\tau$, sec	$10^3\mu_m$	$10^{-5}v$, cm/sec	$10^{17}B$, sec²/cm
		Mephobarbital, 25°		
0.093	1.3	4.5	1.000	390
0.053	1.5	3.3	1.000	426
0.093[a]	2.6	3.4	1.047	360
0.053[a]	3.0	2.1	1.048	397
		Mephobarbital-9-ethyladenine, 25°		
0.217	3.8	11.0	1.023	266
0.155	4.9	9.4	1.013	307
0.105	5.8	8.3	1.009	347
0.052	7.4	6.7	1.001	391
		Thiopental, 10°		
0.161	2.1	8.6	1.054	298
0.130	2.3	6.8	1.056	322
0.103	2.5	4.5	1.050	341
0.057	2.9	2.5	1.046	381
		Thiopental-9-ethyladenine, 25°		
0.200	6.8	19.7	1.014	290
0.150	7.9	16.9	1.010	308
0.100	9.3	14.0	1.010	357
		2-Pyridone, 25°		
0.500	2.3	9.6	1.028	350
0.352	2.7	8.1	1.023	383
0.251	3.3	6.4	1.020	412
0.151	4.0	5.6	1.020	435
0.101	5.3	4.4	1.010	452
		2-Thiopyridine, 10°		
0.576	1.7	9.5	1.076	312
0.393	2.1	8.8	1.068	346
0.298	2.4	7.6	1.063	366
0.199	2.9	6.6	1.055	386
0.102	4.0	4.4	1.051	410
0.047	5.6	3.0	1.040	432

[a] At 10°.

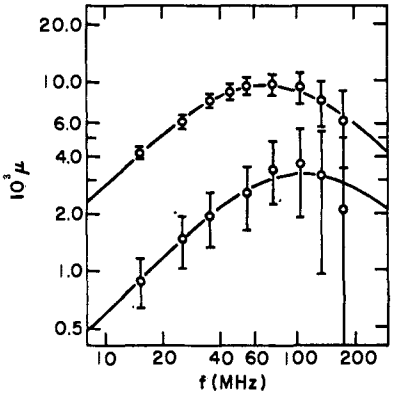

Figure 1. Typical plots of chemical absorption per wavelength, μ_{ch}, as a function of frequency, f. Bottom: 0.053 M mephobarbital in chloroform at 25°. Top: 0.500 M 2-pyridone in chloroform at 25°. The lines are calculated by use of eq 2 and the parameters in Table I. The standard deviations shown correspond to $\pm 2\%$ error in the absorption coefficient, α.

In solutions containing only one solute the relaxation process can be attributed to the reaction

$$A + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A_2 \qquad (4)$$

where A designates mephobarbital, thiopental, 2-pyridone, or 2-thiopyridine. For these cases of self-association the reciprocal relaxation time is[11]

Table II. Rate and Thermodynamic Constants for Hydrogen Bond Dimerization

Reactants	$10^{-9}k_1$ M^{-1} sec^{-1}	$10^{-7}k_{-1}$ sec^{-1}	K M^{-1}	$-\Delta H°$, kcal/mol	$-\Delta S°$, eu	Temp, °C
2-Pyridone	2.2	2.2	100	5.9	10.7	25
2-Thiopyridine	1.5	4.7	32	4.5	9.0	10
			22			25
Mephobarbital	0.75	20	3.7	4.2	12.1	10
	1.1	48	2.3[a]	5.1	15.5	25
				Av 4.7	13.8	
Thiopental	0.57	23	2.5	4.7	14.8	10
			1.7			25
Mephobarbital–9-ethyladenine	3.9	2.0	200[a]	5.9		25
	4.5[b]	2.2[b]		6.6[b]	11.6[b]	
Thiopental–9-ethyladenihe	4.0	0.67	600[a]	9.7		25
	4.3[b]	0.72[b]		9.8[b]	20.2[b]	

[a] Reference 2. [b] Analysis including kinetic coupling.

$$1/\tau = k_{-1} + 4k_1\bar{C}_A \quad (5)$$

where $\bar{C}_A$ is the equilibrium concentration of the monomer. Equation 5 can be squared and rearranged to give

$$1/\tau^2 = k_{-1}^2 + 8k_1k_{-1}C_{0_A} \quad (6)$$

where C_{0_A} is the total concentration of the particular solute.

The amplitude parameter, μ_m in eq 2, is a function of thermodynamic variables only, and for the reaction of eq 4 under the experimental conditions employed is given to a good approximation by[11]

$$\mu_m = (\rho v^2\pi\Gamma/10^3RT)[\Delta V° - \beta\Delta H°/\rho c_P]^2 \quad (7)$$

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 reactions, β is the coefficient of thermal expansion of the solvent, and c_P is the constant pressure specific heat of the solvent. For the self-association reaction of eq 4

$$\Gamma = \frac{1}{8K}\left[\frac{1 + 4KC_{0_A}}{(1 + 8KC_{0_A})^{1/2}} - 1\right] \quad (8)$$

where K is the dimerization association constant. In nonaqueous solvents, $\Delta V° \ll \beta\Delta H°/\rho c_P$ so that $\Delta H°$ for the reactions can be calculated from μ_m at each concentration. Although the sign of $\Delta H°$ cannot be ascertained from ultrasonic data because it is obtained as a square root, $\Delta H°$ can be safely assumed to be negative for all systems under consideration here.

According to eq 6, a plot of $1/\tau^2$ *vs.* C_{0_A} will have a slope of $8k_1k_{-1}$ and an intercept of k_{-1}^2. The plot for 2-thiopyridine at 10° is given in Figure 2 and the values of the forward and reverse rate constants and the equilibrium constant obtained from a least-squares analysis of the data are given in Table II together with the average $\Delta H°$ value for the reaction calculated by use of eq 7. Because of the smallness of the intercept of the $1/\tau^2$ *vs.* C_{0_A} plot (see Figure 2), the rate and equilibrium constants cannot be considered more reliable than ±30%, and the estimated error in $\Delta H°$ is ±0.5 kcal/mol.

A similar plot of $1/\tau^2$ *vs.* C_{0_A} for the thiopental data at 10° is also shown in Figure 2. A much narrower concentration range was covered in this case, relative to 2-thiopyridine, due to the very small amplitude

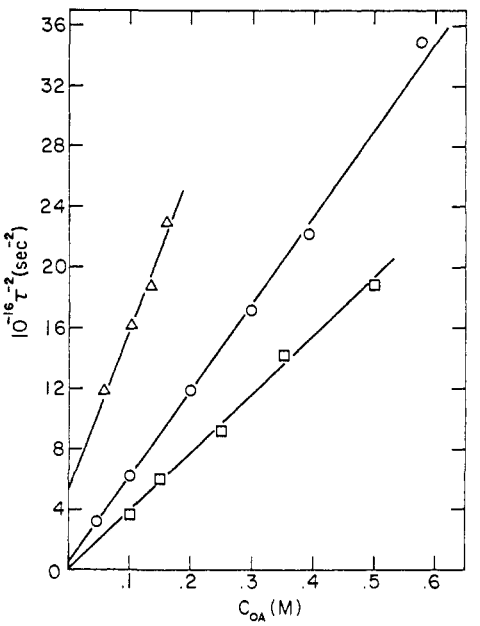

Figure 2. Square of the reciprocal relaxation time, τ^{-2}, as a function of the concentration, C_{0_A}, for 2-thiopyridine at 10° (○), thiopental at 10° (△), and 2-pyridone at 25° (□) in chloroform. The lines were obtained from a least-squares analysis of the data.

($A\tau$ in eq 1) of the process at low concentrations and to the extreme rapidity of the reaction at concentrations greater than 0.16 *M*. At high concentrations at 10° the relaxation frequency ($=1/2\pi\tau$) was too high (>100 MHz) to be estimated with any degree of accuracy even by the weighted least-squares method. This was the case for all concentrations at 25° where the amplitude of the relaxation process was measurable. This problem arises due to the over-all instability of the complex which causes the relaxation time to be exceedingly short. One favorable consequence of the unstable nature of the thiopental dimer is that the reverse rate constant, k_{-1}, for the reaction is relatively large so that a well-defined intercept of the plot in Figure 2 is obtained. However, the very limited concentration range covered causes the rate and equilibrium constants to be no more reliable than for 2-thiopyridine (±30%). These constants together with the average $\Delta H°$ value (±0.5 kcal/mol) for the self-association of thiopental are included in Table II.

The equilibrium constant for the self-association of mephobarbital at 25° was determined by Kyogoku and coworkers from infrared studies.[2] A saturated solution of mephobarbital in chloroform at 25° is only approximately 0.1 *M*; due to the small amplitude of the relaxation effect below 0.05 *M*, ultrasonic data were only obtained at two concentrations. In this case meaningful rate constants cannot be obtained from a plot of $1/\tau^2$ *vs.* C_{0_A}. However, by use of the equilibrium constant of Kyogoku and coworkers and eq 5, the rate constants can be calculated at each concentration. Due to the very small amplitude ($A\tau$) of the relaxation process at the low concentration at 25°, a reliable $\Delta H°$ was only obtained for the 0.093 *M* mephobarbital solution at this temperature. If this $\Delta H°$ is used to estimate the association constant for the dimerization of mephobarbital at 10°, rate constants can also be calculated from the two measured relaxation times at this lower temperature. Standard enthalpy changes can be estimated for both solutions at 10° (since a larger amplitude is obtained at the lower temperature), and the average of these $\Delta H°$ values together with the $\Delta H°$ value for the 0.093 *M* solution at 25° and the average rate constants obtained at both temperatures are included in Table II. Allowing for possible error in the equilibrium constant, the average rate constants at 25° are probably precise to ±20% while those at 10° are somewhat less reliable due to the additional uncertainty in $\Delta H°$. The Arrhenius activation energies for the forward and reverse rate constants can be estimated as 4 and 9 kcal/mol, respectively.

A plot of $1/\tau^2$ *vs.* C_{0A} for solutions of 2-pyridone at 25° has too small an intercept to be measured (see Figure 2). Therefore, an independent estimate of the association constant is necessary before the kinetic parameters can be obtained from the ultrasonic data. Absorbancy measurements were made of 2-pyridone solutions over as wide a concentration range as possible at a wavelength where the change in absorbance with concentration was large (327 mμ). As shown in Figure 3, a plot of optical density *vs.* concentration is linear over the concentration range 10^{-5}–6×10^{-4} *M* and shows increasingly large deviations from this initial linearity up to the highest concentration studied, 1×10^{-2} *M*. If the line through the linear section of the curve at low concentrations is extended to the region of high concentration (see Figure 3) the difference between the extrapolated and the observed absorbance, Δa, can be measured and related to the concentration of 2-pyridone dimer, $\bar{C}_{A_2}$, by the equation[14]

$$\epsilon_A C_{0_A} - a = \Delta a = (\bar{C}_{A_2})\Delta\epsilon \qquad (9)$$

A 1-cm path length has been assumed, and $\Delta\epsilon$ is the molar difference extinction coefficient, $2\epsilon_A - \epsilon_{A_2}$, where ϵ_A is the extinction coefficient of the monomer and ϵ_{A_2} is that of the dimer. The dimerization association constant, *K*, can therefore be written as

$$K = \frac{\Delta a/\Delta\epsilon}{(C_{0_A} - 2\Delta a/\Delta\epsilon)^2} \qquad (10)$$

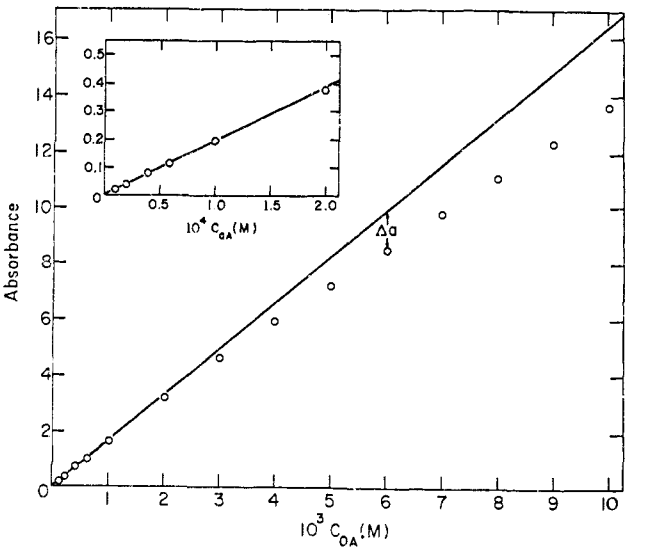

Figure 3. Plot of absorbance at 327 mμ *vs.* concentration for solutions of 2-pyridone in chloroform at 25°. All absorbance values refer to a path length of 1 cm. The actual measured absorbance was always less than 2. The straight line through points at low concentrations (see inset in upper left-hand corner), where no appreciable amount of dimer exists, is extended to regions of high concentration and Δa is the difference between the extrapolated absorbance (solid line) and the actual absorbance (○).

If a valuc is assumed for $\Delta\epsilon$, *K* can be calculated at each concentration using the measured values of Δa. The best value of $\Delta\epsilon$ was taken to be that which minimized the standard deviation (as per cent) in *K*. The value of *K* obtained in this manner is given in Table II together with the forward and reverse rate constants obtained by use of *K* and eq 5. The error in the value reported for *K* and in the average values of the rate constants is estimated to be ±20% (although the standard deviations are considerably less than this), while that in the average enthalpy change (also given in Table II) is ±0.5 kcal/mol.

For equimolar mixtures of 9-ethyladenine with mephobarbital or thiopental the data are also consistent with the assumption of a single relaxation process. The relaxation processes associated with the self-association of mephobarbital and thiopental could not be detected, presumably because of their relatively small amplitudes. The maximum concentration of the mephobarbital dimer relative to that of the mixed dimer was about 1% while that of the thiopental dimer was less than 0.3% if the equilibrium constants of Kyogoku and coworkers[2] are assumed for the formation of the two mixed dimers and the mephobarbital dimer, and the constant obtained in this work is assumed for the dimerization of thiopental. (The dimerization constant for thiopental at 25° calculated by use of $\Delta H°$ and the estimated constant at 10° is included in Table II.) Kyogoku and coworkers[15] found the self-association constant of 9-ethyladenine in chloroform to be 3.0 M^{-1} at 25° so that the per cent concentration of 9-ethyladenine dimers is about the same as for mephobarbital and thiopental in solutions of the mixtures.

As a first approximation, we neglect the self-

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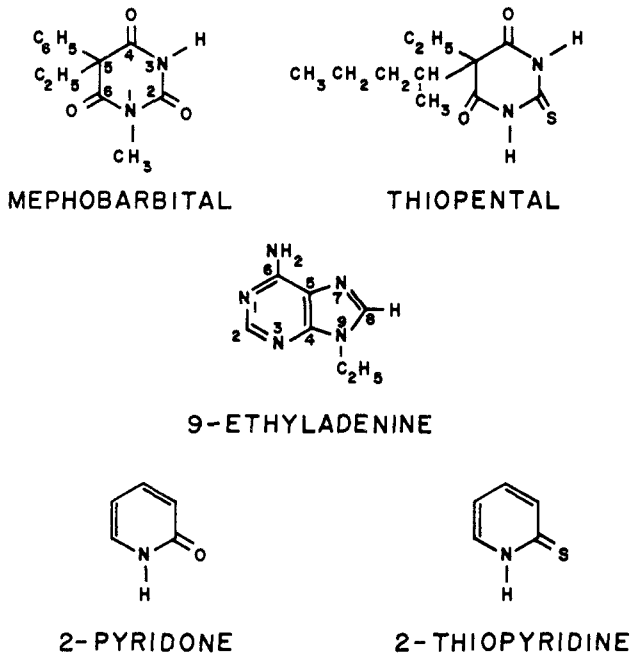

Figure 4. Structural formulae of molecules used in these studies of hydrogen bonding. The ring-numbering system is shown for the six-membered rings on mephobarbital and for 9-ethyladenine.

dimerization of mephobarbital and thiopental and consider only the reaction

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB \qquad (11)$$

where A designates mephobarbital or thiopental and B designates 9-ethyladenine. The reciprocal relaxation time for this reaction is[11]

$$1/\tau = k_{-1} + k_1(\bar{C}_A + \bar{C}_B) \qquad (12)$$

where $\bar{C}_A$ and $\bar{C}_B$ represent the equilibrium concentrations of the monomers. The known equilibrium constants were used to evaluate $\bar{C}_A$ and $\bar{C}_B$ for both mixtures at each concentration. By use of the association constants for the two mixed dimers, k_{-1} was eliminated from eq 12 and k_1 was calculated for the dimerization of mephobarbital–9-ethyladenine and thiopental–9-ethyladenine at each concentration. The average values of the four rate constants are given in Table II. Standard enthalpy changes can be calculated using eq 7 with

$$\Gamma = \frac{1}{2K}\left[\frac{1 + 2KC_0}{(1 + 4KC_0)^{1/2}} - 1\right] \qquad (13)$$

where C_0 is the total concentration of either of the components of a particular equimolar mixture. The calculated value of $\Delta H°$ for each reaction is included in Table II.

A more exact treatment of the problem requires that the self-association of the monomers be included in the analysis. As previously discussed the self-association of 9-ethyladenine probably can be neglected for reactions occurring in this time range. However, the relaxation times found for the self-association of mephobarbital and thiopental and those found with solutions containing equimolar mixtures of these compounds with 9-ethyladenine are sufficiently similar to indicate that some kinetic coupling occurs. An exact analysis of this two-step mechanism was carried out using the equations previously derived,[4] and the rate constants and standard enthalpy changes obtained for mephobarbital–9-ethyladenine and thiopental–9-ethyladenine are included in Table II. The estimated uncertainty in the rate constants obtained is ±30% (coupled case) while that in the average $\Delta H°$ values is ±0.5 kcal/mol. Within the experimental uncertainty all of the rate constants and enthalpy changes are identical for both treatments of the data, although inclusion of the coupling is preferable in principle.

Discussion

The kinetic and thermodynamic constants presented in Table II characterize the behavior of a number of compounds which associate by forming hydrogen bonds of the type N–H···O, N–H···S, and N–H···N. Although chloroform is able to form weak hydrogen bonds, solvation effects should not be of great importance in the mechanism of dimerization. The molecular structures of the compounds are depicted in Figure 4. The two simplest compounds studied were 2-pyridone and 2-thiopyridine which differ only in the substitution of a sulfur atom in the latter compound for oxygen at C-2 on the heterocyclic ring. The enthalpy of formation of the 2-pyridone dimer, −5.9 kcal/mol, is about twice as large as the heat of formation of a single N–H···O=C bond in chloroform,[3] in agreement with the expected involvement of two hydrogen bonds in the dimeric structure. The corresponding enthalpy change in dioxane, −1.7 kcal/mol,[13] is considerably less negative due to the greater hydrogen-bonding capability of dioxane relative to chloroform. Since sulfur is less electronegative than oxygen the N–H···S hydrogen bond would be expected to be weaker, and this is reflected in the lower association constant and more positive enthalpy change for the 2-thiopyridine dimerization relative to 2-pyridone. The large decrease in the standard entropy change for formation of these dimers is approximately that expected for the loss in translational entropy accompanying dimerization. The somewhat less negative value found for 2-thiopyridine may reflect the greater librational entropy of the less stable dimer.

The average standard enthalpy change for the self-association of mephobarbital is the same as that for thiopental (see Table II). Due to the presence of a methyl group on N-1, mephobarbital has only two sites for dimer formation while thiopental has four, two of which involve sulfur. Molecular models indicate that all of these sites are sterically possible and the preferred structures of the two self-dimers cannot be assessed from the thermodynamic data. The standard entropy changes for the self-association of the barbiturates are slightly more negative than those for the pyridine derivatives. This may reflect a loss of rotational entropy of the side groups on the barbiturates upon dimerization, in addition to the loss of translational entropy.

The formation of a cyclic dimer between 9-ethyladenine and mephobarbital would be expected to involve an N–H···N and an N–H···O hydrogen bond. Since 9-ethyladenine dimers only involve N–H···N

bonds and mephobarbital only N–H···O bonds, a simple additivity rule would predict that the enthalpy of formation of the mixed dimer is one-half the sum of the enthalpies of the self-association reactions. The enthalpies of formation of 9-ethyladenine and mephobarbital self-dimers are −4.0 and −4.7 kcal/mol, respectively, so that the predicted enthalpy of formation of the mixed dimer is −4.4 kcal/mol, as compared to the measured value of −6.6 kcal/mol. The fact that an additive relationship is not found emphasizes the fact that the stability of hydrogen bonds involves electronic effects which are not only dependent upon the three atoms directly involved in hydrogen bonding. The involvement of electronic effects in preferential hydrogen bonding in structures such as DNA, RNA, and the barbiturates has been pointed out and discussed by Kyogoku and coworkers.[2,15]

The standard enthalpy change for formation of the dimer between 9-ethyladenine and thiopental is unusually large. A possible explanation for this large enthalpy change is that three hydrogen bonds are formed in the dimer involving sulfur, oxygen, and one of the N–H hydrogens on the thiopental and N-7, an amino group hydrogen, and the C-8 hydrogen of 9-ethyladenine (see Figure 4). Katz and Penman[16] have suggested on the basis of nuclear magnetic resonance studies that the C-8 hydrogen of 9-ethyladenine and 9-ethylguanine can form weak hydrogen bonds with dimethyl sulfoxide. Molecular models indicate that a complex with three hydrogen bonds is sterically feasible. The unusually negative standard entropy of association suggests a quite rigid complex is formed, which again is consistent with a structure involving three hydrogen bonds.

Recently Kim and Rich[17] have reported the structure of a crystalline complex containing phenobarbital and a derivative of adenine in which each phenobarbital is doubly hydrogen bonded to two molecules of the adenine derivative. However, a bromine atom on the adenine derivative sterically blocks one of the most favorable hydrogen-bonding sites involving N-7. In contrast to the crystal structure, a 1:1 stoichiometry was found for the complex formed between 9-ethyladenine and phenobarbital in dilute chloroform solutions. Thiopental has the same number of potential hydrogen-bonding sites as phenobarbital, the only differences being the substitution of sulfur for oxygen at position 2 on the ring and an α-methylbutyl group for a phenyl group at position 5 (see Figure 4). A plot of $1/\tau^2$ *vs.* C_0 (eq 6) for solutions containing equimolar mixtures of thiopental and 9-ethyladenine at 25° was a straight line passing near the origin; this behavior is consistent with a 1:1 stoichiometry. However, at 35° a considerable amount of curvature occurred which suggests that higher order complexes, perhaps a 2:1 complex similar to that found by Kim and Rich in their X-ray investigation, are comparable in stability to the 1:1 complex at the higher temperatures.

A summary of the available kinetic data for the formation and dissociation of hydrogen-bond-stabilized dimers was given in the previous paper of this series.[4] In this work, additional rate data have been obtained in chloroform for the formation of dimers which associate by means of a wide variety of hydrogen bonds. Included in these are the first rate constants associated with N–H···S hydrogen bonds. Regardless of the type of hydrogen bond, all of the association rate constants are about $10^9\ M^{-1}\ \text{sec}^{-1}$ which is the approximate value expected for a diffusion-controlled process.[18] The quantitative differences between rate constants at a given temperature can be attributed to experimental error, and to differences between the diffusion coefficients of the reactants, the effective reaction radii, and steric factors. As previously discussed,[4] the fact that the forward rate is diffusion controlled implies that the rate constant for the formation of the first intermolecular hydrogen bond after the reactants have diffused together is 10^{11}–$10^{12}\ \text{sec}^{-1}$.

The dynamic properties of most of the types of hydrogen bonds involved in biological systems have now been studied. The measured rate constants put some limitations on the rates of important biological processes, for example, DNA replication and protein conformational changes. Most of these results have been obtained in relatively inert solvents but such systems may be appropriate models for the biological environments where hydrogen bonding is of importance. Recently, the rate constant characteristic of the formation of a single intramolecular hydrogen bond in the α helix of poly-L-ornithine in water–methanol (85:15) has been found to be about $10^{11}\ \text{sec}^{-1}$,[19] in good agreement with the estimate obtained in this work for intermolecular hydrogen-bond formation in relatively inert solvents. Work is currently in progress to investigate the role of solvents with strong hydrogen-bonding capabilities in the dynamics of solute hydrogen bonding.

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