TABLE I

CORRELATION TIMES AND ACTIVATION ENERGIES FOR REORIENTATION PROCESSES IN FERROCENE AND SOME OF ITS DERIVATIVES

	Compound	Correlation time τ_c, sec.	τ_0, sec.	Activation energy, kcal./mole
I	Ferrocene	68°K., 2.7×10^{-6} 78°K., 1.0×10^{-6}	1.2×10^{-13}	2.3
II	Diferrocenylmercury	75°K., 4.4×10^{-6} 80°K., 1.2×10^{-6}	4.8×10^{-15}	3.1
III	Monocarboxylferrocene	75°K., 6.5×10^{-6} 80°K., 1.3×10^{-6}	4.1×10^{-17}	3.9
IV	Monoperdeuterioacetylferrocene	78°K., 1.9×10^{-6} 82°K., 1.3×10^{-6}	1.6×10^{-17}	4.1
V	Acetylferrocene	(63°K., 4.3×10^{-6} 69°K., 3.4×10^{-7}	9.6×10^{-19} 5.9×10^{-15}	3.7)[a] 4.6
VI	Diperdeuterioacetylferrocene	132°K., 1.3×10^{-6} 135°K., 1.1×10^{-6}	6.0×10^{-16}	5.0

[a] This represents the component for the reorientation processes in the CH_3 group alone.

this type of motion would require the least amount of energy. This situation may be compared to the pure rotational spectra in the infrared which are observed at very long wave lengths in ferrocene.[20] The correlation times τ_c at various temperatures, the constants τ_0 and the activation energies for the motional processes which narrow the line shape are given in Table I for the cases which permitted such calculations.

The correlation times given in Table I are seen to decrease with increasing temperature. This is to be expected if the reorientation is an activated process; that is, the reorientation rate increases as thermal energy is absorbed from the surroundings by the reorienting group. The activation energy for reorientation of the unsubstituted cyclopentadienyl ring (Cp) has its smallest value in the ferrocene molecule where steric and potential hindrances to the reorientation processes are most likely to be at a minimum in comparison to the substituted rings. In diferrocenylmercury where two ferrocene molecules are linked by a mercury atom the activation energy for the unsubstituted Cp ring is larger than that in ferrocene, indicating the restriction of the reorientation process by the steric and potential barriers imposed by the mercury atom. It is seen from Table I that the activation energy increases systematically in going from compounds I to IV, that is, from ferrocene to diperdeuterioacetylferrocene.

(20) E. R. Lippincott and R. D. Nelson, *Spectrochim. Acta*, **10**, 307 (1958).

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The Enthalpy of Interaction of 9-Methyladenine and 1-Methylthymine in Water[1,2]

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The solubility of 9-methyladenine and 1-methylthymine has been determined in pure water and in water solutions of the second compound over a temperature range of 20–40°. An enhancement of solubility is noted when mixed solutions are used. The enhancement is interpreted as a direct consequence of the interaction of these compounds in water. Simple theoretical considerations enable one to estimate the enthalpy of interaction from the solubility measurements. A value of -7.3 ± 0.2 kcal./mole for complex formation is found. This value suggests that purine–pyrimidine interactions in nucleic acids are somewhat stronger than previous rough guasses of -5 ± 2 kcal./mole of complex.

Introduction

The helical ladder structure of naturally occurring deoxyribonucleic acid is a consequence of purine and pyrimidine base pairing.[3] The stability of this configuration is dependent primarily upon the energy of interaction between the base pairs. Little experimental data exist for the energy of interaction of nucleic acid base pairs. The aqueous solvent system virtually eliminates the determination of low concentrations of a hydrogen bond complex by spectroscopic techniques. Calorimetric measurements have been made by Sturdevant, Rice and Geiduschek on the acid denaturation of DNA,[4] but these total heat effects must be interpreted in the light of ionization, base pair interactions and electrostatic charge interactions.[5] In order to delineate the magnitude of these effects, accurate information is first needed on the heats of interaction of the base pairs. The estimate used by Rice, Wada and Geiduschek for an average enthalpy of formation (per base pair) of 5000 ± 2000[6] calories points out the desirability for more refined experimental determinations.

We have studied the model system base pair of 9-methyladenine (9-MA) and 1-methylthymine (1-MT). In these compounds the methyl group takes the place of the ordinary deoxyribose attachment. The relatively low solubility in water of these derivatives almost precludes a direct calorimetric and activity coefficient study. However, the solubility of these compounds in water and in solutions of each other does provide a route for the measurement of heats of interaction in water. A related technique has been used in estimating enthalpy of interaction of urea with the peptide groups of diketopiperazine.[7] However, the low solubility of the purine and pyrimidine base compounds required a more sensitive measuring technique than had been previously employed.

Theory.—The heat of solution $\bar{H}_2 - H_2^S$ is related to the equilibrium solubility of the compound in terms of the temperature derivative of the solubility S_2, expressed for experimental convenience in terms of weight per cent, and a concentration derivative of the activity coefficient γ at the given temperature of saturation. Williamson[8] gives various forms of this relation. For our purposes we shall write

$$\bar{H}_2 - H_2^S = -R \left(\frac{\partial \ln S_2}{\partial(1/T)}\right)_{sat} \left[1 + \left(\frac{\partial \ln \gamma}{\partial \ln w_2}\right)_T\right] \quad (1)$$

where w_2 represents the weight per cent concentration

(1) This work was supported by the Division of General Medical Sciences, Public Health Service.

(2) Part of a thesis submitted by D. B. Martin in partial fulfillment of a Master of Science Degree, University of Colorado, 1962.

(3) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737, 964 (1953).

(4) J. M. Sturdevant, S. A. Rice and E. P. Geiduschek, *Discussions Faraday Soc.*, **25**, 138 (1958).

(5) S. A. Rice, A. Wada and E. P. Geiduschek, *ibid.*, **25**, 130 (1958).

(6) M. M. Davis, *Ann. Report Prog. Chem.*, **43**, 1 (1946).

(7) S. J. Gill, J. Hutson, J. R. Clopton and M. Downing, *J. Phys. Chem.*, **65**, 1432 (1961).

(8) A. T. Williamson, *Trans. Faraday Soc.*, **40**, 421 (1944).

near saturation. For solutions of slightly soluble nonelectrolyte compounds we expect the activity coefficient derivative to be no more than a very small fraction of unity and therefore for such conditions disregard that term.[9]

The heat of solution of the adenine derivative in water is designated by $\bar{H}_A{}^W - H_A{}^S$, and in a water–methylthymine solvent system by $\bar{H}_A{}^{WT} - H_A{}^S$. These heats are determined by solubility measurements as indicated in eq. 1. The difference between these respective determinations is attributed to the enthalpy of interaction of methyladenine and methylthymine in solution. Suppose a fraction f of the methyladenine interacts with the methylthymine in water, then the heat of solution may be represented by

$$\bar{H}_A{}^{WT} - H_A{}^S = (1 - f)(\bar{H}_A{}^W - H_A{}^S) + f(\bar{H}_{AT}{}^W - \bar{H}_T{}^W - H_A{}^S) \quad (2)$$

The expression in the last parentheses to the right denotes the enthalpy change in treating solid methyladenine with aqueous methylthymine to give the methyladenine–methylthymine complex (AT) in water. This equation reduces to

$$\bar{H}_A{}^{WT} - \bar{H}_A{}^W = f(\bar{H}_{AT}{}^W - \bar{H}_T{}^W - \bar{H}_A{}^W) = f\Delta H_0 \quad (3)$$

where ΔH_0 is the heat of interaction in water. The left-hand side is experimentally determined from the solubility measurements of methyladenine in water and water–methylthymine. Analogous equations can be written for the case where methylthymine is saturated in water and in water–methyladenine solution.

The fraction f of methyladenine–methylthymine complex formation in water solution is approximated by

$$f = (S_A{}^{WT} - S_A{}^W)/S_A{}^{WT} \quad (4)$$

where $S_A{}^W$ and $S_A{}^{WT}$ are the solubilities of methyladenine in water and water–methylthymine at the same temperature. It is assumed in writing this equation that the various solution components have the same activity coefficients and that the complex AT is a dimer. The first condition is reasonable for dilute solutions; the second is made plausible from the specific dimer interaction known for adenine and thymine derivatives in polynucleotides[10] and in crystal structure determinations.[11]

Combining eq. 4 and 1 we can rewrite eq. 3 in terms of experimental quantities as

$$\Delta H_0 = \frac{1}{f} R \left[\left(\frac{\partial \ln S_A{}^{WT}}{\partial(1/T)} \right) - \left(\frac{\partial \ln S_A{}^W}{\partial(1/T)} \right) \right] = -R \frac{1}{f} \left[\frac{\partial \ln (S^{WT}/S_A{}^W)}{\partial(1/T)} \right] = -R \frac{1}{f} \left[\frac{\partial \ln (1/1 - f)}{\partial(1/T)} \right] = R \frac{1}{f} \frac{\partial \ln (1 - f)}{\partial (1/T)} = -R \frac{\partial \ln [f/(1 - f)]}{\partial (1/T)} \quad (5)$$

$$\Delta H_0 = -R \frac{\partial \ln [(S_A{}^{WT} - S_A{}^W)/S_A{}^W]}{\partial (1/T)} \quad (6)$$

The partial derivative here implies saturated equilibrium conditions.

We see from eq. 6 that ΔH_0 can be estimated from solubility measurements of methyladenine in water and in methylthymine–water solutions at various temperatures. The accuracy of the determination will depend primarily upon how accurately the difference in solubilities in the two solvent systems can be measured, since the difference would be expected to be smaller than the direct solubility of methyladenine, $S_A{}^W$, in water.

(9) Williamson shows that even in the case of urea, which has a saturated molarity of 20 at 25°, the activity correction is about 5%. For dilute saturated solutions of non-electrolytes the correction should be correspondingly smaller.

(10) R. F. Steiner and R. F. Beers, Jr., "Polynucleotides," Elsevier Press, New York, N. Y., 1961, Chapt. 8.

(11) K. Hoogsteen, *Acta Cryst.*, **12**, 822 (1959).

Experimental Procedure

A differential refractometer was developed for the purpose of making the solubility measurements.[12] This instrument has a special thermostated cell in which the solubility of a compound in water, or a difference in the solubility in water and in another solvent, can be measured accurately. The temperature of the cell was controlled and measured to ±0.02°. Materials were saturated within the cell; the concentration of the solution was followed until constant readings were obtained for each temperature. This took approximately 8 hours. The approach to equilibrium was made both by raising and lowering the temperature. In the case of methyladenine a small hysteresis effect could not be eliminated. However the up and down temperature solubility curves were parallel. In the refractometer a difference in concentration is measured by the displacement of a slit image. The displacement was shown to be linear with concentration for a given component in water and in the presence of complexing compounds. This provides the necessary calibration constants for translating measured deflections into concentration readings. Since the deflection is proportional to the concentration of the given component, further calibration was strictly unnecessary to apply eq. 6. However, calibration constants were established so that solubilities could be listed.

The technique for measuring the difference in solubility illustrates the advantage of the differential refractometer. For the case where we wanted to determine the difference in the solubility of methyladenine in water and in a solution of methylthymine and water, the center compartment of the refractometer was filled with methylthymine solution and the outer compartment with water. A deflection of the slit image occurs due to the solvent differences. This gives a reference or zero position. Now excess methyladenine was added to both the center and outer compartments and stirred to reach equilibrium as indicated by a constant position of the slit image. The deflection from the reference zero is proportional to the concentration difference of the saturated solutions. The calibration constant was established from known unsaturated solutions and checked at various temperatures.

The 9-methyladenine and 1-methylthymine were obtained from Cyclo Chemicals, Los Angeles, Calif. Solubility and ultraviolet spectra tests on recrystallized materials remained constant indicating purity of compounds as supplied. In addition, no extraneous impurity could be detected by paper chromatography. The reproducibility of the solubility measurements for separate determinations also validates the purity of the materials.

Doubly distilled water was used in all experiments. Measurements of *p*H were taken on the solutions and found to lie in the range of 6–7. No attempt was made to buffer the solutions. Ionization of the compounds is expected to occur only at extreme *p*H values. Purity of the solid compound in equilibrium with mixed solutions was confirmed by measurements of its ultraviolet spectrum.

Results and Discussion

The solubility of 9-methyladenine at various temperatures and in different solvent mixtures is shown in Table I. An estimate of the uncertainty of the values, as indicated from replicate runs, is 1%. A definite enhancement of solubility is noted when methylthymine is present; the magnitude depends upon the concentration, given as weight per cent.

TABLE I

SOLUBILITY OF 9-METHYLADENINE

Solvent	Weight per cent 20°	25°	30°	35°	40°
H_2O	0.367	0.447	0.541	0.651	0.778
0.25% 1-MT	.444	.523	.617	.725	.850
0.50% 1-MT	.533	.613	.702	.810	.934

Because both methyladenine and methylthymine have relatively low but similar solubilities in water, it was possible to determine the solubility of methylthymine in pure water and in the presence of an aqueous solution of methyladenine. The results are given in Table II.

TABLE II

SOLUBILITY OF 1-METHYLTHYMINE

Solvent	Weight per cent 25°	30°	35°	40°	45°
H_2O	0.547	0.632	0.726	0.834	0.962
0.25% 9-MA	0.619	0.700	0.792	0.896	...

(12) S. J. Gill and D. B. Martin, *Anal. Chem.*, **35**, 118 (1963).

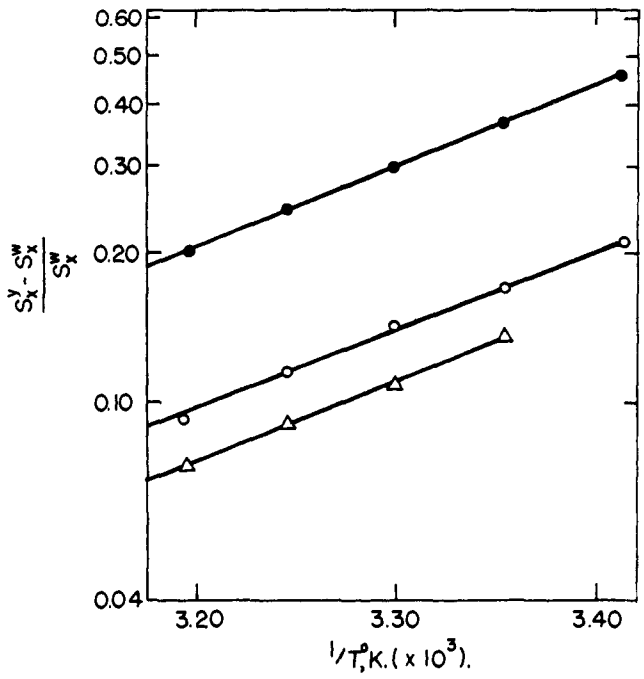


Fig. 1.—Plot of eq. 6 for comparison of theory with data and calculation of ΔH_0: (—●—●—), x = A, y = 0.50% WT; (—○—○—), x = A, y = 0.25% WT; (—△—△—), x = T, y = 0.25% WA.

Logarithmic plots of the solubilities in water against $1/T$ are found to be linear over the temperature range covered experimentally. The heats of solution into water as determined by least squares analysis are given for $\bar{H}_A^W - H_A^S$ and $\bar{H}_T^W - H_T^S$ by 6860 ± 42 cal./mole and 5300 ± 30 cal./mole, respectively. The excellent linearity of these plots suggests that self-association effects, if present at all, are small.

To calculate the enthalpy of interaction in solution, the data were plotted according to eq. 6. The results are shown in Fig. 1. The linearity of the plots supports the assumptions made in deriving eq. 6. Furthermore, the slopes of these plots are nearly identical, which is another requirement of eq. 6. The calculated heats of interaction ΔH_0, as determined by a least squares analysis, are shown in Table III. The differences in these values perhaps reflect the possible errors inherent in neglecting activity coefficients and in the experimental measurements of the solubility. We take an average value of −7.3 ± 0.2 kcal. as the enthalpy of interaction to form the methyladenine–methylthymine complex in the presence of water.

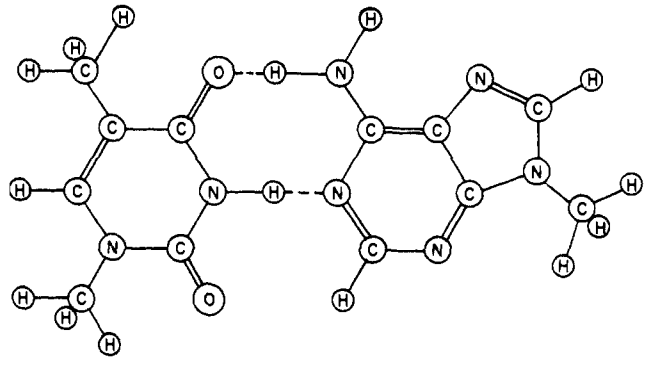
TABLE III

HEAT OF INTERACTION FOR COMPLEX FORMATIONS

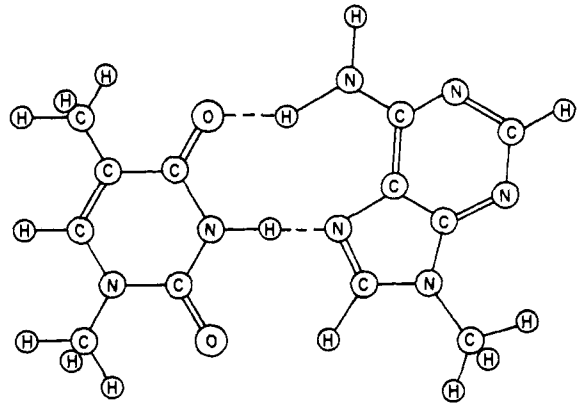
Solvent	Solute	ΔH_0, cal./mole-dimer	Probable error, cal./mole-dimer
0.25% 1-MT	9-MA	−7480	150
.50% 1-MT	9-MA	−7470	99
.25% 9-MA	1-MT	−6970	133

The relatively large value noted for the heat of interaction suggests that a complex is formed through specific interaction between the purine and pyrimidine bases. The data obtained are compatible with simple dimer formation as judged by a consistent equilibrium constant, calculated at a given temperature from the data in Tables I and II. From the structure of 9-MA and 1-MT it would appear that hydrogen bond formation would play a major role in the establishment of the dimer. Additional stabilization of the dimer complex in water might be achieved by hydrophobic bonding. Another possible interaction, which seems less likely due to the structure determination of the solid complex, might involve π-orbital stacking of a purine and pyrimidine base to give a stable dimer. However, it is difficult to see why complex formation with this interaction would be primarily dimeric.

The structure of a hydrogen bonded complex in solution might follow from the Crick and Watson hypothesis as



or the Hoogsten determination of the solid complex as



Both complexes have two hydrogen bonds of the same form —O···H—N— and —N—H···N=. If we consider these bonds forming from the methyladenine and methylthymine hydrogen-bonded in water, then a rough estimate of ΔH_0 may be made from known values of these hydrogen-bonded species.

From values listed by Prigogine[13] we obtain a heat of complex formation in water of −(7 to 2) kcal. This value is essentially the same as the estimate of Rice, Wada and Geiduschek for base pair enthalpy interaction for either the guanine–cytosine pair or the adenine–thymine pair. The experimental values obtained here are nearest to the highest rough estimates which can be made, *i.e.* −7 kcal./mole.

From these results it would seem that the enthalpy of interaction between the cytosine–guanine base pair, which has the possibility of three hydrogen bonds according to the Crick and Watson concept, would be at least half again as much as the adenine–thymine value. This would give something of the order of −11 kcal./mole of the cytosine–guanine complex with respect to water solutions. An estimate for the average base pair enthalpy of formation in water for nucleic acid with equal amounts of adenine–thymine and cytosine–guanine pairs suggests a value in the range of −9 kcal.

(13) J. Prigogine, "The Molecular Theory of Solutions," Interscience Publishers, Inc., New York, N. Y., 1957, p. 419.