$$1/C = 1C^{i} + 1/C^{d}(1 + dq^{1}/dq) \quad (2)$$

where q^1 is the charge due to specifically adsorbed anions and q is the excess charge on the metal. Since q^1 is negative for anions, the effect of the diffuse layer on the measured capacity is eliminated when $dq^1/dq \approx -1$ and the second term in (2) disappears.[44] The results, therefore, can be tentatively explained in terms of enhanced specific adsorption of anions in mixed solvents containing a preponderance of water. This is an interesting result which should have some correlation with the thermodynamic properties of the mixed solvent system.

The nonideality of the variation of the dielectric constant with composition reported by Doucet, *et al.*,[30] is paralleled by similar positive deviations of the viscosity, density, and heat of mixing, which have been reported by Cowie and Toporowski.[45] The maximum deviations occur at 30–40 mole % DMSO and suggest the probable existence of a stable DMSO hydrate with a 2:1 DMSO to water ratio or at least strong hydrogen-bonded association between the two molecules. It is probable, therefore, that the solvation energy of the PF_6^- ion would pass through a minimum at this solvent composition, resulting in enhanced specific adsorption. Studies of the effect of solvent composition on specific adsorption of anions in this system would therefore provide an interesting tool for investigation of the contribution of the solvation energy to the adsorption energy if this interpretation is correct.

(44) Precisely this situation occurs in the case of nitrate ion adsorption from KNO_3 solutions where dq^1/dq is very close to -1 when $q \gtrsim 2$ μ-coulombs/cm^2.[19] Under these conditions the measured capacity is a close approximation to the inner layer capacity.

(45) J. M. G. Cowie and P. M. Toporowski, *Can. J. Chem.*, **39**, 2240 (1961); see also J. Kenttämaa and J. J. Lindberg, *Suomen Kemistilehti*, B33, 98 (1960).

Conclusions

1. Electrocapillary and double-layer capacity measurements have been reported for the DMSO solvent system. The results show a strong resemblance to the corresponding aqueous solutions.
2. The interfacial tension at the Hg–DMSO interface in the absence of specific adsorption is 370.5 ± 0.2 dynes/cm at 25° compared with 425.4 dynes/cm for the Hg–water interface.
3. A large capacity hump occurs in KPF_6 solutions at $q \approx 8$ μcoulombs/cm^2. The positive shift in the ecm in DMSO solutions in consistent with Macdonald's theory that such humps are due to reorientation of positively (toward the metal) oriented solvent dipoles. Similar results for formamide and N-methylformamide, however, are not consistent with this theory. The minimum capacity on the cathodic side is ~ 7 μf/cm^2.
4. As found in aqueous solutions, anions are specifically adsorbed in the order $I^- > Br^- > Cl^- > NO_3^- > ClO_4^- > PF_6^-$, and cations are not appreciably adsorbed.
5. Specific adsorption of anions from mixed DMSO–water solutions is enhanced by strong interaction between DMSO and water in the range 20–30 mole % DMSO.

An Infrared Study of Hydrogen Bonding between Adenine and Uracil Derivatives in Chloroform Solution

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Abstract: The association constants K for hydrogen-bonded dimer formation in chloroform solution by 9-ethyladenine and by 1-cyclohexyluracil, as well as for the formation of the 1:1 hydrogen-bonded complex of the two bases, have been measured by infrared spectroscopy over the temperature range 4–58°. At 25°, K_{AU} for the mixed dimer is 30 times larger than K_{AA} for the 9-ethyladenine dimer and 15 times larger than K_{UU} for the 1-cyclohexyluracil dimer. From the temperature dependence of association constants, $\Delta H°$ for association is found to be -4.3 ± 0.4 kcal/mole of dimer for cyclohexyluracil, -4.0 ± 0.8 kcal for ethyladenine, and -6.2 ± 0.6 kcal for the mixed dimer. The entropy differences $\Delta S°$ are -11.0 ± 1, -11.4 ± 2, and -11.8 ± 1.2 eu, respectively. The structures of the dimers in solution are discussed on the basis of their infrared spectra.

In the double-stranded structures of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), adenine specifically forms hydrogen bonds with thymine (or uracil) and guanine with cytosine. These specific bondings are believed to be the molecular basis of information transfer by nucleic acids. Much work has been done to find the basis of this specificity. Crystallographic studies have shown that specific hydrogen bonds are formed even between the constituent purines and pyrimidines.[1-4] Recently Hamlin, Lord, and Rich[5] have found by infrared spectroscopic methods that 1-cyclohexyluracil and 9-ethyl-

(1) K. Hoogsteen, *Acta Cryst.*, 12, 822 (1959); 16, 907 (1963).
(2) F. S. Mathews and A. Rich, *J. Mol. Biol.*, 8, 89 (1964).
(3) L. Katz, K. Tomita, and A. Rich, *ibid.*, 13, 340 (1965).
(4) A. E. V. Haschemeyer and H. M. Sobell, *Proc. Natl. Acad. Sci. U. S.*, 50, 872 (1963).
(5) R. M. Hamlin, Jr., R. C. Lord, and A. Rich, *Science*, 148, 1734 (1965).

adenine form a hydrogen-bonded dimer with each other much more strongly than with themselves when their dilute chloroform solutions are mixed, and further that certain guanosine derivatives interact specifically with cytidine derivatives in similar fashion in the same solvent.[6]

A similar phenomenon was observed by Kuechler and Derkosch[7] for isopropylidene–trityladenosine and isopropylidene–trityluridine in carbon tetrachloride solutions. More recently Katz and Penman[8] and Shoup, Miles, and Becker[9] have found (by proton magnetic resonance studies) that nucleosides also specifically interact with each other in dimethyl sulfoxide solutions. All of these studies show that single base residues interact by means of specific hydrogen bonding in a solution. Association of purines and pyrimidines in aqueous solutions has also been suggested by Ts'o and his associates[10] from their vapor pressure measurements and proton magnetic resonance spectra, although they do not attribute the binding to hydrogen bonds.

In spite of many studies of the interaction, no work has been done on the quantitative determination of the strength of the specific pairing. In the present study, association constants for the complex dimer observed by Hamlin, *et al.*,[5] were obtained at various temperatures, and the bonding energy between ethyladenine and cyclohexyluracil has been determined therefrom. We chose chloroform as a solvent for the reason that the bases are sufficiently soluble in it, and the hydrogen-bonded interaction between the solutes and the solvent is very weak.

Experimental Methods

Theory of the Procedure. The method used by Lord and Porro[11] in the study of the dimerization of caprolactam has been applied to the problem of the self-dimerization of adenine and uracil and extended to the determination of the association constants of a heterodimer.

At those concentrations where adenine or uracil molecules form a single type of dimer, the association constant K may be written as

$$K = \frac{C_d}{C_m^2} \tag{1}$$

where C_m and C_d are monomer and dimer concentrations in moles per liter. The initial concentration of the solute C_0 is determined by the weight of solute dissolved per liter. It is related to C_m and C_d by

$$C_0 = C_m + 2C_d \tag{2}$$

The molecule of 1-cyclohexyluracil has one imino group and that of 9-ethyladenine has one amino group. If these compounds form cyclic dimers with themselves, the absorbance A of the bands due to the *nonbonded* imino or amino group should be

$$A = a_m C_m l \tag{3}$$

where a_m is the extinction coefficient in liters per mole per centimeters at the frequency of maximum monomer absorbance, and l is the path length in centimeters. From (1), (2), and (3) it follows that

$$A = \left(\frac{1}{2K}\right)a_m^2 l^2\left(\frac{C_0}{A}\right) - \left(\frac{1}{2K}\right)a_m l \tag{4}$$

If A is plotted against C_0/A, a straight line should be obtained. The unknown quantities a_m and K can be computed from the slope s and the intercept i of this line as follows

$$a_m = -\frac{s}{il} \tag{5}$$

$$K = \frac{s}{2i^2} \tag{6}$$

An advantage of this procedure is that a_m does not have to be assumed independent of temperature.

The heat of association $\Delta H°$ and the entropy change $\Delta S°$ can be obtained from the measurements of association constants at various temperatures by applying the van't Hoff equation

$$\log K = -\Delta H°/2.303RT + \Delta S°/2.303R$$

If ethyladenine and cyclohexyluracil are mixed together in chloroform, three kinds of dimers should exist in the solution: adenine–adenine, uracil–uracil, and adenine–uracil. The association constants, K_{AA}, K_{UU}, and K_{AU}, corresponding to each dimer are defined as

$$K_{AA} = \frac{C_{AA}}{C_A^2},\ K_{UU} = \frac{C_{UU}}{C_U^2},\ \text{and}\ K_{AU} = \frac{C_{AU}}{C_A C_U} \tag{7}$$

where C_A and C_U are monomer concentrations and C_{AA}, C_{UU}, and C_{AU} are dimer concentrations. The initial concentration C_0 of the mixture is defined as

$$C_0 = C_{0A} + C_{0U}$$

and thus

$$C_0 = C_A + C_U + 2C_{AA} + 2C_{UU} + 2C_{AU} \tag{8}$$

If *equimolar* amounts of both compounds are mixed, $C_0 = 2C_{0A} = 2C_{0U}$ and $C_{0A} = C_A + 2C_{AA} + C_{AU} = C_U + 2C_{UU} + C_{AU} = C_{0U}$.

If only a cyclic dimer is formed, the relation between absorbance and monomer concentration expressed by eq 3 may be kept for the nonbonded band of ethyladenine where C_m is replaced by C_A.

From eq 3, 7, and 8 and the definition of C_{0A}, it follows that

$$A = \frac{1}{(\alpha K_{AU} + 2K_{AA})}\left[\frac{a_m^2 l^2}{2}\left(\frac{C_0}{A}\right) - a_m l\right] \tag{9}$$

where α is defined as C_U/C_A. If K_{AA} has been measured and a provisional value of α is assumed, a_m and K_{AU} are given by

$$a_m = -\frac{2s}{il} \tag{10}$$

$$K_{AU} = \frac{2}{\alpha}\left(\frac{s}{i^2} - K_{AA}\right) \tag{11}$$

where s is the slope and i the intercept of the straight line given by a plot of A *vs.* C_0/A. From these values α can then be computed and if necessary the calculation repeated.

Experimental Procedures. 9-Ethyladenine and 1-cyclohexyluracil were obtained from Cyclo Chemical Co., Los Angeles, Calif. Ethyladenine was recrystallized from chloroform. Cyclohexyluracil was used without further purification. Deuterated samples were prepared by dissolving nondeuterated samples in hot heavy water and successive lyophilization of the solution.

Chloroform and chloroform-*d* were used as solvents. The chloroform (reagent grade, Fisher Scientific Co.) was distilled after being dried with phosphorus pentoxide overnight and then passed through an alumina gel column 25 cm long. Chloroform-*d* was purchased from New England Nuclear Corp., Boston, Mass. It seemed to contain a slight amount of exchangeable deuterium, which caused exchange at the amino or imino group of the solutes in dilute solutions. The chloroform-*d* was therefore purified by passing it through an alumina gel column 5 cm in length.

All spectra were observed with a Perkin-Elmer Model 521 double-beam infrared spectrophotometer. In the intensity measurements the slit width was fixed at 125 μ, which corresponded to a 3-cm^{-1}

(6) See, for example, J. Pitha, R. N. Jones, and J. Pithova, *Can. J. Chem.*, **44**, 1044 (1966); Y. Kyogoku, R. C. Lord, and A. Rich, *Science*, **154**, 518 (1966).

(7) E. Kuechler and J. Derkosch, *Z. Naturforsch.*, **21b**, 209 (1966).

(8) L. Katz and S. Penman, *J. Mol. Biol.*, **15**, 220 (1966).

(9) R. R. Shoup, H. T. Miles, and E. D. Becker, *Biochem. Biophys. Res. Commun.*, **23**, 194 (1966).

(10) For example, P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289 (1963); M. V. Schweizer, S. I. Chan, and P. O. P. Ts'o, *ibid.*, **87**, 5241 (1965).

(11) R. C. Lord and T. J. Porro, *Z. Elektrochem.*, **64**, 672 (1960).

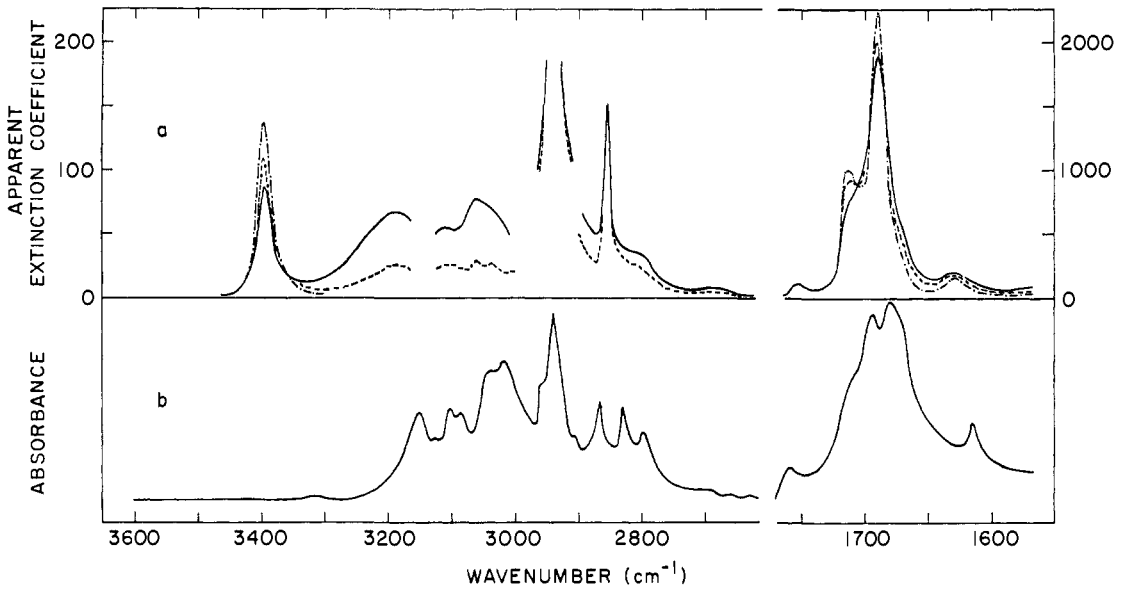


Figure 1. Infrared spectra of 1-cyclohexyluracil. (a) Chloroform solutions plotted as apparent extinction coefficient log $(I_0/I)/C_0l$ in l./mole cm *vs.* wavenumber in cm^{-1}: ———, 0.1997 *M* in $CDCl_3$ (0.2-mm NaCl cell in 3-μ region and 0.028-mm NaCl cell in 6-μ region); – – – –, 0.0413 *M* in $CDCl_3$ (1.0-mm quartz cell in 3-μ region and 0.1-mm NaCl cell in 6-μ region); – · – ·, 0.00082 *M* in $CHCl_3$ (50-mm quartz cell and a variable thickness cell of 2.5 mm). (b) Solid in Nujol and hexachloropropene mulls: plotted as absorbance log I_0/I *vs.* wavenumber in cm^{-1}.

spectral slit width at 3400 cm^{-1}. The same cell was used for measurements of both solvent and solution spectra, and absorbances of the solution were calculated with the aid of the solvent curves as base lines.

In order to test the equilibrium over a wide range, cells of fused silica (American Instrument Co. and Beckman Instruments) of thickness ranging from 1 mm to 10 cm and sodium chloride cells (including one of variable thickness) ranging from 0.028 to 2 mm were used.

For absorbance measurements at several temperatures a circulating hot-water thermostatic bath (Precision Scientific Co., Inc., Chicago, Ill.) was used, slightly modified to enable the circulation of ice water. The fused silica cells were held by the insulated cell holder constructed by Porro,[11] and a similar device was constructed for sodium chloride cells.

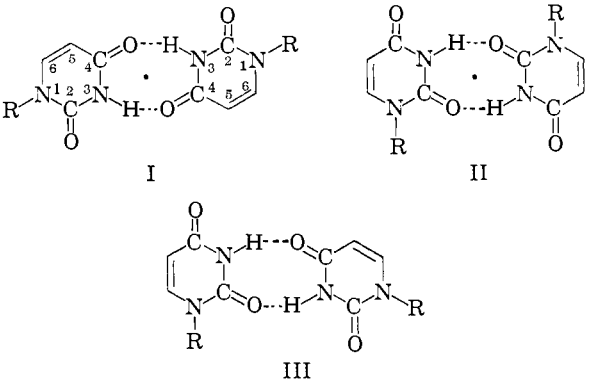
The temperature difference between the bath and the cell was checked by a copper-constantan thermocouple. It was 4° with water at 0° in the bath and gradually decreased at higher temperature. It was almost zero at 58°. For measurements at 4°, dry nitrogen was flushed around the cell to avoid the deposit of atmospheric water vapor on the surface of the cell. Spectra of the solutions were first taken at 4°, and then the temperature was gradually elevated for successive spectra. It took 4-hr for a series of runs from 4 to 58°. In working up the data at the different temperatures, correction was made for the volume changes of the solutions on the assumption that the changes were calculable from the temperature dependence of the molar volume of pure chloroform. These corrections were small (less than 5% at the extreme temperatures) but significant.

Spectra of the solids were taken in Nujol or hexachloropropene mulls at room temperature (21°).

Results and Discussion

1-Cyclohexyluracil. In the spectrum of the saturated solution (~0.2 *M*) of 1-cyclohexyluracil in chloroform a sharp band is observed at 3395 cm^{-1} and broad bands at 3184, 3110, 3050, 2835, and 2800 cm^{-1}. Since all these bands disappear upon deuterium substitution at the 3-N position, they are associated with the NH stretching vibrations in the species present. The band at 3395 cm^{-1} increases, and the other bands decrease in intensity with dilution as shown in Figure 1a. In the spectrum of the solid sample (Figure 1b) no band is observed near 3395 cm^{-1}, and strong bands are found at 3150, 3100, 3034, 2827, and 2800 cm^{-1}. From these facts the band at 3395 cm^{-1} is assigned to the nonbonded NH stretching vibration of the monomer, and the others are related to the bonded NH stretching vibrations. (See Table I for assignments.)

At lower concentrations it is assumed that only monomers and dimers exist in solution. Two different kinds of dimer structure are conceivable, that is, open and cyclic ones. Two forms are possible for the open dimer, one of which is connected with the carbonyl oxygen atom at the 2-C position and the other with that at the 4-C position. For a cyclic dimer three forms (I–III) can be written.



I II III

If a dimer is open, it also contains a nonbonded NH. On the assumption that the dimer is open and the nonbonded NH extinction coefficient is the same as that of the monomer, eq 3 is to be written as

$$A = a_m(C_m + C_d)l \qquad (12)$$

By combining eq 1 and 2 with (12), an equation analogous to (4) is obtained as discussed by Lord and Porro[11]

$$A = \left(\frac{1}{4}\right)\left(\frac{1}{K} - C_0\right)a_m{}^2l^2\left(\frac{C_0}{A}\right) - \left(\frac{1}{4K} - C_0\right)a_ml \qquad (13)$$

Table I. The Frequencies of Absorption Bands and Their Assignments (in wavenumber cm^{-1})[a]

1-Cyclohexyluracil			9-Ethyl adenine			1:1 mixture	
H	D	S	H	D	S	H	D
			3527			3527	
			3485			3485	
				3470			3470
			3416			3415	
3395						3395	
			3312			3327	
					3305		
			3250		3240	3256	
					3220		
			3200		3195	3210	
3184		3150					
		3125			3140		
3110		3100			3100	3110	
		3082					
3064	3064		3062			3064	3064
3050		3034			3050	3050	
3036	3036	3014	3034			3040	3040
					2977		
2940	2940	2950	2942	2942	2950	2940	2940
		2931			2934	2900	
		2904					
			2884	2885	2890		
2861	2861	2866			2875	2860	2860
2835		2827					
2800		2800	2800		2822	2800	
					2720		
2680					2694	2675	
				2640	2642		2640
					2584	2605	
	2548			2560			2560
	2526						2525
	2501						
				2495			2495
				2483			2480
							2450
				2410			2425
1752	1730	1763				1752	1730
1710	1708	1710				1710	1710
		1695					
1687	1678	1678				1688	1680
1670	1653	1670				1675	1650
			1642		1675	1640	
			1629			1629	
1629	1625			1612			1615
			1600		1600	1600	
			1586			1586	
			1575	1575	1575	1575	1575

[a] H, nondeuterated compound in chloroform-*d* solution; D, deuterated compound in chloroform solution; S, solid. The assignments (cm^{-1}) of the bands in solution spectra are as follows. 1-Cyclohexyluracil (nondeuterated compound): 3395, free NH str; 3184, bonded NH str; 3064 and 3036, ring CH str; 2940 and 2861, cyclohexyl CH str; 3110, 3050, 2835, 2800, and 2680 are related to the bonded NH str; 1710, 1687, and 1670, free and bonded C=O str; 1629, ring str; (deuterated compound): 2548, 2526, and 2501 are related to free ND str; 1708, 1678, and 1653, free and bonded C=O str; 1625, ring str. 9-Ethyladenine (nondeuterated compound): 3527, free NH_2 antisym str; 3485, free NH (bonded H) str; 3416, free NH_2 sym str; 3312, bonded NH (free H) str; 3062 and 3034, ring CH str; 2942 and 2884, ethyl CH str; 3250, 3200, and 2800 are related to the bonded NH_2 str; 1642, bonded NH_2 scissor plus ring str; 1629, free NH_2 scissor plus ring str; 1600, ring str plus bonded NH_2 scissor; 1586, ring str plus free NH_2 scissor; 1575, ring str; (deuterated compound): 3470, free NH(D) str; 2640, free ND_2 antisym str; 2560, free ND (bonded D) str; 2495 and 2483; free ND_2 sym str; 2410, bonded ND (free D) str; 1612 and 1575, ring str.

This equation shows that the relation between A and C_0/A is not linear for the case of an open dimer. If C_0 is sufficiently small with respect to $1/K$, however, a linear relation will be realized. Hence the fact that observed data follow a linear relation would be no guarantee that the dimer is cyclic. A more reliable way of distinguishing between the two forms is to measure $-\Delta H°$ for the association of a dimer, which should be about twice as large for the cyclic dimer as for the open dimer.[11,12]

For solutions in the range 0.01–0.07 M, absorbances of the band at 3395 cm^{-1} were measured with the 1-mm cell at six temperatures between 4 and 58°. By plotting A against C_0/A, straight lines were obtained at each temperature, and from these a_m and K were calculated by eq 5 and 6. The results are given in Tables II and III.

Table II. Temperature Dependence of Extinction Coefficients (in l./mole cm)[a]

1-Cyclohexyluracil		9-Ethyladenine			1:1 mixture		
	a_m at		a_m at			a_m at	
Temp, °C	3395 cm^{-1}	Temp, °C	3527 cm^{-1}	3416 cm^{-1}	Temp, °C	3527 cm^{-1}	3404 cm^{-1}
4	138	5	109	204	5	104	226
13	137	13	107	202	14	104	223
25	135	24	104	200	25	103	222
33	134	36	102	196	35	102	220
45	134	48	99	193	47	99	219
57	131	59	96	186	58	97	218

[a] Estimated random error: ±6% in a_m for 1-cyclohexyluracil and 9-ethyladenine, ±10% for 1:1 mixture.

The plot of log K *vs.* $1/T$ gave a good straight line, from which $\Delta H°$ and $\Delta S°$ were obtained (Table IV). The value of $-\Delta H°$, 4.3 kcal/mole of dimer, is about 1.5 times as large as the heat of formation of a single N—H···O=C bond measured in chloroform.[13] Therefore, it may be concluded that the dimer is probably bonded with two hydrogen bonds. The fact that $-\Delta H°$ is not twice as large as that for a single NH··· O=C bond may be attributed to the steric restrictions imposed on the bonds in a cyclic dimer.

If three forms of the cyclic dimer, as shown above, coexist in a solution, eq 4 becomes

$$A = \frac{1}{2(K_I + K_{II} + K_{III})}\left[a_m^2 l^2\left(\frac{C_0}{A}\right) - a_m l\right] \quad (14)$$

where K_I, K_{II}, and K_{III} are the association constants corresponding to each form of the cyclic dimer. If one of the three forms is predominant in a solution (*e.g.*, $K_I \gg K_{II}, K_{III}$), or if the relative numbers of the three forms are the same at every temperature, then eq 14 reduces to eq 4. The fact that a straight line was obtained by the plot of log K *vs.* $1/T$ shows that one or the other of these cases is the true situation.

In order to get further information on the structure of the dimer, the carbonyl stretching bands were studied. In a dilute chloroform solution of cyclohexyluracil two strong bands were observed at 1710 and 1687 cm^{-1}. The 1710-cm^{-1} band is considered[14–16]

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(13) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., 1960.
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(16) R. C. Lord and G. J. Thomas, Jr., submitted for publication.

Table III. Temperature Dependence of Association Constants (in l./mole)

	1-Cyclohexyluracil		9-Ethyladenine			1:1 mixture		
		K_{UU} at		K_{AA} at			K_{AU} at	
	Temp, °C	3395 cm^{-1}	Temp, °C	3527 cm^{-1}	3416 cm^{-1}	Temp, °C	3527 cm^{-1}	3404 cm^{-1}
Association constants	4	10	5	4.8	4.2	5	139	274
	13	8.1	13	3.9	3.7	14	104	195
	25	6.1	24	3.1	3.1	25	67	140
	33	4.7	36	2.3	2.6	35	51	98
	45	3.8	48	1.7	2.0	47	32	67
	57	2.8	59	1.1	1.4	58	23	47
Solvent	$CDCl_3$		$CDCl_3$			$CHCl_3$		
Concn range, M	0.07–0.01		0.06–0.01			0.01–0.002		
Estd random error in K, %	±10		±10			±20		

Table IV. Thermodynamic Functions[a]

	Form of dimer						
	Uracil–uracil	Adenine–adenine			Adenine–uracil		
Wavenumber of measurements, cm^{-1}	3395	3527	3416	Mean	3527	3404	Mean
$-\Delta H^\circ$ of dimer, kcal/mole	4.3 ± 0.4	4.2	3.8	4.0 ± 0.8	6.3	6.1	6.2 ± 0.6
$-\Delta S^\circ$, eu	11.0 ± 1	12.1	10.7	11.4 ± 2	12.8	10.8	11.8 ± 1.2

[a] The indicated limits of error are consistent with the estimated random errors of Table II and III and do not include allowance for systematic error except for the adenine–adenine system. Here the plot of log K *vs.* $1/T$ (Figure 3) is somewhat curved, and the error estimate in ΔH° and ΔS° has been increased.

to arise mainly from the 2-C carbonyl and the 1687-cm^{-1} band from the 4-C carbonyl stretching vibrations coupled with the NH bending mode, since the 4-C carbonyl bond, being conjugated with the C=C bond, should have lower frequency of vibration than the 2-C carbonyl bond.

When the concentration of the solution is increased, the peak intensities of both these bands decrease and the intensities at about 1700 and 1670 cm^{-1} increase, as the formation of hydrogen bonds lowers both the 4-C and 2-C carbonyl frequencies. Since both bands show decreased intensity in concentrated solution, both carbonyl groups appear to be involved in hydrogen bonding. However, hydrogen bonding between one carbonyl group and the NH group may affect the frequencies and the intensities of the both carbonyl stretching bands if their vibrations are coupled with the NH bending mode or with each other. Therefore from the spectral changes around 1700 cm^{-1} it is not safe to conclude that there is more than one kind of dimer in the solution.

The spectrum of the solid around 1700 cm^{-1} resembles that of the concentrated solution except for the crystal-field splitting at 1690 and 1675 cm^{-1}. The relative intensities among the bonded NH stretching bands around 3000 cm^{-1} in the spectrum of the solid are about the same as those of the solution. Cyclohexyluracil molecules probably form a cyclic dimer of type I in the solid state, as has been found by X-ray studies on the related compounds 1-methyluracil[17] and 1-methylthymine,[18] whose infrared spectra in the solid states resemble each other and that of cyclohexyluracil. The cyclic dimer of similar structure must predominate in solution.

9-Ethyladenine. The spectrum of a dilute solution of 9-ethyladenine shows sharp bands at 3527 and 3416 cm^{-1} (Figure 2). These bands become weak in concentrated solution and cannot be observed in the spectrum of the solid. They are therefore assigned to the antisymmetric and symmetric stretching vibrations of the nonbonded NH_2 group. As the concentration of the solution increases, new bands appear first at 3485 and 3312 cm^{-1}, and later at 3250, 3200, 2884, and 2800 cm^{-1}. These bands disappear on deuteration of the amino group and must originate from the bonded NH_2 group. In the solid spectrum there is no band above 3350 cm^{-1}, and the strongest band is observed at 3050 cm^{-1}, whereas the bonded NH_2 bands at 3485 and 3312 cm^{-1} are strongest in the solution spectra.

When ethyladenine forms a dimer, one of the two NH bonds of the amino group is bonded with the partner molecule and the other is free. The stretching frequency of this nonbonded NH is expected to appear between the antisymmetric and symmetric stretching bands of the free amino group. This suggests assignment of the band at 3485 cm^{-1} to the free NH and that at 3312 cm^{-1} to the bonded NH stretching modes. In the spectrum of partially deuterated ethyladenine in dilute solution, the NH stretching band of the half-deuterated amino group is observed at 3470 cm^{-1}, in support of the assignment.

In the solid state, ethyladenine molecules are presumably bonded to adjacent molecules by both hydrogens of the NH_2 group. An X-ray diffraction study of crystalline 9-methyladenine[19] showed that all molecules are bonded through the NH_2 groups to form chain polymers. If the solid of ethyladenine has a similar crystal structure, the free NH band of the amino group should not be observed in its infrared spectrum, and,

(17) D. W. Green, F. S. Mathews, and A. Rich, *J. Biol. Chem.*, 237, PC3573 (1962).
(18) K. Hoogsteen, *Acta Cryst.*, 16, 28 (1963).
(19) R. F. Stewart and L. H. Jensen, *J. Chem. Phys.*, 40, 2071 (1964).

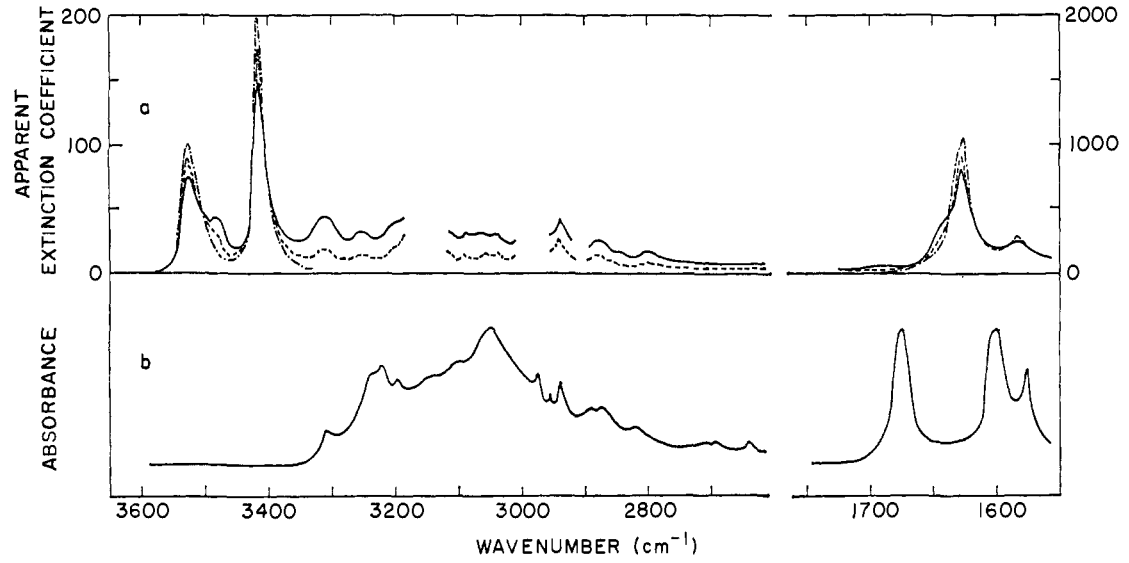


Figure 2. Infrared spectra of 9-ethyladenine. (a) Chloroform solutions: ———, 0.1048 M in $CDCl_3$ (0.5-mm NaCl cell in the 3-μ region and 0.05-mm NaCl cell in the 6-μ region); - - - -, 0.0413 M in $CDCl_3$ (1.0-mm quartz and 0.1-mm NaCl cells); – · – ·, 0.00082 M in $CHCl_3$ (50-mm quartz cell and a variable thickness cell of 2.5 mm). (b) Solid in Nujol and hexachloropropene mulls.

in fact, there is no band around 3480 cm^{-1}. However, there are many strong bands in the spectrum of the solid centered at 3050 cm^{-1}, which probably arise from the bonded amino group in polymer chains.

In dilute solution ethyladenine gives a strong band at 1629 cm^{-1} and a medium band at 1586 cm^{-1}. On deuteration of the amino group only a single band remains at 1612 cm^{-1}. These two bands are therefore assigned to the coupled vibrations of the NH_2 scissors vibration and the ring-stretching motions.[14,16] As the concentration of the solution increases, shoulder bands appear at 1642 and 1600 cm^{-1}, which must be related to the bonded amino groups. In the spectrum of the solid there are two strong bands at 1675 and 1600 cm^{-1}, the former probably due to the pure amino scissors mode and the latter to the ring stretching vibration. The scissors frequency is expected to be higher in the solid because of stronger hydrogen bonding. In solution the observed changes due to concentration in the spectral region near 1600 cm^{-1} are consistent with those around 3000 cm^{-1}.

There is no evidence bearing on the structure of the dimer in dilute solution. From the molecular structure of ethyladenine both open and cyclic dimers are possible. Thermodynamic considerations, however, indicate that the formation of a cyclic dimer is much more probable than that of an open dimer, if entropy changes on dimerization are nearly the same for both cases and the heat of formation due to hydrogen bonds in a cyclic dimer is twice as great as that of an open dimer.

On the assumption of cyclic dimers, a_m and K have been determined from the two nonbonded amino bands. As can be seen in Figure 2, the band of the monomer at 3527 cm^{-1} is overlapped by the band at 3485 cm^{-1}, and there is also some interference with the 3416-cm^{-1} peak by the 3312-cm^{-1} band. Measured absorbances were corrected by subtracting the contributions of these adjacent bands on the assumption that the bands are symmetrical about their centers. Straight lines were obtained when the corrected absorbances were plotted against C_0/A. However, the

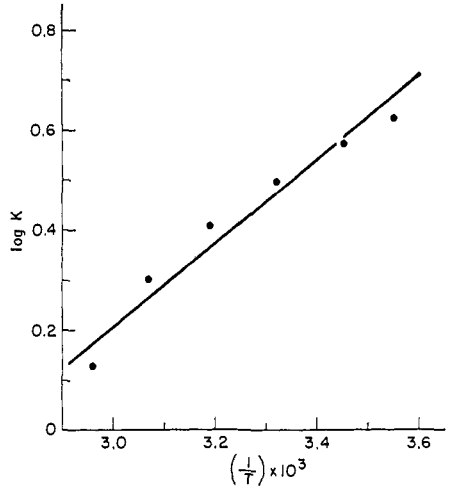


Figure 3. Plot of log K *vs.* $1/T$ for a chloroform-*d* solution of 9-ethyladenine at 3416 cm^{-1}.

plot of log K *vs.* $1/T$ is curved and the deviations from linearity are larger than the expected experimental errors (Figure 3). To check this result, the temperature-dependence experiments were repeated for the concentration range from 0.01 to 0.002 M, but these concentrations were too dilute to measure the effects of association. The concentration range for which the K's in Table III were obtained is the lowest at which we could measure the association constants of ethyladenine by this method.

Three structures are possible for the cyclic dimer of ethyladenine (IV–VI). For example, in the crystal structure of adenine hydrochloride[20] and in the helical form of polyadenylic acid,[21] two adenine residues are held together by two hydrogen bonds from the amino group to the imidazole nitrogen 7-N (structure V). The crystal of 9-methyladenine[19] is formed from dimeric units of structure VI.

(20) W. Cochran, *Acta Cryst.*, 4, 81 (1951).
(21) A. Rich, D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, 3, 71 (1961).

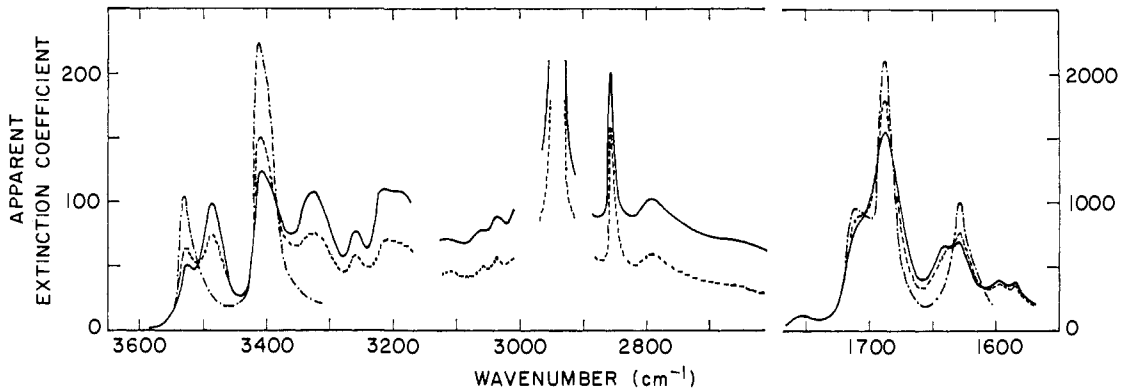
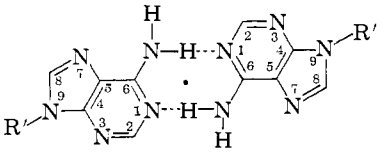
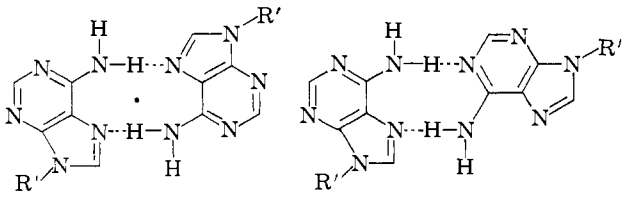


Figure 4. Infrared spectra of a 1:1 mixed solution of 9-ethyladenine and 1-cyclohexyluracil in chloroform: ———, 0.1059 *M* (total) in $CDCl_3$ (0.5-mm NaCl cell in the 3-μ region and 0.05-mm NaCl cell in the 6-μ region); - - - -, 0.0413 *M* (total) in $CDCl_3$ (1.0-mm quartz and 0.1-mm NaCl cells); - · - ·, 0.00082 *M* (total) in $CHCl_3$ (50-mm quartz cell and a variable thickness cell of 2.5 mm).

If these forms coexist in a solution, K obtained from eq 4 is $K_{IV} + K_V + K_{VI}$ and, if these association constants depend differently on temperature, the plot of log $(K_{IV} + K_V + K_{VI})$ *vs.* $1/T$ will not give a straight line. The thermodynamic functions for ethyladenine in Table IV were obtained from the mean line which minimized the deviation from the plotted points. These values are correspondingly less reliable than those for cyclohexyluracil and for the mixed dimer.



IV

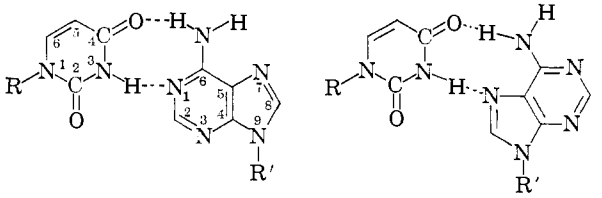


V VI

The 1:1 Mixture of Cyclohexyluracil and Ethyladenine. When equimolar solutions of cyclohexyluracil and ethyladenine are mixed together, the bonded NH stretching bands become very strong (Figure 4). A decided change in intensity is also observed in the range 1750 to 1550 cm^{-1}. Both these changes show that the number of associated molecules increases in the mixed system.

On the same basis as discussed for ethyladenine it is assumed that cyclic dimers predominate in the mixed solution. In support of this assumption we cite the following facts. As shown in Figure 4 the nonbonded NH band of cyclohexyluracil at 3395 cm^{-1} is overlapped by the band of the nonbonded amino group of adenine at 3416 cm^{-1}. However, when 9-ethyladenine associates with 1-cyclohexyl-5-bromouracil,[22] the nonbonded NH band of uracil appears at 3385 cm^{-1} and can be observed separately from the bands of the amino group in adenine. In this case the intensity of the nonbonded uracil band relative to the nonbonded amino group bands remains constant over the concentration range 0.01–0.001 *M*. The same phenomenon is observed for the 1:1 mixture of 6-methylamino-9-ethylpurine and 1-cyclohexyluracil,[22] where the nonbonded NH band of purine appears at 3450 cm^{-1} separately from the nonbonded uracil band at 3395 cm^{-1}. These facts show that the imino group of uracil and the amino group of adenine are employed for dimer formation *at a constant ratio*, and hence the dimer must be cyclic.

Four mixed cyclic dimer structures between adenine and uracil are readily drawn. The amino group of adenine can form a hydrogen bond with the carbonyl oxygen atom of uracil at position 2 or 4, and the imino group of uracil can bond with either the 1-N or 7-N atom of adenine. Form VII with thymine presumably occurs in DNA.[23] In the crystal structures of the complexes 9-methyladenine–1-methylthymine[1] and 9-ethyladenine–1-methyluracil,[2] the bonding is between the amino group of adenine and the 4-C carbonyl group of the pyrimidine, as well as between the imidazole nitrogen 7-N of adenine and the 3-N hydrogen of the pyrimidine (structure VIII). However, in the crystal complex adenosine–5-bromouridine[4] the adenine amino group bonds to the 2-C carbonyl of uracil, with the other bond as in structure VIII. The crystal complex 9-ethyladenine–1-methyl-5-bromouracil[3] has a mixture of both structures.



VII VIII

By assuming either that the enthalpy of dimerization of each dimer is the same or that only one dimer has appreciable concentration in solution, eq 9 can be used to calculate the association constant of the dimer adenine and uracil. Data from the NH_2 antisymmetric stretching band of adenine at 3527 cm^{-1}, which is free of interference, can be employed in eq 9, but the NH_2

(22) Y. Kyogoku, R. C. Lord, and A. Rich, *Proc. Natl. Acad. Sci. U. S.*, in press.

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

symmetric stretching at 3416 cm^{-1} cannot be so used because of overlapping with the NH stretching band of uracil. Absorbance of the overlapped band, however, should depend on the monomer concentrations of adenine and uracil (provided the dimer is cyclic), and therefore this band was used to check the result given by the band at 3527 cm^{-1}. The absorbance due to these bands was corrected by subtracting the estimated contributions of the neighboring dimer bands at 3485 and 3327 cm^{-1}.

In the calculation of K_{AU} from eq 9 and 11, the values of K_{AA} for ethyladenine in Table III were used, and C_U/C_A was assumed to be 1. C_U/C_A is close to unity in dilute 1:1 solutions and the error of K_{AU} from this assumption is small. The real K_{AU}, however, must be slightly larger than that obtained here, as K_{UU} is larger than K_{AA} at every temperature.

The plot of log K_{AU} against $1/T$ gave a straight line with a slope yielding $\Delta H°$ equal to -6.2 kcal/mole of dimer. This value of $-\Delta H°$ is too large to be attributed to the breaking of a single hydrogen bond and confirms the existence of the cyclic dimer in a solution. However, this experiment does not allow us to choose among the possible structures described above.

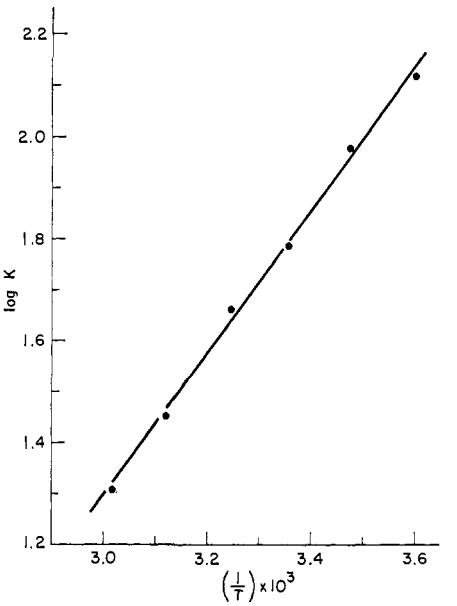


Figure 5. Plot of log K *vs.* $1/T$ for a mixed solution of 9-ethyladenine and 1-cyclohexyluracil at 3527 cm^{-1}.

Conclusions

It is concluded that ethyladenine and cyclohexyluracil form cyclic dimers in chloroform with themselves and with each other from comparison of the infrared spectra of their solutions and solids and also from the equilibrium constants. One of the several plausible cyclic forms appears to be predominant in solutions of the uracil–uracil and adenine–uracil dimers, though which one could not be determined. On the other hand, more than one form of cyclic dimer may be present in the solutions of ethyladenine.

It is shown quantitatively that adenine and uracil associate in chloroform solution much more easily with each other than with themselves. The association constant between adenine and uracil, K_{AU}, is 15 times larger than K_{UU}, which is about twice as large as K_{AA} at 25°. These values mean that, if equal volumes of 0.01 M solutions of adenine and uracil are mixed at 25°, 25.8% of each dissolved substance forms the complex dimer, 3.0% of uracil forms the uracil–uracil dimer, 1.6% of adenine forms the adenine–adenine dimer, and 71.2% of uracil and 72.6% of adenine are left as monomer. Kuechler and Derkosch[7] have obtained dissociation constants for adenosine and uridine derivatives in carbon tetrachloride at room temperature. Their results show the same tendency among the constants as described above, though their values (expressed as *association constants*) are 6 times larger than those obtained here. The difference is presumably due to the difference in solvents.[24]

The entropy changes, $-\Delta S°$, for the formation of the three kinds of dimers (AA, UU, and AU) have almost equivalent values, 11 eu, which fact suggests that the structures of the three dimers are closely similar. They are believed to be cyclic, because the values of $-\Delta H°$ obtained are too large for the formation of a single hydrogen bond. The $-\Delta S°$ for the formation of the cyclic dimer of caprolactam in carbon tetrachloride has been determined as 9.0 eu,[11] which is not greatly different from that obtained here.

The heat of formation $-\Delta H°$ for the adenine–uracil dimer implied by the larger association constant exceeds that for the adenine–adenine or uracil–uracil dimer by 1.5–2.0 kcal/mole of dimer. We believe that this is the first quantitative evidence for the general belief that adenine and uracil form stronger hydrogen bonds in the mixed dimer than in the simple dimers.

The $-\Delta H°$ obtained, however, is not simply the enthalpy difference resulting from the formation of hydrogen bonds. It is, of course, the entire heat of formation of solvated dimer from solvated monomers. In view of the expected small differences between the enthalpies of solvation of dimer and of monomers, $-\Delta H°$ does approximate reasonably well to the enthalpy (and also the energy) of the hydrogen bonds formed.

The difference of 1.5–2.0 kcal between $\Delta H°_{AU}$ and $\Delta H°_{AA}$ (or $\Delta H°_{UU}$) is rather small but, of course, has an important effect on the association constants. We had also expected that this difference would be apparent in qualitative changes in the bonded NH frequencies of the AU dimer, an expectation that was not realized. Comparison of the bands in Figures 1, 2, and 4 will show that the differences are quantitative rather than qualitative. We conclude that the bonds formed in the AU complex are favored by steric and other factors only to a slight extent, but that this has a marked effect on the association constants because of the relative values of $\Delta H°$ and $\Delta S°$.

The differences between solvation effects in chloroform and in water are probably far greater than our observed $\Delta H°$ differences. Nonetheless, the specificity of hydrogen bonding in chloroform solution is the same as that seen in naturally occurring polynucleotides, in that adenine derivatives specifically bond to uracil (or thymine) derivatives and guanine to cytosine derivatives.[6,8,9] The fact that this selectivity occurs even in a nonaqueous solution may not be entirely fortuitous, since the interior of the double-stranded complementary DNA or RNA is also "nonaqueous"

(24) R. E. Merrifield and W. D. Phillips, *J. Am. Chem. Soc.*, 80, 2778 (1958).

with the hydrogen bonds of the base pairs enclosed in a domain of the stacked unsaturated rings. Recent experiments on countercurrent distribution of DNA from bacteria show that newly replicated DNA is more soluble in a nonaqueous phase than in an aqueous phase.[25] This also suggests that these experiments in nonaqueous solvents may have more relevance to behavior in biological systems than would be anticipated otherwise.

(25) C. Kidson, *Biochim. Biophys. Acta*, in press.

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Iodine-Catalyzed Isomerization of *n*-Heptenes. Thermodynamic Data for the Positional and Geometrical Isomerization and the *cis* Effect in the Entropy Difference of Geometrical Isomer Pairs

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Abstract: The equilibrium compositions of the iodine atom catalyzed isomerization of 1-, 2-, and 3-heptenes have been studied in the gas phase over a temperature range from 148.5 to 333.5°. The equilibrium constants for the positional and geometrical isomerization obtained from gas-chromatographic analysis show straight lines in a van't Hoff plot of log K against $1/T(°K)$. Least-squares fits of the data yield (with standard errors) differences in heats of formation and entropies between the geometrical and positional isomers for a mean temperature of 510°K. In addition, the measured equilibrium constants have been fitted to quadratic equations of the form $\log K = (a/T^2) + (b/T) + c$. The results show that the experimental data are best represented with linear equations, and that for both geometrical isomer pairs $\Delta C_p°$ (400–600°) equals 0 ± 0.5 cal/deg mole. Refined values for partial group contributions (increments) to the heat of formation and entropy of olefins as well as isomer corrections thereof are given. These data confirm previous estimates and they demonstrate the anticipated *cis* effect in the entropy differences of various geometrical isomer pairs. This effect can be attributed to differences in barriers to free rotation of the end groups in the *cis* configurations of the isomers.

The value of the method of the iodine-catalyzed, gas-phase isomerization of olefins in obtaining kinetic parameters as well as accurate thermodynamic data through the measurement of equilibrium constants has been demonstrated by Benson and co-workers on a large number of olefins.[1]

The same method and a new experimental setup was used to obtain the equilibrium constants in the *n*-heptene series. Direct experimental literature data on heats of formation, entropies, and specific heats are available only for 1-heptene[2,3] whose heat of hydrogenation[4] and heat of combustion[5] have been measured. API values,[2] calculated from experimental data on the lower olefins, and comparative estimates are available through the hexenes and for 1-heptene only. A value of 16.8 kcal/mole[6] for $\Delta H_f°(299°)$ of *cis*-2-heptene has been reported from calculations in the homologous series of hydrocarbons.

This study is primarily concerned with the differences in heats of formation ($\Delta\Delta H_f°$) and entropies ($\Delta S°$) between the geometrical isomer pairs of 2-heptene and 3-heptene. From previous equilibrium studies on 2-butene,[1a,b] 2-pentene,[1f] and 1,3-pentadiene,[1e] it was to be expected that the differences in enthalpies for the 2- and the 3-heptenes be the same, while the entropy differences between the geometrical isomer pairs should reflect differences in barriers to internal rotation of the end groups in the *cis* configuration of the molecule. Based on the reported variation between 2-butene[1b] and 2-pentene,[1f] the difference in entropy of the *cis–trans* pair of 3-heptene is estimated to be 1.2 cal/deg mole larger than that of the 2-heptenes.

In addition this work gives $\Delta\Delta H_f°$ and $\Delta S°$ between the *trans* configurations of 2-heptene and 3-heptene. It is reasonable to expect that *trans* forms of various positional isomers should not only have the same enthalpy but very nearly the same entropy content as well, considering that any differences in entropy would have to arise mainly from variations in barriers to internal rotation of the end groups in the molecules.

Finally, accurate data for the differences in enthalpies and entropies of the *n*-heptene isomers, when combined with earlier work reported in the literature, will result

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(4) G. B. Kistiakowsky, J. R. Ruhoff, H. A. Smith, and W. E. Vaughan, *J. Am. Chem. Soc.*, **58**, 137 (1936).

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(6) M. Kh. Karapet'-yants, *Khim. i Tekhnol. Topliva*, **9**, 22 (1956).