Hydrogen Bonded Complexes of ϵ-Caprolactam

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The ultraviolet absorption band of a complex between 9-ethyladenine and ϵ-caprolactam has been observed at a wavelength longer than that of the absorption band for the 9-ethyladenine monomer. Absorbance values (at 277.5 mμ) of solutions that contained 9-ethyladenine and different concentrations of ϵ-caprolactam in cyclohexane were determined at different temperatures. Linear plots were utilized to determine the apparent association constant (K′) of the 9-ethyladenine-caprolactam complex over the range of 25° to 60°. The K′ values for the complex of 4-aminopyrimidine and ϵ-caprolactam were determined for the same temperature range from the absorbance of cyclohexane solutions at 282.5 mμ. The K′ values of the two complexes are the same at 25°, but ϵ-caprolactam is more strongly bonded to 9-ethyladenine than to 4-aminopyrimidine at elevated temperatures. The synthesis of 4-amino-1-ethylbenzimidazole hydrochloride was performed. An attempt to detect a complex between ϵ-caprolactam and 4-amino-1-ethylbenzimidazole in a cyclohexane solution was not successful.

The dimerization of a cyclic lactam in a nonpolar solvent leads to the formation of a stable complex with two hydrogen bonds in a ring of eight atoms (1). The formation of dimers of δ-valerolactam (1,2) and ϵ-caprolactam (3) has been observed, and a 1:1 complex of ϵ-caprolactam and 2-aminopyrimidine has been reported (4). The detection of the 2-aminopyrimidine-lactam complex by ultraviolet absorption spectrophotometry (5) suggests that this technique may be used to demonstrate additional ϵ-caprolactam complexes with heterocyclic amines.

The effect of different concentrations of ϵ-caprolactam on the absorbance of solutions of 9-ethyladenine or 4-aminopyrimidine in cyclohexane was used to determine the apparent association constant (K′) of the complex at a particular temperature. The procedure is a modification of the method of Benesi and Hildebrand (6). Equation (1) is based on the assumption that a 1:1 complex is formed. The total concentrations of ϵ-caprolactam and 9-ethyladenine (or 4-aminopyrimidine) are $(L)_o$ and $(P)_o$, and the concentration of the complex is (D):

$$K' = (D)/[(L)_o - (D)][(P)_o - (D)] \quad (1)$$

$$A = \epsilon_p b[(P)_o - (D)] + \epsilon_D b(D) \quad (2)$$

The term $(L)_o - (D)$ in Eq. (1) is approximately equal to $(L)_o$, if the solutions are prepared with a low $(P)_o$ in comparison with $(L)_o$. In Eq. (2) the absorbance of the solution is represented by A, the molar absorbance of the purine or pyrimidine monomer is represented by ϵ_p, the molar absorbance of the complex is ϵ_D, and the length of the light path is b. The molar absorbance of ϵ-caprolactam was omitted from Eq. (2) because it was found to be zero from 277 to 300 mμ. Equation (3) may be obtained from Eqs. (1) and (2).

$$\frac{(D)}{(P)_o} = \frac{K'(L)_o}{1 + K'(L)_o} = \frac{A - \epsilon_p b(P)_o}{[\epsilon_D - \epsilon_p]b(P)_o} \quad (3)$$

$$\frac{A - \epsilon_p b(P)_o}{(L)_o} = K'\epsilon_D b(P)_o - K'A \quad (4)$$

Equation (3) was rearranged to give Eq. (4); the latter equation shows that a plot of $[A - \epsilon_p b(P)_o]/(L)_o$ *versus* A at constant $(P)_o$ is a straight line with a slope of $-K'$ at a particular temperature (5). It is important to note that the value of ϵ_D is not required to prepare the linear plots based on Eq. (4). Since the direct determination of ϵ_D may be quite difficult, the application of Eq. (4) is a relatively convenient procedure for the estimation of K′ values. Furthermore, the ϵ_D may be estimated from the intercept of these plots; the ϵ_D value should be independent of temperature within the experimental error.

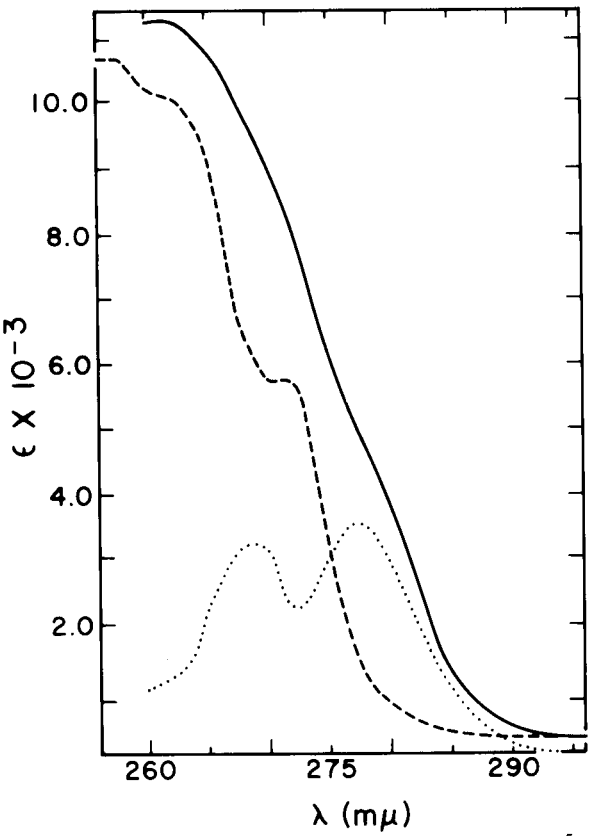


Figure 1. The absorption spectrum of 1.1 x 10^{-5} *M* 9-ethyladenine in cyclohexane, with no lactam added and in the presence of ε-caprolactam at 23°. ————————, lactam = 0; ————————, lactam = 0.094 *M*; ·····, the difference spectrum of the purine and lactam mixture *minus* the spectrum of the purine with no lactam.

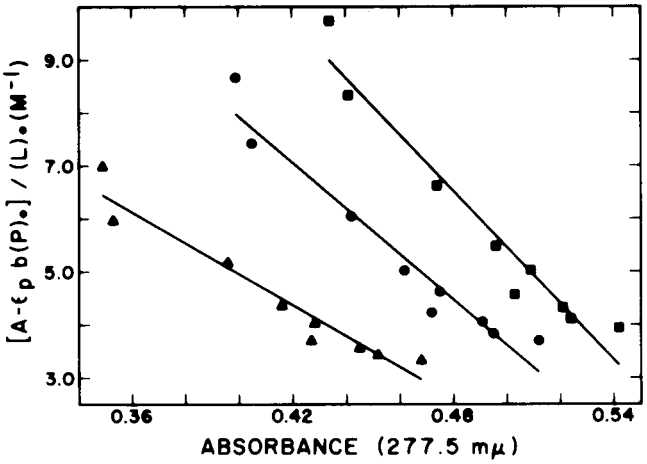


Figure 2. Plots of $[A - \epsilon_p b(P)_o]/(L)_o$ *versus* the absorbance at 277.5 mμ of a 1.1 x 10^{-5} *M* solution of 9-ethyladenine in cyclohexane with different concentrations of ε-caprolactam from 0.027 *M* to 0.094 *M*. The lines at 57° (▲), at 42° (●), and at 31° (■) were determined by an analysis of least squares.

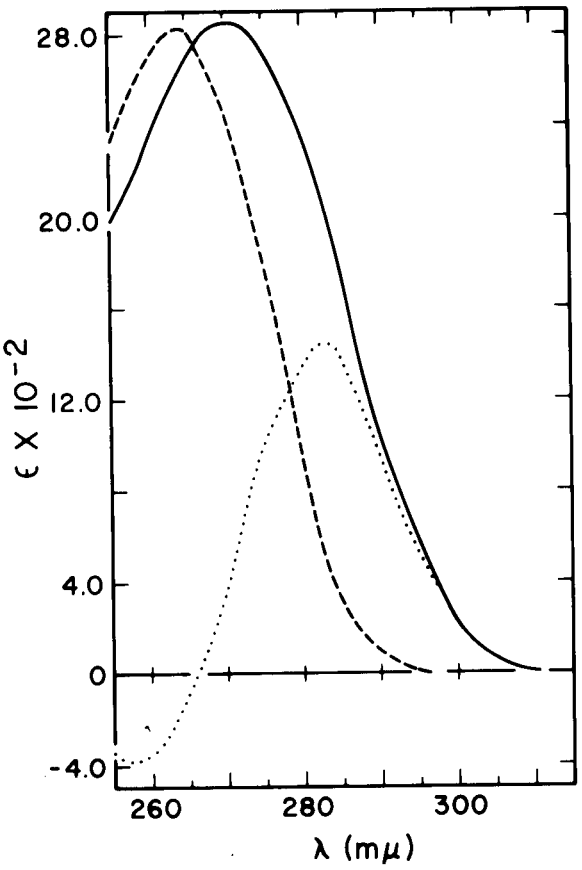


Figure 3. The absorption spectrum of 6.3 x 10^{-5} *M* 4-aminopyrimidine in cyclohexane with no lactam added and in the presence of ε-caprolactam at 23°. ————————, lactam = 0; ————————, lactam = 0.094 *M*; ·····, the difference spectrum of the pyrimidine and lactam mixture *minus* the spectrum of the pyrimidine with no lactam.

EXPERIMENTAL

Thiocytosine (Cyclo Chemical Corp.) was converted to 4-aminopyrimidine by the hydrogenolysis method of Brown (7), recrystallized from ethyl acetate, and dried under vacuum, m.p. 150-152°; uv λ max (ethanol) 270 mμ (ε = 2880). The ε-caprolactam (Calbiochem Organic Chemicals) was recrystallized from ethyl acetate, washed with cyclohexane, and dried under vacuum, m.p. 68-68.5°. The cyclohexane was purified with activated charcoal (Nuchar, Matheson Coleman and Bell) under reflux for twenty minutes [cyclohexane:charcoal, 3:1 (v/v)]. The solvent was filtered through diatomaceous earth (Celite), and distilled from sodium-lead alloy (dri-Na, Baker).

Solutions of a known concentration of 9-ethyladenine (Cyclo Chemical Corp.) in cyclohexane were prepared by an indirect procedure because of the low solubility of the purine in the hydrocarbon solvent. A sample of the purine was dried to constant weight, and a primary stock solution in dry ethanol was prepared. Aliquots of known volumes of the ethanol solution were pipetted into volumetric flasks, and the ethanol was removed under vacuum.

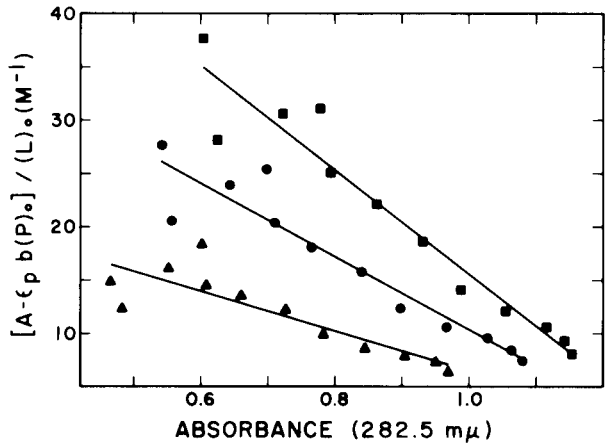


Figure 4. Plots of $[A - \epsilon_p b(P)_o]/(L)_o$ *versus* the absorbance at 282.5 mμ of a 5.9 x 10^{-5} *M* solution of 4-aminopyrimidine in cyclohexane with different concentrations of ϵ-caprolactam from 0.0054 *M* to 0.094 *M*. The lines at 57° (▲), at 42° (●), and at 31° (■) were determined by an analysis of least squares.

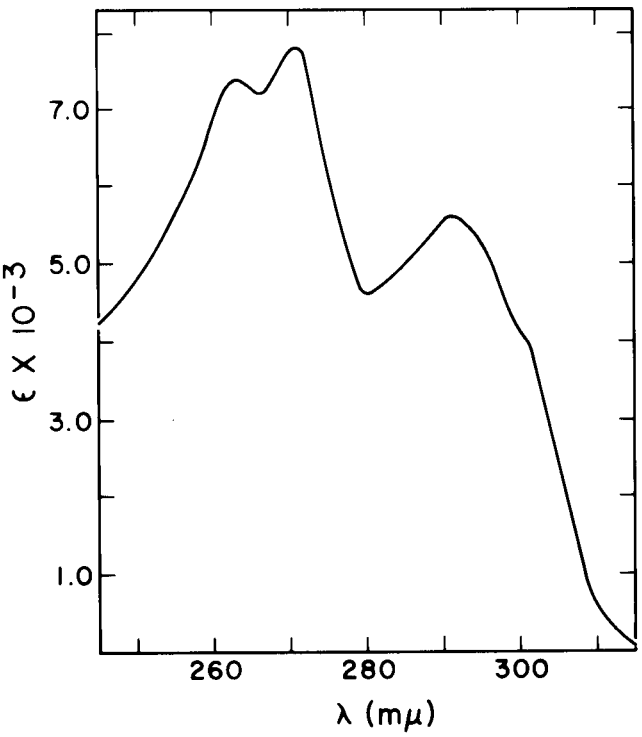


Figure 5. The absorption spectrum of 4.3 x 10^{-6} *M* 4-amino-1-ethylbenzimidazole in cyclohexane, with no lactam added and in the presence of 0.080 *M* ϵ-caprolactam at 23°. The two spectra are exactly the same.

TABLE I

Apparent Association Constants for the 9-Ethyladenine and ϵ-Caprolactam Complex in Cyclohexane (a)

t (°C)	K′ (M^{-1})	ϵ_D (b) (M^{-1})
25	60.0 ± 5.6 (c)	5680
31	53.4 ± 4.8	5400
36	47.2 ± 4.3	5550
42	43.1 ± 4.0	5510
47	38.0 ± 3.4	5450
52	34.2 ± 3.4	5410
57	29.7 ± 2.9	5670
60	28.6 ± 3.8	5380

(a) The experiment was performed with 1.1 x 10^{-5} *M* 9-ethyladenine and ϵ-caprolactam from 0.027 to 0.094 *M*. The molar absorbance of the purine monomer at 277.5 mμ is 1610 M^{-1}. (b) The ϵ of the adenine-lactam complex. (c) The optimal value and standard deviation of K′ at each temperature was computed from an analysis of least squares.

TABLE II

Apparent Association Constants for the 4-Aminopyrimidine and ϵ-Caprolactam Complex in Cyclohexane (a)

t (°C)	K′ (M^{-1})	ϵ_D (b) (M^{-1})
25	56.8 ± 4.4 (c)	2270
31	48.8 ± 4.1	2250
36	41.3 ± 3.6	2240
42	34.5 ± 3.7	2250
47	28.9 ± 3.5	2260
52	23.6 ± 3.7	2310
57	18.4 ± 3.4	2390
60	15.2 ± 3.4	2480

(a) The experiment was performed with 5.9 x 10^{-5} *M* 4-aminopyrimidine with ϵ-caprolactam from 0.0054 *M* to 0.094 *M*. The molar absorbance of the pyrimidine monomer at 282.5 mμ is 685 M^{-1}. (b) The ϵ of the aminopyrimidine-lactam complex. (c) The optimal value and standard deviation of K′ at each temperature was computed from an analysis of least squares.

The volumetric flasks were exhaustively dried under high vacuum to remove traces of ethanol in the residue, and the flasks were filled to volume with dry cyclohexane. The flasks were shaken to dissolve the 9-ethyladenine.

Aliquots of the stock solutions of 9-ethyladenine or 4-aminopyrimidine in cyclohexane were pipetted into dry volumetric flasks from a L/l Repipet at 21°, different volumes of a concentrated ε-caprolactam stock solution were added, and the flasks were filled to a known volume with cyclohexane.

The ultraviolet absorption spectra were determined at room temperature with a Beckman Model DK1 recording spectrophotometer and a 50 mm light path. The ultraviolet absorbance at the wavelength of the maximum in a difference spectrum (and a fixed slit width) was determined with a Gilford Model 240 spectrophotometer and a Sargent Model MR recorder set for an expanded absorbance scale. For the absorbance measurements (fixed wavelength) at different temperatures, the solutions were placed in a water-jacketed 100 mm cylindrical quartz cell (American Instrument Co.) connected to a circulating water bath. The probe of a thermistor thermometer (YSI Model 42 SC), connected to a Sargent Model SRG recorder, was inserted into the cell jacket. Temperature drift-absorbance scans at fixed wavelength were performed for each solution from 60° to 25°; the water bath was heated above 60°, and ice was added to the bath to provide a bath temperature drift to 25° in about 30 minutes. The absorbance change (at a fixed wavelength) and the temperature change were continuously recorded in each experiment. A temperature calibration curve was prepared to correct for the temperature difference between the water in the cell jacket and the cyclohexane solution.

It was assumed that the change in the volume of the solutions due to thermal expansion could be calculated from the molar volume of pure cyclohexane at different temperatures. Literature values of the specific gravity of pure cyclohexane between 10° and 70° were obtained (8), and a regression curve of the temperature dependence of specific gravity was determined by least squares analysis. Between 25° and 60° the change in the specific gravity is less than 5%. The absorbance at particular temperatures in intervals of about 5° on the paper strip chart was determined, and the solute concentrations were corrected for the specific gravity of cyclohexane.

4-Amino-1-ethylbenzimidazole Solutions in Cyclohexane.

The compound, 1,2-diamino-3-nitrobenzene (Aldrich Chemical Co.), was converted to 4(7)-nitrobenzimidazole by the method of Fisher and Joullié (9). The benzimidazole derivative was heated with ethyl iodide and potassium hydroxide in dry ethanol (10). A precipitate was removed from the reaction mixture at 25°, and the filtrate was concentrated on a rotary evaporator. The residue was dissolved in 2.5 *M* sodium hydroxide (aqueous), and the solution was extracted with ethyl ether in a liquid-liquid continuous extractor. The aqueous fraction, which contains unreacted 4(7)-nitrobenzimidazole, was discarded. The ethyl ether fraction was concentrated to a syrup, and the syrup was treated with boiling petroleum ether (11) to extract the 1-ethyl-7-nitrobenzimidazole (m.p. 75-76°). The identity of this compound was confirmed by the preparation of 1-ethyl-3-methyl-7-nitrobenzimidazole iodide by the method of Mizuno *et al.* (12). The methiodide salt was identified by paper chromatography and a qualitative iodine analysis of a chromatographically purified sample.

The petroleum ether insoluble residue was dissolved in hot benzene and treated with activated charcoal. The benzene solution was cooled, dried with anhydrous sodium sulfate, and the product was recrystallized from benzene and petroleum ether to yield 1-ethyl-4-nitrobenzimidazole, m.p. 69-71°, the R_f is 0.31 for thin layer chromatography on ChromAR Sheet 1000 (Mallinckrodt Chemical Co.) with a 2% methanol in chloroform solvent.

A sample of 1-ethyl-4-nitrobenzimidazole was dissolved in dry ethanol and mixed with 10% palladium on charcoal catalyst. The mixture was shaken with hydrogen gas for 25 minutes at room temperature, and the catalyst was removed by filtration. The filtrate was partially concentrated, and concentrated hydrogen chloride was added drop-wise with stirring until the precipitation was complete. The white precipitate was separated by filtration, and additional product was obtained by treatment of the filtrate with ethyl ether. The precipitates were pooled, washed with ethanol and ethyl ether, and dried under vacuum to yield 4-amino-1-ethylbenzimidazole hydrochloride; descending paper chromatography on Whatman 3MM paper with a 1-butanol:glacial acetic acid:water solvent (5:2:3, v/v) in descent gave an R_f of 0.78; uv λ max (hydrochloric acid in water, *p*H 2.0) 267 mμ (ε = 5110), 274 mμ (ε = 5370), and 287 mμ (ε = 3260); λ max (sodium hydroxide in water, *p*H 12) 265 mμ (ε = 7810); λ max (tris buffer in water, *p*H 8.3) 265 mμ (ε = 7760). The ultraviolet spectrum is very similar to the spectrum of 4-amino-1-(β-D-ribofuranosyl)-benzimidazole (13). (The spectrum of 7-amino-1-ethylbenzimidazole in acid, λ max (hydrochloric acid in water, *p*H 2.0) 274 mμ, does not show the two sharp absorption bands of the 1-alkyl-4-aminobenzimidazole derivatives).

Anal. Calcd. for $C_9H_{11}N_3 \cdot 1.65$ HCl: C, 48.81; H, 5.76; N, 18.99; Cl, 26.44. Found: C, 48.85; H, 5.73; N, 18.92; Cl, 26.58.

The hydrochloride was dissolved in aqueous sodium hydroxide, the *p*H was adjusted to 12, and the solution was extracted with chloroform. The chloroform solution of 4-amino-1-ethylbenzimidazole was treated with anhydrous sodium sulfate, filtered, and aliquots were transferred to volumetric flasks. The chloroform was removed under vacuum, dry cyclohexane was added to certain flasks, and aqueous tris buffer (*p*H 8.3) was added to the other flasks to determine the concentration.

Results.

The ultraviolet spectrum of a solution of 1.1 x 10^{-5} *M* 9-ethyladenine in cyclohexane is given in Figure 1; the absorption band is composed of three vibronic components at 256 (ε = 10,700), 263 and 271.5 mμ. The absorbance of 9-ethyladenine at 256 and 277.5 mμ is proportional to concentration at 23° in cyclohexane from 4.5 x 10^{-7} *M* to 1.3 x 10^{-5} *M* 9-ethyladenine, but positive deviations from linearity of a Beer-Lambert plot are observed at higher concentrations. Thus, dimerization of 9-ethyladenine may not occur at concentrations less than 1.3 x 10^{-5} *M*. The absorbance of a 1.1 x 10^{-5} *M* solution of 9-ethyladenine in cyclohexane was determined at 277.5 mμ in the temperature range from 57° to 25°. When the concentration was corrected for the thermal expansion of the solvent, the absorbance of 9-ethyladenine was found to be independent of temperature for this experimental interval. Thus, the spectrum reported in Figure 1 represents the molar absorbance of the 9-ethyladenine monomer from 25° to 57°.

The spectrum of a solution of 1.1 x 10^{-5} *M* 9-ethyladenine and 9.4 x 10^{-2} *M* ε-caprolactam in cyclohexane was determined. A shift of the absorption band envelope to longer wavelengths, with a maximum at 262 mμ was observed for the purine in the presence of the lactam. The spectrum of 9-ethyladenine was subtracted from that of the purine-lactam mixture; the difference spectrum reveals maxima at 269 and 277.5 mμ (Figure 1).

In the temperature drift experiments, cyclohexane solutions of 9-ethyladenine at a fixed concentration of 1.1 x 10^{-5} *M* were prepared with different concentrations of ε-caprolactam from 2.7 x 10^{-2} *M* to 9.4 x 10^{-2} *M*. The absorbance of each solution was recorded continuously at a maximum in the difference spectrum (277.5 mμ) with a fixed slit width of 0.45 mm, and the

temperature was recorded from 60° to 25°. The solute concentrations were corrected for the thermal expansion of the solution, and the linear plots were prepared at 5° or 6° temperature intervals. The method of least squares analysis was used to determine the standard deviation and the -K′ value for each temperature. Several linear plots are shown in Figure 2, and the data are summarized in Table I.

The absorbance (at 282.5 mμ) of a solution of 4-aminopyrimidine in cyclohexane was found to be proportional to the concentration between 2.1 x 10^{-5} *M* and 2.1 x 10^{-4} *M*. The ultraviolet absorption spectrum of 4-aminopyrimidine in dry cyclohexane reveals a maximum at 264 mμ (Figure 3); the addition of ϵ-caprolactam to the 4-aminopyrimidine solution produces a shift in the absorption maximum to 270 mμ. The absorbance at the wavelength of the maximum in the difference spectrum of the complex and the pyrimidine monomer (282.5 mμ, slit width = 0.50 mm) was used for the determination of K′. The absorbance of solutions of 5.9 x 10^{-5} *M* 4-aminopyrimidine and 5.4 x 10^{-3} *M* to 9.4 x 10^{-2} *M* ϵ-caprolactam was determined from 60° to 25°; the linear plots for the 4-aminopyrimidine-lactam complex are given in Figure 4. The value of $(D)/(P)_0$, determined with Eq. (3), varies from 0.23 to 0.84 at 25° in the 4-aminopyrimidine experiments.

The effect of a change in $(P)_0$ on the estimate of K′ was determined with an experiment performed at 1.64 x 10^{-5} *M* 4-aminopyrimidine and nine lactam concentrations from 5.4 x 10^{-3} *M* to 8.0 x 10^{-2} *M*. The K′ value at 31° was found to be 50.2 ± 6.1, which agrees with the results of Table II within the experimental error.

The spectrum of 4-amino-1-ethylbenzimidazole in cyclohexane is given in Figure 5. The spectrum was determined in a solution which was adjusted to a concentration of 0.08 *M* ϵ-caprolactam. Figure 5 shows that the spectrum of the aminobenzimidazole is not altered by the addition of the lactam to the cyclohexane solution.

Discussion.

The ultraviolet absorption spectrum of 6 x 10^{-5} *M* 9-ethyladenine in isooctane has been reported by Gratzer and McClare (14). Since deviations from a linear absorbance relationship with concentration in a hydrocarbon solvent are observed at all concentrations above 1.3 x 10^{-5} *M*, the spectrum reported at 6 x 10^{-5} *M* (14) may include contributions from homodimers of adenine. The perturbation of the spectrum with ethanol (14) is qualitatively similar to the effect of ϵ-caprolactam on 9-ethyladenine.

The absorbance of a 0.094 *M* solution of ϵ-caprolactam in cyclohexane (100 mm path) was found to be 0.11 at 269 mμ. This observation, taken with the results of Figures 1 and 3, demonstrates that the most reliable wavelengths which could be used to estimate complex formation are 277.5 mμ for the adenine experiments and 282.5 mμ for the 4-aminopyrimidine. It was not readily possible, therefore, to apply the multiple wavelength technique of Johnson and Bowen (15).

The precision of the fixed wavelength ultraviolet method for K′ is enhanced by the temperature independence of the molar absorbance of the 9-ethyladenine monomer. This observation may be compared with the results in the infrared region of the spectrum which show an 8% increase in the molar absorbance of 9-ethyladenine for a temperature decrease from 59° to 24° (16). In contrast with the infrared technique, however, a constant ultraviolet ϵ_p value could be utilized in the determination of K′ at all the temperatures reported in Table I and II.

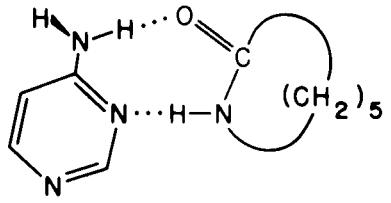
The linear treatment of Eq. (4) for the determination of K′ at a particular temperature, is only applicable when $(P)_0$ is held constant. Furthermore, Conrow *et al.* (17) have demonstrated that a small experimental error in $(P)_0$ may lead to large errors in K′. To improve the precision and reproducibility of $(P)_0$, a fixed stop cylinder and piston pipettor was utilized to deliver the solutions of 9-ethyladenine and 4-aminopyrimidine. With this technique, it was possible to reproduce $(P)_0$ within ± 1%.

A solution of ϵ-caprolactam in cyclohexane will contain different concentrations of the lactam monomer (L) and the lactam dimer (L_2) at different temperatures. Since the dimerization was not considered in the derivation of Eq. (4), K′ can not be interpreted as an equilibrium constant for the association of P (9-ethyladenine or 4-aminopyrimidine) and the monomer of ϵ-caprolactam. The physical significance of K′ is explicable in terms of the following equation (4):

$$P + L_2 \rightleftharpoons L + L + P \rightleftharpoons D + L \qquad (5)$$

The overall reaction from L_2 to D is characterized by the K′ values at different temperatures. It will not be possible to calculate the equilibrium constant for the association of L and P in a particular reaction until (L) values have been determined for the solutions of ϵ-caprolactam in cyclohexane at different temperatures. Nevertheless, the K′ values are of interest because the stability of the 9-ethyladenine-caprolactam complex can be compared with that of the 4-aminopyrimidine-caprolactam complex. The results in Table I and II show that at 60° the K′ value for the formation of the 9-ethyladenine-lactam complex is substantially higher than the corresponding K′ value of the 4-aminopyrimidine-lactam complex.

In the formation of the 9-ethyladenine-caprolactam complex, the N–H group of ϵ-caprolactam may be bonded to the N-7 position of the purine ring or the N-1 position of the purine (18). Thus, the molecular structure of the 9-ethyladenine-lactam complex is unknown at the present time. The following model for the complex of 4-aminopyrimidine and ϵ-caprolactam, however, may be the only structural representation that is consistent with current concepts of hydrogen bond formation.



De Maeyer *et al.* (4) have suggested the existence of an eight atom ring with two hydrogen bonds in the complex of 2-aminopyrimidine and ϵ-caprolactam. The crystal structure of a complex between 2-amino-9-ethylpurine and 5-fluoro-1-methyluracil has been determined by x-ray diffraction (18). The structure of the hydrogen-bonded ring of the purine-uracil complex is essentially the same as that proposed for the complex of 4-aminopyrimidine and ϵ-caprolactam.

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