

Enthalpies of Solution of the Nucleic Acid Bases.

2. Thymine in Water

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An adiabatic solution calorimeter was used to measure enthalpies of solution in water of 8 samples of thymine for which analytical data are reported. Our best values for the enthalpy of solution and the change in heat capacity are

$$\Delta H^\circ(\infty, 298.15 \text{ K}) = (24.32 \pm 0.70) \text{ kJ} \cdot \text{mol}^{-1}$$

$$\Delta C_p^\circ = (106 \pm 26) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}, 298 \text{ K} < T < 328 \text{ K}.$$

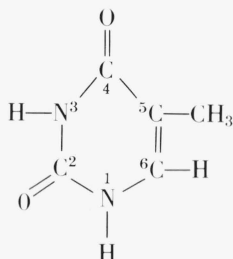
These were used to calculate $\Delta S^\circ = (53.1 \pm 3.6) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ for the entropy of solution, and $C_p^\circ = (256 \pm 26) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ for the apparent molal heat capacity at infinite dilution. No change in the enthalpy of solution with concentration was observed in the range of 5 to 35 mmol \cdot kg⁻¹.

Key words: Calorimetry; 5-methyl-2,4 (1H, 3H)-pyrimidinedione; 5-methyluracil; nucleic acid bases; thermochemistry; thymine; density, enthalpy of solution.

1. Introduction

This is the second in a series of papers on enthalpies of solution of the bases of the nucleic acids. The first paper reported on the enthalpy of solution of adenine in water [1]¹ and described many procedural details also followed in this work. Emphasis is placed on the characterization of the samples in order to establish realistic uncertainty limits for the measured values.

Thymine, a pyrimidine base, has the following structural formula [2]:



It is slightly soluble in water ($\sim 4 \text{ g} \cdot \text{L}^{-1}$ at 298 K [3,4,5]). It sublimes and decomposes before it melts. The only measurements of the enthalpy of solution reported previously were by Alvarez and Biltonen [6].

¹ Figures in brackets indicate the literature references at the end of this paper.

In this work enthalpies of solution of four samples from three commercial sources (including a portion of the sample used by Alvarez and Biltonen) were compared. Enthalpies of solution were also measured for samples that were products of sublimation and of recrystallization from water and from ethyl alcohol. Measurements were made over the temperature range 297 to 328 K and over the concentration range 5 to 35 mmol \cdot kg⁻¹.

2. The Thymine (Thy) Samples

Enthalpies of solution were measured for four commercial samples² as received or after vacuum drying at 373 K. The following information about these samples was obtained from labels, catalogs, and brochures supplied by the manufacturers or distributors:

Thy 1. Calbiochem, Cat. No. 6090, Grade A, 100 g received about 1970—exact date unknown. *Label information:* Lot 900490; Analysis: Nitrogen, 22.08 percent; Spectra at pH 7, 250/260 0.69; 280/260 0.53; λ max 264 m μ , ϵ max 7830; Chromat. Homogeneous.

Thy 2. 9.4 g received from R. L. Biltonen, July 1974, from sample used in [6]. Distributed by Mann Research Laboratories, Cat. No. 2802, Lot R-1282, Paper Chr.: Homogeneous.

² The information presented in this paper is in no way intended as an endorsement nor a condemnation of any of the materials or services used. Commercial sources are named only for specific identification.

Thy 3. E-M Laboratories, Cat. No. 8209, for biochemistry, Lot 4955541, 100 g received August 1947. *Brochure information:* Type analysis: assay, 98 percent (ref. to dried substance). Optical properties, Measured values pH 7: λ max 264.5; E_{250}/E_{260} 0.67 ± 0.02 ; E_{280}/E_{260} 0.53 ± 0.01 ; E_{290}/E_{260} 0.09 ± 0.02 . Thin layer chromatography, Layer: TLC plates Silica Gel F₂₅₄ pre-coated. Solvent: 1-Propanol/water (70/30). *R_f* value: ~ 0.75 .

Thy 4. Same as Thy 3 except E-M Laboratories Lot 4958785, 10 g received January 1975.

Portions of Thy 3 were further purified in this laboratory as follows:

Thy 3a. Approximately 22 g of Thy 3 was dissolved in 0.8 L of boiling distilled H₂O and several grams of decolorizing activated charcoal was added. The hot solution was filtered into a heated filtration flask through a Nalgene Buchner funnel with No. 1 Whatman paper under No. 50 Whatman paper which was covered by a mat of filter paper pulp. The filtrate was reheated and passed through the same filter three times to remove traces of the charcoal and pulp. The final filtrate was transferred to a crystallizing dish and cooled slowly at about 295 K. The needlelike crystals which formed were filtered through No. 1 Whatman paper and then redissolved in about 0.4 L of boiling distilled H₂O. As before, the hot solution was filtered and cooled slowly. The crystals which formed in the second recrystallization were filtered and dried at 340 K at atmospheric pressure for about 72 h. The yield was 6.6 g.

Thy 3b. Approximately 8 g of Thy 3 was dissolved in 0.5 L of hot 90 percent ethyl alcohol (EtOH) and filtered through the filter described above. After cooling slowly, the large star-shaped crystals which formed were filtered on No. 1 Whatman paper. These crystals were redissolved, filtered, and recrystallized a second time. After filtering, this product was dried as described for Thy 3a. The yield was 1.2 g.

(Thy 3a and b were further dried under vacuum at 375 K for 134 h during volatile matter observations to be discussed in section 2.1.1.)

Thy 3d. 5 g of Thy 3 was slowly vacuum-sublimed according to the procedures described for Ade 1c and 5c [1]. The maximum temperature was about 410 K but most of the sublimation occurred at 390 K. After 6 days the sublimation was stopped before all of the sample had sublimed. The sublimed sample and the residue of sublimation were removed from the sublimation vessel in a glove box guarded from atmospheric H₂O. Calorimetric samples were also transferred to the sample holder in this glove box. The sublimed sample was light and fluffy, and static-charge problems were encountered in transfers of the samples.

Thy 3e. This was the unsublimed residue from the sublimation of Thy 3d.

All of the samples were white powders; Thy 1 had the finest granules and Thy 2 had a grey cast and was more inclined to stick together in lumps than the other samples.

2.1 Characterization of Samples

The purity of the bases of the nucleic acids cannot be determined from freezing temperature measurements because they decompose before melting. A less desirable alternative is to determine impurities in the samples and to describe the properties of the samples by various chemical and physical methods. In this laboratory the density of the thymine

samples was measured, volatile matter was determined by vacuum drying, H₂O by Karl Fischer titration, and impurities by paper and thin layer chromatography (TLC). Greater detail about these methods was given previously [1]. Analyses of the elemental compositions, emission spectra, and x-ray diffraction patterns were obtained from other laboratories. The heat capacity of the crystalline samples at 298 K was also measured.

2.1.1. Density, Volatile Matter, and H₂O

The benzene displacement method of measuring densities was described [1]. The insolubility of thymine in benzene was confirmed by observing that there was (1) no change in the thymine mass as a result of the density measurement and (2) no visible residue when the benzene from the density measurement was evaporated to dryness. There was difficulty in wetting the samples with the benzene, and errors due to trapped air may have resulted. The measured densities were as follows: 1.341, 1.328, 1.372, and 1.344 g·mL⁻¹ for Thy 1, 2, 3, and 4 (approximate sample mass: 0.6, 0.4, 1.3, and 0.7 g), respectively. The nearly linear relationship between the measured density and the mass of sample indicates a probable systematic error (such as air entrapment); however, the mean density value of 1.35 g·mL⁻¹ was used to calculate the buoyancy correction factor, 1.0007, for this work. This density is less than that of (1.455 \pm 0.005) g·mL⁻¹ obtained on single crystals by the floatation method [7].

Samples of Thy 1 vacuum-dried at 340 K (see [1] for details) were at essentially constant mass after 4 h with a loss of only 0.5 mg·g⁻¹; at 375 K, similar samples appeared to be at constant mass after about 8 h with a loss of 1.0 mg·g⁻¹; Thy 3 showed similar losses. However, Thy 2 did not reach constant mass at 340 K; even after approximately 170 h the rate of mass loss was 0.6 mg·g⁻¹·h⁻¹. It was observed that Thy 4 lost mass at the constant rate of only 0.1 mg·g⁻¹·h⁻¹ for approximately 170 h. The recrystallized samples, Thy 3a and 3b, showed a constant rate of mass loss of 0.025 mg·g⁻¹·h⁻¹. It is assumed that these constant rates are all sublimation losses and not H₂O or other impurities in the samples since the rates remained essentially constant from the beginning of the heating. The different rates are probably related to the different surface areas since Thy 2 which had the highest sublimation rate was the finest powder, and the coarse products of recrystallization had the lower sublimation rates.

Karl Fischer titrations of water in samples of Thy 1, 2, 3, and 4 indicated 0.12, 0.18, 0.12, and 0.16 mass percent H₂O, respectively. A Karl Fischer automatic titrator with a 1-mL automatic buret and recorder were used in the titrations. The reagent was calibrated before and after each group of measurements by addition of 1 mL of distilled H₂O to the 50 mL of ACS spectroscopic methanol which was the solvent

for the 50-mg samples of thymine. Approximately 30 min was required for complete solution. Dry nitrogen was continuously passed over the solution during the titrations. The estimated limit of detection under these conditions is about 0.02 mg of H₂O.

A sample of Thy 4 which had been vacuum-dried at 375 K for 12 h, showed no gain in mass when exposed to the atmosphere (~ 35% relative humidity) for 24 h. This agreed with the findings of Falk [8] that no hydrates of thymine are formed at relative humidities as high as 93 percent. Therefore, because thymine was not hygroscopic, the calorimetric samples were transferred to the sample holder in the laboratory atmosphere except the products of sublimation where the transfers were made in the glove box.

2.1.2. Other Analytical Work

Emission spectroscopic analyses³ of Thy 1, 2, 3, and 4 indicated the same background limits of detection as for the adenine samples [1]. In addition, the following impurities were found in Thy 1 (in mass %): Si, 0.15; Fe, 0.05; and traces of Cu and Mg. The possible presence of these impurities is assumed to contribute no significant error to the enthalpy of solution.

The duplicate elemental analyses⁴ of Thy 1, 2, 3, and 4 as received are given in table 1. The H values for all of the samples agree with the theoretical value for anhydrous thymine; the C, O, and N for Thy 1, 3, and 4 are a little

high; and the C, O, and N for Thy 2 are significantly lower than those for anhydrous thymine. None of our other analytical data provides an explanation for the latter results which certainly indicate an undefined impurity. The analyses do, however, eliminate the possible presence of hydrates in the samples in significant amounts.

The guidelines set forth in a National Academy of Sciences (NAS) publication [9] were followed in our TLC and paper chromatographic work. Details of our procedures and preliminary tests of sensitivities and backgrounds were described in the first paper of this series [1].

Approximately 32 mg of Thy 1, 2, 3, and 4 were each dissolved in 25 mL of hot NH₄OH (aq, 2 mol·L⁻¹). 5 μL aliquots were taken for the spottings which contained about 6 μg of thymine. A "standard" reference solution in NH₄OH (aq, 1 mol·L⁻¹) was prepared which contained 5 mmol·L⁻¹ of adenine, cytosine, thymine, and uracil; this standard was run with each group of the thymine solutions.

R_f values = (distance traveled by major component/distance traveled by the solution front) are given in table 2 for the four thymine samples on glass TLC plates coated with MN300F Cellulose (with fluorescent indicator), on Whatman No. 1 chromatography paper, P-1, and on Whatman No. 40 chromatography paper, P-40, in the four carrier solutions listed. The NAS values from similar systems are also listed for comparison. Our values on P-40 (also used by NAS) are as much as 0.1 *R_f* unit higher than the NAS value—the results using P-1 are in better agreement with those of NAS. However, these are in acceptable agreement for independent measurements.

³ Analyses by J. A. Norris, Center for Analytical Chemistry, National Measurement Laboratory.

⁴ Analyses by Micro-Analysis, Inc., Wilmington, DE 19808.

Table 1. Elemental analyses of thymine samples

Substance	Empirical Formula	Molecular Mass g/mol	C	H	O	N (Kjeldahl)	Sulfated Ash	E
Theoretical Composition:								
Thy	C ₅ H ₆ N ₂ O ₂	126.1146	47.62	4.80	25.37	22.21	-----	100.00
Thy·H ₂ O	C ₅ H ₈ N ₂ O ₃	144.1298	41.67	5.59	33.30	19.44	-----	100.00
Analyses:								
Thy 1	-----	-----	48.09	4.76	25.89	22.37	0.24	101.35
	-----	-----	47.92	4.72	25.65	22.12	0.26	100.67
Thy 2	-----	-----	46.49	4.72	24.71	21.39	0.03	97.34
	-----	-----	46.62	4.62	24.53	21.49	0.08	97.34
Thy 3	-----	-----	47.87	4.72	25.48	22.57	0.00	100.64
	-----	-----	47.93	4.81	25.57	22.39	0.03	100.73
Thy 4	-----	-----	47.77	4.82	25.56	22.64	0.01	100.80
	-----	-----	47.95	4.74	25.27	22.83	0.00	100.79

The four carrier (or tank) solutions, A, B, C, and D, are described in the footnote in table 2. With solution A, the standard reference solution produced three distinct spots: one at the R_f for adenine, one at the R_f for uracil, and one at the R_f 's for thymine and cytosine which were undistinguishable in this solution. With solutions B and C cytosine and uracil were undistinguishable at nearly the same R_f , but thymine and adenine formed separate spots. With solution D all three of the pyrimidine bases were undistinguishable; only adenine formed a separate spot.

The four thymine samples gave the same R_f values within the reading uncertainty of approximately $\pm 0.02 R_f$ unit. No spots were visible except those of the major component in any of the chromatograms of the thymine solutions. We assume that 5 percent impurity would have been detected for

any of the compounds listed on pages 153 and 154 of the NAS publication [9]. From these observations we estimate that the purity of the four thymine samples as received was 95 percent or more.

2.1.3. Calorimetric Characterization

Alvarez and Biltonen [6] reported $(151.4 \pm 0.4) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ for the heat capacity of the crystalline Thy 2 at 298 K. A comparable value, $(150.4 \pm 0.5) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ [10], for Thy 3 was measured by Ernesto Friere using a drop microcalorimeter at the University of Virginia. The latter is considered to be the better value because results presented in this paper indicate that Thy 3 is probably of higher purity.

Table 2. R_f values for the thymine samples in NH_4OH (aq, $2 \text{ mol} \cdot \text{L}^{-1}$) solutions on fluorescent TLC plates and two papers with four carrier solutions, A, B, C, and D.

Thymine Sample No.	A ^a			B ^a			C ^a			D ^a		
	TLC	P-1	P-40	TLC	P-1	P-40	TLC	P-1	P-40	TLC	P-1	P-40
1	0.81	0.79	0.84	0.72	0.68	0.73	0.80	0.80	---	0.88	0.78	0.79
2	.81	.80	.83	.70	.69	.72	.80	.79	---	.91	.75	.78
3	.81	.79	.83	.71	.66	.73	.81	.79	---	.89	.75	.82
4	.81	.79	.82	.70	.70	.72	.81	.78	---	.87	.79	.77
NAS[10]			0.78			0.63			0.72			---

^aThe compositions of the carrier (or tank) solutions was as follows:

Soln A: 5 parts of iso-butyric acid + 3 parts of NH_4OH (aq, $0.5 \text{ mol} \cdot \text{L}^{-1}$).

Soln B: 7 parts of iso-propyl alcohol + 1 part of conc. NH_4OH + 2 parts of H_2O .

Soln C: 7 parts of 95% ethyl alcohol + 3 parts of sodium acetate (aq, $1 \text{ mol} \cdot \text{L}^{-1}$).

Soln D: H_2O adjusted to pH 10 with NH_4OH (≈1 drop of conc. NH_4OH in 300 mL H_2O).

3. Enthalpy of Solution

Measurements of the enthalpy of solution of thymine in water were made in a platinum-lined, adiabatic solution calorimeter which was previously described in detail [11]. The solution capacity is approximately 300 mL, and the volume of the platinum sample container (see [11]) used in this work was 2.7 mL.

A fast stirring rate, $860 \text{ revolutions} \cdot \text{min}^{-1}$, was used to reduce the difficulty in wetting the sample. There was a tendency for the thymine to remain unreacted in air bubbles which stuck to the inner surface of the sample holder. Sometimes the air bubbles released upon opening the sample holder, carried some of the thymine to the surface of the solution where it floated or even bounced above the solution surface causing some of the sample to stick to the cover of the vessel. This problem was greatly reduced after the

installation of a bronze sieve-wire baffle under the surface of the solution. The baffle did not significantly limit the stirring of the solution, but it did inhibit the air bubbles from carrying thymine above the solution surface. Occasionally however, some of the material escaped around the edges of the baffle. In a preliminary experiment, a wetting agent (Triton X-100) was added to the initial calorimetric solution, but there appeared to be no improvement in the wetting of the thymine sample.

In 10 of the 26 experiments reported here, some unreacted sample was visible at the end of the experiment—either stuck on the vessel cover, floating on the surface of the solution, or trapped in air bubbles adhering to the sample holder surface. However, the quantity of unreacted material in these experiments was estimated to be $< 1 \text{ mg}$ and the deficiency in the heat of reaction was apparently within the calorimetric uncertainty. In a private communication, R. L.

Biltonen stated that similar problems were encountered in their work [6], and an experiment was rejected if there was visible unreacted sample at the end of an experiment. However, their samples were in the 20- to 30-mg range and visible material would represent a larger fraction of the total heat of reaction than in our work where the samples were in the 0.2- to 1.3-g range. Biltonen also suspected some improvement in the completeness of reaction after the samples were vacuum dried; we found that this made little if any difference. Further observations regarding factors influencing completeness of reaction will be described in the discussion of the experimental data later in this section.

In each experiment there was an electrical calibration before and after the chemical reaction. In most of the experiments electrical energy was added during the endothermic reactions of thymine in water to prevent a decrease in the calorimeter temperature (hence, loss of adiabatic conditions). However, in a few experiments where the heat absorbed during the reaction was expected to be less than 100 J or the reaction was expected to be slow, electrical energy was not added during the chemical reaction. The energy absorbed was compensated for by the stirring energy, thus maintaining the calorimeter temperature and adiabatic conditions. Details of procedures and calculations were described previously [11, 12].

Measurements of the pH of stirred thymine solutions (25 mmol · kg⁻¹) in equilibrium with air (as were the calorimetric solutions) indicated a pH of 5.6 with an estimated uncertainty of 0.1 pH unit for both Thy 1 and Thy 3. The meter was calibrated with a standard buffer at pH 7.0 and was checked at the end of the measurements.

Uncertainties in this paper are at the 95 percent confidence level except as noted. The calorimetric experiments were completed between June and September 1976 although the preparational and analytical work began nearly 2 years earlier. The Table of Atomic Weights 1975 [13] was used in calculating the molecular masses used in this work: Thy, 126.1146; and H₂O, 18.0152. The calibrations of the quartz oscillator thermometer system and of the reference standards for electrical energy measurements are the same as described in the first paper of this series [1]. For conversions of energy units, 1 thermochemical calorie = 4.184 joules.

3.1. Calorimetric Experiments

The data for enthalpy of solution measurements of 8 thymine samples, described in section 2, are given in table 3. The calorimetric measurements were made on Thy 1, 2, and 4 as received except that they were vacuum dried for 14 h at about 340 K and then for 10 h at about 373 K; Thy 3 was used as received without vacuum drying. Portions of Thy 3 were twice recrystallized from distilled H₂O (Thy 3a), and from 90 percent ethyl alcohol (Thy 3b), and sublimed under

vacuum at about 400 K (Thy 3d) with an unsublimed residue from the sublimation (Thy 3e). The calorimetric samples were transferred to the platinum sample holder in the atmosphere (relative humidity = 0.35 ± 0.10 and temperature = 295.5 ± 1.0 K) except the sublimation products, Thy 3d and 3e, which were transferred in a glove box containing a desiccant.

There was a significant difference in the reaction rates of the various samples in H₂O. It can be seen in table 3 that at 313 K the reaction period for Thy 1, 2, and 3e was 2 to 4 h, for Thy 3, 3a, and 3b, about 0.5 h; and for Thy 3d and 4, 1 to 2 h. At lower temperatures the reaction periods were longer. The reaction periods in the experiments marked with an asterisk, where a small amount of unreacted sample was visible at the end of the measurements, were no longer than comparable experiments in which the reaction was apparently complete; thus incomplete reaction is not the cause of the different reaction rates. The last three experiments with Thy 3 show that the sample size has little effect on the duration of the reaction period.

In preliminary experiments using Thy 1 (not given in table 3) it became apparent that high stirring speed (860 rpm) and relatively high temperature of reaction (~ 310 K) greatly reduced the amount of undissolved material remaining at the end of the experiments. However, the problem was greater with Thy 2 which was sticky and tended to form agglomerates even after vacuum drying. The sample was sieved and only a small amount of Thy 2 was visible at the end of the three experiments given in table 3. The samples were between standard sieve sizes 35 and 100 in Expts. No. 1165 and 1166, and less than standard sieve size 100 in Expt. No. 1167. In Expt. No. 1166 and subsequent experiments the sieve-wire baffle was installed under the solution surface; this reduced the probability of sample being carried to the surface by air bubbles. None of the other samples was sieved. There was no evidence of incomplete reaction at the end of experiments with the two recrystallized samples, with the unsublimed residue of sublimation, or with Thy 4. Our analytical data do not explain these differences in the solution behavior of the various samples.

In table 3, the Experiment Number is a serial number for experiments with this calorimeter and indicates the chronological order of the experiments. The Reaction Period is the elapsed time between initiating the reactions and the beginning of the rating period which follows the reaction. T_{reaction} is the mean of the initial and final temperatures of reaction. The estimated calorimetric uncertainty for an experiment, Cal. Unc., is based on the duration of the reaction, the magnitude of the temperature change from the reaction, and the standard deviation of the slope of the rating period following the reaction (see [1] for details). The heat of the solution reaction, Q_{reaction} , is given by the following equation:

$$Q_{\text{reaction}} = \Delta T_{\text{reaction}} \left(\frac{\epsilon_i + \epsilon_f}{2} \right) - q_{\text{vap}}$$

where the electrical energy equivalents (in $\text{J} \cdot \text{K}^{-1}$) of the initial and final systems are ϵ_i and ϵ_f , the temperature change due to the solution reaction is $\Delta T_{\text{reaction}}$, and the heat of vaporization of water into the air space in the sample holder upon opening is q_{vap} . $\Delta T_{\text{reaction}} = \Delta T - EIt \left(\frac{\epsilon_i + \epsilon_f}{2} \right)^{-1}$ where ΔT (not given in table 3) is the net temperature change resulting from the endothermic solution reaction and the electrical energy added, EIt . $q_{\text{vap}} = \Delta H_{\text{vap}} \left(V - \frac{s}{d} \right) (1 - RH)$ where ΔH_{vap} is the enthalpy of vaporization of water per unit volume at the mean temperature of reaction, V is the internal volume of the sample holder, s is the mass of sample, d is the density of the sample, and RH is the relative humidity of the atmosphere in which the sample was transferred to the sample holder (for the transfer in the dry box, $RH = 0$, in the laboratory atmosphere, $RH = 0.35 \pm 0.10$). The enthalpy of solution per gram at the temperature of reaction and at the concentration of the measurements is $\Delta H_m(T) = -Q_{\text{reaction}} (\text{Sample mass})^{-1}$. The methods of calculating the electrical energy equivalents, ϵ , the corrected temperature change, ΔT , and the electrical energy added during the reaction, EIt , were described previously [11, 12].

The change in the enthalpy of solution with temperature, ΔC_p , was obtained from the data for $\Delta H(T)$ and T_{reaction} for

Expt. Nos. 1152–1158 which are plotted in figure 1. A least squares fit of the data for $\Delta H(T)$ versus $(T_{\text{reaction}} - 298.15 \text{ K})$ to a linear equation in the range $296 \text{ K} < T < 328 \text{ K}$, resulted in the intercept, $(24.07 \pm 0.15) \text{ kJ} \cdot \text{mol}^{-1}$, and the slope $(0.1056 \pm 0.0099) \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$. The uncertainties are the standard errors and the standard error of the estimate is $0.27 \text{ kJ} \cdot \text{mol}^{-1}$. The value, $\Delta C_p = (106 \pm 26) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$, was used in computing the correction given in table 3 to obtain $\Delta H(298.15 \text{ K})$.

In figure 2 are plotted the data for $\Delta H(298.15 \text{ K})$ versus concentration for the 26 experiments listed in table 3. The radius of a circle (or a circle circumscribed by the other geometric figures) is equal to the estimated calorimetric uncertainty for the experiment. There appears to be no significant change in the enthalpy of solution in the concentration range of 5 to 35 $\text{mmol} \cdot \text{kg}^{-1}$; this is most evident from the open circles (Thy 3) where the one sample was used over the range.

The Karl Fischer titrations (see sec. 2.1.1) indicated little difference in the H_2O content of the four commercial samples (Thy 1, Thy 2, Thy 3, and Thy 4) as received. In the calorimetric experiments given in table 3, all samples had been vacuum dried and were assumed to contain no H_2O , except Thy 3 which was used as received. A correction, $0.20 \text{ kJ} \cdot \text{mol}^{-1}$, for 0.83 mol percent (0.12 mass %) H_2O was later applied to the values of $\Delta H(298.15 \text{ K})$ for Thy 3 given in table 3, but it is not shown in figure 2. There appears to be no significant difference between the enthalpies of solution for the recrystallized samples (the upper and lower half-shaded circles) and the parent material (open circles); this

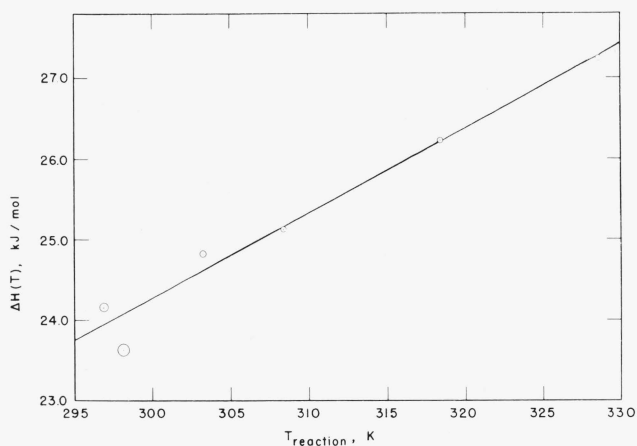


FIGURE 1. Plot showing the relationship between the temperature of reaction and the enthalpy of solution of thymine in H_2O .

The line [slope = $\Delta C_p = (106 \pm 26) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$] represents the fit of the data by a least squares method. The estimated calorimetric uncertainty (see text) is equal to the radius of a circle.

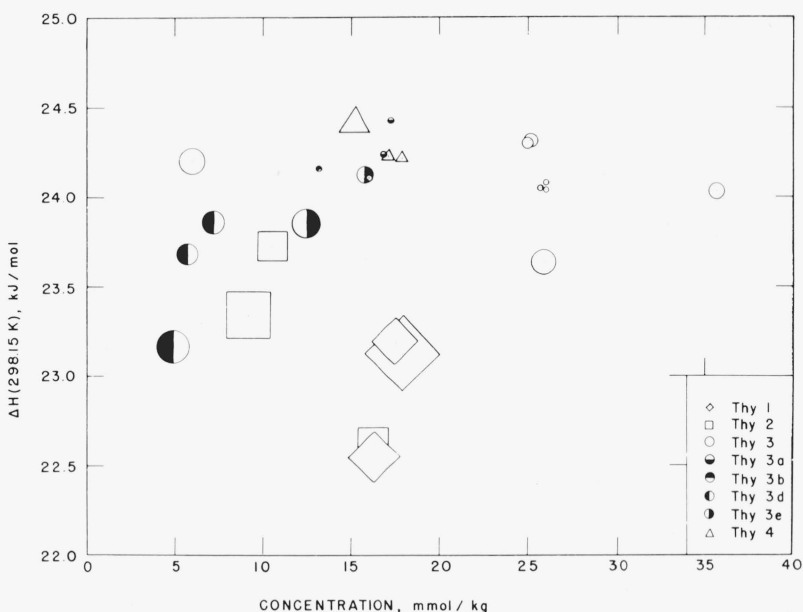


FIGURE 2. Plot showing the effect of concentration on the enthalpy of solution of various thymine samples in H_2O at 298.15 K.

The estimated calorimetric uncertainty (see text) is equal to the radius of a circle or a circle circumscribed by the other geometric figures.

probably indicates the Thy 3 was of high purity. However, the values for Thy 1 (diamonds), Thy 3 (squares), and the sublimed sample, Thy 3d (left half-shaded circles), are lower than most of the others; their calorimetric uncertainties are larger primarily because of the longer reaction periods.

If the sublimed sample, Thy 3d (left half-shaded circles), were drier than the others shown in figure 2, its enthalpy of solution should have a *higher* value than the others. Because lower values were measured, we conclude that some decomposition occurred during sublimation or the sublimed material was of a lower degree of crystallinity or a different tautomeric form as was found for sublimed adenine [1]; however, the magnitude of the difference in the enthalpies of solution of sublimed and unsublimed thymine ($\sim 1 \text{ kJ} \cdot \text{mol}^{-1}$) was about one-fifth that for adenine. The two experiments with the unsublimed residue of sublimation, Thy 3e (right half-shaded circles), are borderline values and provide no conclusive information.

The one low value for Thy 3 (Expt. No. 1154) was probably the result of incomplete reaction. Some undissolved material remained at the end of the experiment, the slope of the rating period following the reaction was about 1 percent less than those of the preceding and subsequent rating periods; and the length of the reaction period was only approximately two-thirds that of the following similar experiment at a slightly lower temperature. Therefore, this experiment was omitted

from the averages. The measurements with Thy 4 (triangles) agree well with those of Thy 3, 3a, and 3b.

On the assumption that the highest values for the enthalpy of solution represent the purest samples and the most complete reaction, we take as our best value the mean of the results of eight Thy 3 experiments (corrected for H_2O and omitting Expt. No. 1154), two Thy 3a experiments, and three Thy 4 experiments. Thus $\Delta H (\infty, 298.15 \text{ K}) = (24.32 \pm 0.09) \text{ kJ} \cdot \text{mol}^{-1}$. The uncertainty is the experimental imprecision at the 99 percent confidence level or 3.0 (standard deviation of the mean). An estimated uncertainty of $0.61 \text{ kJ} \cdot \text{mol}^{-1}$ (2.5%) is added for undetected impurities in the spectroscopic or chromatographic analyses and the unexplained differences in the elemental analyses. The sum of these uncertainties is $0.70 \text{ kJ} \cdot \text{mol}^{-1}$ (3%) which is taken as the overall uncertainty for the enthalpy of solution.

Alvarez and Biltonen [6] have shown that the enthalpy of solution of thymine in water is independent of concentration in the range, 0.8 to $8 \text{ mmol} \cdot \text{kg}^{-1}$, and we have found this to be true from about 5 to $35 \text{ mmol} \cdot \text{kg}^{-1}$. The ionization constant for the N_3 proton [2] is about 4 orders of magnitude larger than the H^+ concentration of the final solutions in these measurements. Therefore, we assume there is no significant amount of ionization in the range of these measurements, and the enthalpies of solution can be taken as the values at infinite dilution for unionized thymine.

Table 3. Data for the measurements of the enthalpies of solution in water of various thymine samples.

Expt. No.	Sample Mass x10 ⁵	H ₂ O Mass-300g	Final Concentration x10 ⁶	T _{reaction}	Reaction Period	Cal. unc. x10 ²	Electrical Energy Equivalent-1730 J/K		-ΔT _{reaction} x10 ²	E _{it} x10 ²	n _{exp} x10 ²	-Q _{reaction} x10 ²	ΔH(T)		Corr. to T = 298.15 K	ΔH(298.15 K)
							Initial	Final					J/g	J/mol		
Thy 1: Calbiochem Lot 900490																
1168	66723	2.434	17494	313.402	198	38	7.94	7.12	7563	87483	20	13121	196.65	24800	1611	23189
1169	68209	2.414	17884	313.384	237	65	7.65	7.22	7708	86848	20	13372	196.04	24724	1609	23115
1170	62018	2.394	16262	313.402	227	44	6.48	6.76	6852	86230	20	11878	191.52	24154	1611	22543
Thy 2: Mann Lot R-1282																
1165	34971	2.349	9171	314.132	117	50	5.82	5.07	4012	86318	23	6940	198.45	25027	1688	23339
1166	40129	2.394	10522	313.470	102	34	6.26	5.85	4658	86878	22	8064	200.95	25343	1618	23725
1167	62203	2.349	16313	313.404	202	36	7.21	6.60	6886	86975	20	11941	191.97	24210	1582	22628
Thy 3: E-M Lot 4955541																
1152	99116	2.404	25989	318.457	37	6	7.92	8.54	11871	86138	23	20612	207.96	26226	2144	24082
1153	97813	2.384	25649	308.330	67	7	6.30	6.05	11231	85472	14	19486	199.22	25124	1075	24049
1154	98724	2.419	25885	298.109	97	28	5.04	4.71	10667	85591	8	18499	187.38	23631	- 4	(23635)
1155	95734	2.429	25100	296.860	152	17	4.23	4.33	10410	-----	8	18352	191.70	24176	- 136	24312
1157	98989	2.359	25960	303.236	107	12	5.17	5.17	11242	87580	11	19495	196.94	24837	537	24300
1158	99211	2.384	26016	328.511	27	6	10.62	11.31	12333	86119	36	21434	216.04	27246	3206	24040
1159	136090	2.434	35680	313.374	37	18	7.93	6.85	15937	88924	16	27672	203.34	25644	1608	24036
1160	61066	2.369	16014	313.413	22	6	6.13	5.97	7184	86782	20	12451	203.89	25714	1612	24102
1161	19428	2.394	5094	313.189	32	30	6.10	5.33	2302	-----	23	3973	204.50	25790	1588	24202
Thy 3a: Twice recrystallized from H₂O																
1175	64086	2.394	16804	313.437	27	6	6.98	7.05	7568	89606	20	13127	204.83	25832	1614	24218
1176	65771	2.359	17248	298.402	47	9	5.35	4.58	7356	89131	9	12754	193.92	24456	27	24429
Thy 3b: Twice recrystallized from EtOH																
1178	50214	2.354	13169	313.440	22	6	6.83	6.52	5922	87606	21	10263	204.38	25776	1615	24161
Thy 3d: Sublimed																
1179	18425	2.339	4832	313.228	39	43	6.69	5.72	2104	-----	35	3617	196.31	24757	1592	23165
1180	27427	2.399	7192	313.602	72	24	7.34	6.87	3212	-----	35	5544	202.14	25492	1632	23860
1181	21587	2.404	5660	313.231	67	25	6.49	6.00	2512	-----	35	4327	200.44	25279	1592	23686
Thy 3e: Sublimation residue																
1182	60245	2.379	15798	313.175	117	20	6.67	6.54	7092	-----	32	12283	203.88	25713	1587	24126
1183	47593	2.364	12481	313.214	157	34	6.59	6.07	5550	-----	33	9603	201.77	25446	1591	23855
Thy 4: E-M Lot 4958785																
1171	65155	2.379	17086	313.418	47	10	7.41	6.91	7696	86930	20	13348	204.88	25838	1612	24226
1172	68062	2.394	17847	313.425	52	8	7.32	7.09	8035	88414	20	13939	204.80	25828	1613	24215
1174	57812	2.424	15158	313.439	117	19	6.96	6.46	6880	87665	21	11929	206.34	26023	1614	24408

* Small amount of undissolved sample visible at end of these experiments - either trapped in air bubble in sample container or floating on surface.

^b The sample holder was filled with Thy 3d and 3e in a glove box (relative humidity = 0); it was filled with all other samples in the atmosphere (relative humidity = 0.35 ± .10).

^c Electrical energy was not added during this reaction because the reaction was expected to be slow. However, during a brief period initially the vessel temperature dropped below that of the adiabatic shield and it was necessary to make an approximate correction of 3.06 J for the heat transfer to the vessel until adiabatic conditions were restored.

4. Discussion and Summary

The only direct measurements of the enthalpy of solution of thymine in water previously published were those of Alvarez and Biltonen [6]. They point out that about one-third of their measurements were rejected because of visible undissolved sample in the final solutions. Their value for ΔH (298.15 K), $(22.70 \pm 0.46) \text{ kJ} \cdot \text{mol}^{-1}$, is lower but within the uncertainty limits of the mean of our three measurements on their sample (Thy 2 in table 3), $23.23 \text{ kJ} \cdot \text{mol}^{-1}$ with an average deviation of $0.40 \text{ kJ} \cdot \text{mol}^{-1}$. We experienced the difficulty of incomplete reaction with this material more than with the other samples used in this work. Alvarez and Biltonen [6] found $\Delta C_p = (188 \pm 17) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ for this reaction; in this work, $\Delta C_p = (106 \pm 10) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ was obtained from measurements with another sample, Thy 3, which appears to be of higher purity than Thy 2.

Differences in the enthalpies of solution of various samples of thymine were detected which were not explained by our

analytical results; this was also true for adenine [1]. Better analytical data must be obtained before values for the enthalpy of solution of higher accuracy and smaller overall uncertainty can be expected. Our best values for the enthalpy of solution and for the change in the heat capacity for the reaction of thymine in water are:

$$\Delta H^\circ(\infty, 298.15 \text{ K}) = (24.32 \pm 0.70) \text{ kJ} \cdot \text{mol}^{-1}$$

$$\Delta C_p^\circ = (106 \pm 26) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}, 296 \text{ K} < T < 328 \text{ K}$$

Other useful thermodynamic data can be calculated with these values. Herskovits and Harrington measured solubilities in water of adenine, thymine, cytosine and uracil [4]; Scruggs et al. made similar measurements for all of these except thymine [5]. The values of Scruggs et al. at 298 K are consistently 13 to 15 percent higher than the comparable values of Herskovits and Harrington which may have systematic errors. We have estimated the solubility of thymine in water at 298 K to be $32 \text{ mmol} \cdot \text{kg}^{-1}$ (based on the value of

27.8 mmol·kg⁻¹ [4] with an estimated uncertainty of 10 percent, and at 298.15 K we obtain for the solution process

$$\Delta G^\circ = -RT \ln m = (8.5 \pm 0.8) \text{ kJ} \cdot \text{mol}^{-1}$$

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} = (53.1 \pm 3.6) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}.$$

The apparent molal heat capacity at infinite dilution was calculated using C_p° given in section 2.1.3. as follows:

$$C_{p2}^\circ = \Delta C_p^\circ + C_p^\circ = (256 \pm 26) \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}.$$

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