Enthalpies of Solution of the Nucleic Acid Bases.
5. Adenine in Aqueous Hydrochloric Acid, Aqueous Sodium Hydroxide, Methanol, and Ethanol

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The following values were obtained from measurements of the enthalpy of solution of a well characterized sample of crystalline adenine in various solvents:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( \Delta H(\infty, 298.15\text{K})/\text{kJ mol}^{-1} )</th>
<th>( \Delta C_p/I\cdot\text{mol}^{-1}\cdot\text{K}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl(aq)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st protonation</td>
<td>11.41 (\pm 0.18)</td>
<td>107 (\pm 3^*) at 1 mol HCl·L(^{-1})</td>
</tr>
<tr>
<td>2nd protonation</td>
<td>19.3 (\pm 4.4)</td>
<td></td>
</tr>
<tr>
<td>NaOH(aq)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st proton dissociation</td>
<td>15.21 (\pm 0.06)</td>
<td>126 (\pm 8^*) at 0.9 mol NaOH·L(^{-1})</td>
</tr>
<tr>
<td>2nd proton dissociation</td>
<td>17.26 (\pm 0.50)</td>
<td></td>
</tr>
<tr>
<td>CH(_3)OH</td>
<td>21.6 (\pm 0.9^*)</td>
<td>93 (\pm 14)</td>
</tr>
<tr>
<td>C(_2)H(_5)OH</td>
<td>22.0 (\pm 0.9^*)</td>
<td>240 (\pm 40^*)</td>
</tr>
</tbody>
</table>

* Uncertainty estimated (others are at the 95% confidence level).

Using these measured values the following enthalpies of protonation and proton dissociation were calculated:

\[ \Delta H(\infty, 298.15\text{K})/\text{kJ mol}^{-1} \]

- Addition of 1 proton: \(-22.1 \pm 1.1\)
- Addition of 2 or more protons: \(-14.2 \pm 2.2\)
- Removal of 1 proton: \(37.6 \pm 1.0\)
- Removal of 2 protons: \(95.5 \pm 1.0\)

Keywords: Adenine; enthalpy of dissociation; enthalpy of protonation; enthalpy of reaction; enthalpy of solution; nucleic acid bases; solution calorimetry; thermochemistry.

1. Introduction

The preceding papers in this series [1, 2] described the results of measurements of the enthalpies of solution in water for some of the bases of the nucleic acids. In this work the enthalpies of solution or reaction of adenine (Ade), C\(_5\)H\(_5\)N\(_5\) or 6-amino purine, in other solvents are reported.

Enthalpies of protonation and of proton dissociation of Ade have previously been obtained by various methods including titrations and spectrophotometry at various temperatures and calorimetric titrations [3]. However, the Ade samples used in the earlier work were not characterized as were the samples used in this work. We have measured enthalpies of reaction for Ade in aqueous HCl and in aqueous NaOH. The concentration range for the HCl and NaOH was 0.1 mol·L\(^{-1}\) to more than 6 mol·L\(^{-1}\). At about 5(mol HCl) L\(^{-1}\) there was evidence of a second protonation of Ade; this was suggested by Albert and Brown [4] as occurring at pH < 1. At about 4(mol NaOH) L\(^{-1}\) evidence of a second proton dissociation of Ade was observed; this has not been previously reported.

Adenine was found to be less than half as soluble in ethanol (EtOH) as in methanol (MeOH). A concentration dependence of the enthalpy of solution was observed in the range of about 1 to 8 mmol Ade (kg MeOH)\(^{-1}\).

Values for \(\Delta C_p\) were determined for the reactions of Ade in each of the four solvents.
2. Materials, Apparatus, and Procedures

The adenine sample, Ade 2, previously described in detail [1] was used as received from a commercial source for all experiments reported in this work except one with Ade 5b, a sample recrystallized from aqueous solution [1]. The volatile matter for Ade 2, determined previously and presumed to be H$_2$O, was (0.15 ± 0.10) mass per cent or (1.1 ± 0.7) mole per cent, and for Ade 5b, (0.40 ± 0.05) mass per cent or (2.9 ± 0.4) mole per cent. No other impurities were identified. These materials were assumed to be of high purity since the values for the enthalpy of solution at infinite dilution agreed with each other and were the highest of the six samples compared.

The calorimetric solutions of aqueous HCl and NaOH were taken from stock solutions prepared by dilution of the concentrated solutions with distilled water. The concentrations of the HCl stock solutions were determined by titrations using analytical reagent grade Na$_2$CO$_3$ (dried 1 h at 548 K), with bromphenol blue as an end-point indicator; the solutions were boiled to remove CO$_2$ before adding the final portion of acid. Potassium acid phthalate, NBS Standard Reference Material No. 84 h, was used in titrations to determine the concentration of the NaOH stock solutions with phenolphthalein as the end-point indicator. CO$_2$-free air was bubbled through the solutions during these titrations.

The platinum sample holder with interchangeable cylinders has been described [5]. In this work only the smallest cylinder, of 0.7-mL capacity, was used. It was filled in the laboratory atmosphere controlled at (295.6 ± 0.5)K and (30 ± 10) per cent relative humidity.

The platinum-lined adiabatic solution calorimeter, the measurement procedures, and the methods of calculation have been previously described [5, 6]. The physical constants and calibrations were the same as those given in [1]. The experiments reported here were made intermittently during the period from April 1974 through May 1976. Most of these reactions were endothermic except those in solutions above 2 (mol HCl)L$^{-1}$ which were exothermic. Electrical energy was not added during the endothermic reactions (except as noted in one experiment) to prevent a decrease in the calorimeter temperature, because the energy absorbed was always less than 50 J and the decrease in the calorimeter temperature was less than 0.04 K; thus, heat transfer corrections were usually negligible.

3. Solution of Adenine in Aqueous HCl

The enthalpy of solution of adenine was measured in 24 experiments over the concentration range of 0.1 to 6.6 (mol HCl)L$^{-1}$. The results obtained in these experiments are given in table 1. The column headings are defined as follows:

Expt. No. is a serial number for experiments with this calorimeter and indicates the chronological order of the experiments.

Mass of Ade has been corrected for the H$_2$O in the samples; all weightings were corrected to vacuum.

Mass and Concentration of aqueous HCl solutions used as the calorimetric solutions are given.

$T_{reaction}$ is the mean temperature of reaction.

Reaction period is the elapsed time between the initiation of the reaction and the beginning of the rating period which follows the reaction.

Cal. Unc., the estimated calorimetric uncertainty for an experiment, 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).

Electrical energy equivalents of the initial and final systems, $\epsilon_i$ and $\epsilon_f$, are given.

$\Delta T_{reaction}$ is the corrected temperature change for the reaction (see [5]).

$Q_{reaction}$ is the heat of the solution reaction represented by the following equation:

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

where $q_{vap}$ is the heat of vaporization of the solution into the air space in the sample holder upon opening and

$$q_{vap} = \Delta H_{vap} \left( V - \frac{s}{d} \right) (1 - RH)$$

where $\Delta H_{vap}$ is the enthalpy of vaporization of the solution 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 (for Ade, $d = 1.47$ g·mL$^{-1}$ [1]), and $RH$ is the relative humidity of the atmosphere in which the sample was transferred to the sample holder.

$\Delta H_{m}(T)$ is the enthalpy of solution at the temperature and concentration of the measurement, and

$$\Delta H_{m}(T) = -Q_{reaction} \cdot M \cdot (\text{Mass of Ade})^{-1}$$

where $M$ is the molecular mass of adenine.

Corr. to $T = 298.15$ K is $\Delta C_p(T_{reaction} - 298.15$ K) where $\Delta C_p$ is the thermal coefficient determined for the reaction.

$\Delta H_{m}(298.15$ K) is the enthalpy of solution at the standard temperature or $\Delta H_{m}(T) +$ (Corr. to $T = 298.15$ K).

The value used for $\Delta C_p$, 107 J·mol$^{-1}$·K$^{-1}$, was calculated from Expt. Nos. 953 and 954 in which the HCl concentration was approximately 1 mol·L$^{-1}$. The uncertainty in the $\Delta C_p$ value is estimated to be 2.5 per cent or 3
J·mol¹·K⁻¹ which is twice the sum of the estimated calorimetric uncertainties. At other concentrations the uncertainty may be somewhat greater.

The experiments given in table 1 are listed in order of increasing concentration of the HCl solutions. The adenine samples, 0.2 g or 0.0015 mol, are Ade 2 except in Expt. No. 1005 where Ade 5b was used. In Expt. No. 954 at 308 K, \( q_{\text{vap}} \) was estimated to be \(-0.04\) J and in the other experiments, \(-0.02\) J. The data in table 1 show that the reactions are decreasingly endothermic up to about 2 mol HCl L⁻¹ after which they are increasingly exothermic. No undissolved material was observed in the final solutions.

In figure 1 are plotted the values from table 1 for \( \Delta H_m \) (298.15 K) as a function of HCl concentration. No corrections were made in these experiments for the variations in adenine concentration, 1.4 to 1.8 mmol·kg⁻¹ solution. The adenine concentration dependence of the enthalpy of solution in water reported previously [1] was small with a large uncertainty. Examination of the values for \( \Delta H_m \) (298.15 K) in table 1 for those experiments where the same concentration of HCl solution was used revealed no consistent dependence on Ade concentration. It is assumed that the differences are due to sample inhomogeneity and experimental uncertainty.

The two straight lines shown in figure 1 were obtained by fitting linear equations to the data for \( \Delta H_m \) (298.15 K) and HCl concentration by the method of least squares, and may be represented by the following equations: for the 14 data points below 5 (mol HCl) L⁻¹,
\[ \Delta H_m^{\text{(298.15 K), kJ \cdot mol}^{-1}} = (11.411 \pm 0.080) - (5.194 \pm 0.028)C, \]

and for the 10 data points above 5(mol HCl)L\(^{-1}\),

\[ \Delta H_m^{\text{(298.15 K), kJ \cdot mol}^{-1}} = (19.3 \pm 2.0) - (6.76 \pm 0.34)C, \]

where \( C \) is the concentration of HCl in mol\cdot L\(^{-1}\) and the standard deviations of the fits are 0.17 kJ\cdot mol\(^{-1}\) and 0.55 kJ\cdot mol\(^{-1}\), respectively. The uncertainties are the standard deviations. From the difference in the two intercepts we obtain the enthalpy of the second protonation at infinite dilution, (7.9 \pm 2.0) kJ\cdot mol\(^{-1}\). The change in slope at 5(mol HCl)L\(^{-1}\) is apparently the result of the second protonation previously reported by Albert and Brown [4] at pH < 1.

It should also be noted that the length of the reaction periods given in table 1 fall into three groups: (30 \pm 18) min for the first 14 experiments but omitting the one at 309 K, (73 \pm 15) min for the next 5 experiments, and (128 \pm 25) min for the last 5 experiments. This suggests a third protonation between 5.8 and 6.0 (mol HCl)L\(^{-1}\); however, the uncertainties in the slopes and intercepts do not justify separation of the last two groups.

## 4. Solution of Adenine in Aqueous NaOH

In table 2 are the data for 20 experiments in which the enthalpy of solution of Ade 2 in aqueous NaOH solutions was measured. The description of column headings given in section 3 also applies to table 2. It is assumed that \( q_{\text{vap}} \) is negligibly small in these experiments because if the solution were pure water \( q_{\text{vap}} \) would be less than 0.05J in the worst case (at 309 K); the corrections are smaller than this since the vapor pressure of the NaOH solution is less than that of water.
The uncertainty may be greater at other concentrations.

From the lengths of the reaction periods given in Table 2, it can be seen that all of the reactions were rapid. No undissolved material was visible in the final solutions. The value of the enthalpy of solution decreases as the concentration of the NaOH calorimetric solutions increases. Figure 2 is a plot of $\Delta H_m$ (298.15 K) as a function of NaOH molarity. It shows a change of slope near 4 (mol NaOH)L$^{-1}$ which probably indicates a second proton dissociation not previously reported. The results of fitting linear equations to the data for $\Delta H_m$ (298.15 K) and NaOH concentration from Table 2 by the method of least squares are as follows:

$$
\Delta H_m(298.15 \text{ K}) \text{, kJ \cdot mol}^{-1} = (15.207 \pm 0.029) - (0.364 \pm 0.019)C
$$

and for the 8 points at greater than 4(mol NaOH)L$^{-1}$,

$$
\Delta H_m(298.15 \text{ K}) \text{, kJ \cdot mol}^{-1} = (17.26 \pm 0.21) - (0.889 \pm 0.042)C
$$

where $C$ is the NaOH concentration in mol \cdot L$^{-1}$ and the uncertainties are the standard deviations. The standard deviations of the fits are 0.072 kJ \cdot mol$^{-1}$ and 0.093 kJ \cdot mol$^{-1}$, respectively. From the difference in the intercepts, we obtain the enthalpy of the second proton dissociation at infinite dilution, $(2.06 \pm 0.21)$ kJ \cdot mol$^{-1}$. 

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Table 2. Data from the measurements of the enthalpy of solution of adenine in aqueous NaOH.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Mass of Adenine (g)</th>
<th>Mass of NaOH (g)</th>
<th>Mass Conc. (mol/L)</th>
<th>Reaction period of solution (min)</th>
<th>Calculated concentration (mol/L)</th>
<th>$\Delta H_m$ at 298.15 K (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>959</td>
<td>0.19889</td>
<td>307.371</td>
<td>0.0912</td>
<td>1750.23</td>
<td>1749.37</td>
<td>0.012727</td>
</tr>
<tr>
<td>961</td>
<td>0.21312</td>
<td>307.416</td>
<td>0.0912</td>
<td>1750.29</td>
<td>1750.16</td>
<td>0.013790</td>
</tr>
<tr>
<td>1134</td>
<td>0.19707</td>
<td>308.618</td>
<td>0.197</td>
<td>1744.30</td>
<td>1743.90</td>
<td>0.012696</td>
</tr>
<tr>
<td>962</td>
<td>0.19380</td>
<td>307.368</td>
<td>0.462</td>
<td>1725.49</td>
<td>1725.71</td>
<td>0.012437</td>
</tr>
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<td>1135</td>
<td>0.19241</td>
<td>308.571</td>
<td>0.545</td>
<td>1722.08</td>
<td>1721.82</td>
<td>0.012416</td>
</tr>
<tr>
<td>957</td>
<td>0.19922</td>
<td>307.223</td>
<td>0.919</td>
<td>1699.37</td>
<td>1698.97</td>
<td>0.012974</td>
</tr>
<tr>
<td>958</td>
<td>0.19357</td>
<td>307.204</td>
<td>0.919</td>
<td>1703.13</td>
<td>1703.74</td>
<td>0.013703</td>
</tr>
<tr>
<td>959</td>
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<td>307.259</td>
<td>0.919</td>
<td>1699.38</td>
<td>1698.58</td>
<td>0.012907</td>
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<td>1137</td>
<td>0.19726</td>
<td>325.979</td>
<td>1.998</td>
<td>1725.78</td>
<td>1726.20</td>
<td>0.012256</td>
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<td>1138</td>
<td>0.18930</td>
<td>335.734</td>
<td>3.012</td>
<td>1734.68</td>
<td>1734.78</td>
<td>0.011410</td>
</tr>
<tr>
<td>1140</td>
<td>0.19024</td>
<td>346.960</td>
<td>4.015</td>
<td>1755.71</td>
<td>1756.18</td>
<td>0.011058</td>
</tr>
<tr>
<td>1149</td>
<td>0.19448</td>
<td>350.243</td>
<td>4.096</td>
<td>1766.38</td>
<td>1766.76</td>
<td>0.011051</td>
</tr>
<tr>
<td>1142</td>
<td>0.20667</td>
<td>348.501</td>
<td>4.221</td>
<td>1757.53</td>
<td>1757.96</td>
<td>0.011635</td>
</tr>
<tr>
<td>1141</td>
<td>0.18986</td>
<td>353.112</td>
<td>4.488</td>
<td>1770.04</td>
<td>1770.62</td>
<td>0.010500</td>
</tr>
<tr>
<td>1136</td>
<td>0.21816</td>
<td>313.451</td>
<td>5.064</td>
<td>1618.02</td>
<td>1618.14</td>
<td>0.012850</td>
</tr>
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<td>1137</td>
<td>0.20246</td>
<td>333.433</td>
<td>5.064</td>
<td>1691.02</td>
<td>1691.34</td>
<td>0.011372</td>
</tr>
<tr>
<td>1145</td>
<td>0.19739</td>
<td>363.371</td>
<td>6.078</td>
<td>1786.83</td>
<td>1787.58</td>
<td>0.009697</td>
</tr>
<tr>
<td>1147</td>
<td>0.19118</td>
<td>363.306</td>
<td>6.078</td>
<td>1787.33</td>
<td>1787.60</td>
<td>0.009320</td>
</tr>
</tbody>
</table>

$^{a}$ Corrected for H$_2$O in sample: $(0.15 \pm 0.10)$ mass%.

$^{b}$ The stirring rate was 450 rpm in Expts. No. 959, 960, 962 and 963; 550 rpm in all other experiments.
5. Solution of Adenine in MeOH and in EtOH

The data are given in table 3 for 12 measurements of the enthalpy of solution of Ade 2 in ACS Spectro-grade methanol, and for 2 measurements in ACS absolute ethanol. The column headings described in section 3 also apply to table 3.

The rating period slopes are given in table 3 because they differ markedly from those where the aqueous calorimetric solutions were used. The reaction period was preceded by the initial slope and followed by the final slope. These slopes are obtained by fitting a linear equation to about 550 points. At 298 K, with a stirring rate of 550 rpm, the rating period slopes for the aqueous HCl solutions were between 6.5 and 7.5 μK·s⁻¹ and for the aqueous NaOH solutions, between 5.9 and 6.6 μK·s⁻¹; in both cases the final slope was 0.1 to 0.3 μK·s⁻¹ larger than the initial slope. For the solution reaction in methanol, the final slope was always more than 1 μK·s⁻¹ smaller than the initial slope even with the variations in temperature and stirring rate. This indicates an increase in the evaporation cooling effect after the reaction which is surprising in view of the fact that non-volatile molecules were added to the methanol. The solutions apparently depart greatly from ideal behavior. For the solutions in ethanol, the initial and final slopes were essentially equal.

Some undissolved particles were visible at the bottom in all final solutions in the experiments with methanol and ethanol. The amounts appeared to be independent of both concentration and temperature of reaction. The undissolved material was not analyzed, but in one noncalorimetric experiment about 8 mg or 4 per cent of the sample mass was recovered in a filter. Most of the final solutions were cloudy, but the cloudiness decreased as the reaction temperature increased; at about 342 K, the final solutions were essentially clear. When a cloudy final solution was filtered through Whatman No. 50 hardened filter paper (for fine crystalline precipitates), the filtrate was clear indicating that the cloudiness was caused by finely divided material suspended in the solution. It is unlikely that the suspended material was adenine since its density is nearly twice that of the alcohols. The unreacted material was probably an impurity (or impurities) in the adenine which was soluble in water, but insoluble in the alcohols at the lower temperatures.

The ΔCₚ for the reaction of Ade in methanol was obtained by fitting a linear equation by the least squares method to the data for ΔHₘ(T) and Tₑ for 10 experiments in table 3 (omitting Nos. 1009 and 1012 because the values for ΔHₘ(T) were quite low and leakage of the sample holder was suspected). A plot of these data is shown in figure 3. The slope of the line is ΔCₚ = (92.9 ± 5.3) J·mol⁻¹·K⁻¹; the uncertainty given is the standard deviation and the standard deviation of the fit is 0.18 kJ·mol⁻¹.
Table 1. Data from measurements of the enthalpies of solution of adenine in methanol and in ethanol.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Mass</th>
<th>Final Ade conc.</th>
<th>Rating period slopes</th>
<th>Reaction period</th>
<th>Electrical energy equivalent</th>
<th>-ΔT reaction</th>
<th>q_vap</th>
<th>q_reac</th>
<th>Corr. to T = 298.15 K</th>
<th>ΔH_m (298.15 K)</th>
<th>Corr. to infinite dil.</th>
<th>ΔH_m (298.15 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>966</td>
<td>0.09183</td>
<td>242.003</td>
<td>2.8081</td>
<td>8.2</td>
<td>7.1</td>
<td>298.123</td>
<td>38</td>
<td>0.35</td>
<td>1086.99</td>
<td>1086.83</td>
<td>0.013508</td>
<td>0.18</td>
</tr>
<tr>
<td>967</td>
<td>0.05760</td>
<td>242.018</td>
<td>1.7613</td>
<td>8.0</td>
<td>6.7</td>
<td>298.973</td>
<td>48</td>
<td>1.06</td>
<td>1085.95</td>
<td>1086.59</td>
<td>0.008568</td>
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<td>0.09479</td>
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<td>2.9596</td>
<td>12.4</td>
<td>6.3</td>
<td>318.347</td>
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<td>3.0019</td>
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<td>6.5035</td>
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<td>1071.79</td>
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<td>12.6</td>
<td>298.013</td>
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<td>1072.09</td>
<td>1072.93</td>
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<td>14.0</td>
<td>12.8</td>
<td>298.007</td>
<td>38</td>
<td>0.24</td>
<td>1072.03</td>
<td>1072.29</td>
<td>0.030026</td>
<td>0.16</td>
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<td>8.5247</td>
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<td>12.5</td>
<td>297.981</td>
<td>48</td>
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<td>12.8</td>
<td>298.117</td>
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<td>1072.31</td>
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<td>6.2</td>
<td>301.101</td>
<td>38</td>
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<td>1079.76</td>
<td>1079.72</td>
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<td>3.2224</td>
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<td>4.7</td>
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<td>1118.34</td>
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<td>3.1697</td>
<td>8.4</td>
<td>4.8</td>
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<td>0.20</td>
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<td>1119.12</td>
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<td>0.55</td>
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</table>

Solvent: Spectro ACS Methanol

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Mass</th>
<th>Final Ade conc.</th>
<th>Rating period slopes</th>
<th>Reaction period</th>
<th>Electrical energy equivalent</th>
<th>-ΔT reaction</th>
<th>q_vap</th>
<th>q_reac</th>
<th>Corr. to T = 298.15 K</th>
<th>ΔH_m (298.15 K)</th>
<th>Corr. to infinite dil.</th>
<th>ΔH_m (298.15 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1019</td>
<td>0.05061</td>
<td>236.771</td>
<td>1.5818</td>
<td>5.9</td>
<td>5.8</td>
<td>298.143</td>
<td>24</td>
<td>0.87</td>
<td>1048.20</td>
<td>1049.53</td>
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<tr>
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<td>236.836</td>
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<td>5.0</td>
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<td>0.69</td>
<td>1077.26</td>
<td>1078.62</td>
<td>0.017030</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Solvent: Absolute Ethanol (ACS)

- Corrected for H_2O in sample: (0.15 ± 0.10) mass%.
- The stirring rate was 700 rpm in Expts. No. 1009 through 1013, and 550 rpm in all others.
- Refers to the final temperature of the reaction since only the initial energy equivalent was used in calculating the heat of the reaction; all other experiments refer to the mean temperature of the reaction, and the mean energy equivalent was used.
- Electrical energy (887.961 J) was added during this reaction; the net ΔT = 0.799691 K.
- Corrections for departures of the adiabatic shield from the calorimeter temperature: No. 1009 = 0.034 J, No. 1011 = 0.027 J, and No. 1012 = 0.070 J.
Figure 4 is a plot of the adenine concentration in the final solutions versus $\Delta H_m(298.15 \text{ K})$ before the corrections to the mass of Ade for H$_2$O in the sample were applied. These corrections were small and caused the line shown in figure 4 to be raised 0.02 kJ·mol$^{-1}$ with a negligible change in slope. This line was obtained by fitting a linear equation to the data from the 12 experiments by the method of least squares. For the data which includes the H$_2$O corrections,

$$\Delta H_m(298.15 \text{ K}), \text{kJ} \cdot \text{mol}^{-1} = (21.60 \pm 0.18) - (0.114 \pm 0.038)C$$

where $C$ is the Ade concentration in mmol (kg MeOH)$^{-1}$ and the uncertainties are standard deviations. The standard deviation of the fit is 0.26 kJ·mol$^{-1}$. The slope, 113.7 J·mol$^{-1}$·C$^{-1}$ was used for the corrections to infinite dilution (table 3) which were added to $\Delta H_m(298.15 \text{ K})$ to obtain $\Delta H(\infty, 298.15 \text{ K}) = (21.6 \pm 0.9)$ kJ·mol$^{-1}$; the large uncertainty of 4 per cent was assigned to account for the unidentified, undissolved material in the final solutions.

No corrections were made for the dilution in MeOH of the H$_2$O impurity in the Ade primarily because of the uncertainty in the state of the H$_2$O. In the worst case (Expt. No. 1012) assuming liquid H$_2$O in the Ade, the correction to $\Delta H(\infty, 298.15 \text{ K})$ would be less than 0.1 kJ·mol$^{-1}$ which is much smaller than the assigned uncertainty.

In Expts. No. 1009, 1011, and 1012 (table 3), the heat of the endothermic reaction was large enough to result in small departures of the temperature of the adiabatic shield from that of the reaction vessel. Therefore small corrections for heat transfer from the shield to the vessel were made as previously described [5]. The need for this correction was eliminated in Expt. No. 1010 because precisely measured electrical energy was added during the reaction to prevent a decrease in the temperature of the reaction vessel.

In figure 4, the experiments represented by the two points at the lower right (Expts. No. 1009 and 1012) were suspected of prereaction although the rating period slopes did not support this suspicion. If these two experiments are in error, we would conclude that the enthalpy of solution was independent of concentration in the range of these measurements.

---

**Figure 4.** Plot showing the values for the enthalpy of solution of adenine in methanol at various concentrations of adenine.

The radius of a circle is equal to the estimated calorimetric uncertainty of the experiment (see text). The straight line shown is obtained by fitting a linear equation to the data by the least squares method; the slope is $(114 \pm 38) \text{ J} \cdot \text{mol}^{-1} \text{ (mmol Ade)}^{-1} \text{ (kg MeOH)}$. 

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In preliminary experiments, it was learned that only about one-third as much Ade could be dissolved in ethanol as in an equal volume of methanol. The final solution in Expt. No. 1020 (table 3) was very close to saturation. At such low concentrations, meaningful dilution data were not obtained. However, $\Delta C_p$ for the reaction was about $(0.24 \pm 0.04)$ kJ·mol$^{-1}$·K$^{-1}$ and $\Delta H(298.15 \text{K}) = (22.0 \pm 0.9)$ kJ·mol$^{-1}$; the uncertainties are estimated taking into consideration the unreacted material in the final solutions.

6. Discussion and Summary

The structure of adenine has been given as follows [4]:

![Structure of Adenine]

There are three possible sites for protonation at N1, N3, and N7, and two sites for proton dissociation at N9 and the amino group. A review [3] lists measurements of the enthalpy of protonation at N1 (pH 4) and suggests a second protonation [4] at N7 (pH < 1); enthalpies of proton dissociation at N9 (pH 10) are also listed.

The first work in this series [1] gave our best value for the enthalpy of solution at infinite dilution which may be represented by the following equation (where adenine = H$_2$Ade):

$$
\text{H}_2\text{Ade}(c) + \text{aq} = \text{H}_2\text{Ade}(\text{aq}),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = (33.47 \pm 1.00) \text{kJ} \cdot \text{mol}^{-1}
$$

(1)

From the measurements reported in section 3 of this work we assume the following equations:

$$
\text{H}_2\text{Ade}(c) + \text{H}^+(\text{aq}) = \text{H}_3\text{Ade}^+(\text{aq}),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = (11.41 \pm 0.08)\text{kJ} \cdot \text{mol}^{-1}
$$

(2)

$$
\text{H}_2\text{Ade}(c) + 2\text{H}^+(\text{aq}) = \text{H}_4\text{Ade}^{2+}(\text{aq}),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = (19.3 \pm 2.0) \text{kJ} \cdot \text{mol}^{-1}
$$

(3)

(Some evidence is mentioned in section 3 that eq (3) may represent a triple protonation rather than the addition of the two protons as indicated here.) From the measurements in section 4,

$$
\text{H}_2\text{Ade}(c) + \text{OH}^-(\text{aq}) = \text{H}\text{Ad}e^-(\text{aq}) + \text{H}_2\text{O}(l),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = (15.207 \pm 0.029) \text{kJ} \cdot \text{mol}^{-1}
$$

(4)

$$
\text{H}_2\text{Ade}(c) + 2\text{OH}^-(\text{aq}) = \text{Ade}^{2-}(\text{aq}) + 2\text{H}_2\text{O}(l),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = (17.26 \pm 0.21) \text{kJ} \cdot \text{mol}^{-1}
$$

(5)

By subtracting eq (1) from eq (2) and from eq (3) we obtain

$$
\text{H}_2\text{Ade}(\text{aq}) + \text{H}^+(\text{aq}) = \text{H}_3\text{Ade}^+(\text{aq}),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = -(22.1 \pm 1.0) \text{kJ} \cdot \text{mol}^{-1}
$$

(6)

$$
\text{H}_2\text{Ade}(\text{aq}) + 2\text{H}^+(\text{aq}) = \text{H}_4\text{Ade}^{2+}(\text{aq}),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = -(14.2 \pm 2.2) \text{kJ} \cdot \text{mol}^{-1}
$$

(7)

where the uncertainties are the square root of the sum of the squares of the individual uncertainties.

Equation (6) is equivalent to the protonation of N1 as given in [3]. The value obtained for this reaction by Christensen et al. using calorimetric titrations is $-(20.12 \pm 0.08)$ kJ·mol$^{-1}$ [7]; the purity of their sample was not determined. Equation (7) has not been previously measured although evidence for it was given by Albert and Brown [4].

Parker [8] has selected a "best" value for $\Delta H_N$ which may be represented by the equation,

$$
\text{H}^+ + \text{OH}^- = \text{H}_2\text{O}(l),
$$

$$
\Delta H(\infty, 298.15 \text{K}) = -55.84 \pm 0.10 \text{kJ} \cdot \text{mol}^{-1}
$$

(8)

If the sum of eq (1) and eq (8) is subtracted from eq (4), we obtain

$$
\text{H}_2\text{Ade}(\text{aq}) = \text{H}\text{Ad}e^-(\text{aq}) + \text{H}^+, \quad \Delta H(\infty, 298.15 \text{K}) = (37.58 \pm 1.00) \text{kJ} \cdot \text{mol}^{-1}
$$

(9)

which is equivalent to the value, $(40.38 \pm 0.21)$ kJ·mol$^{-1}$, reported for the dissociation of the N9 proton by Christensen et al. [7] using a sample of unknown purity. Similarly, if the sum of eq (1) and twice eq (8) is subtracted from eq (5) we obtain

$$
\text{H}_2\text{Ade}(\text{aq}) = \text{Ade}^{2-}(\text{aq}) + 2\text{H}^+, \quad \Delta H(\infty, 298.15 \text{K}) = (95.47 \pm 1.04) \text{kJ} \cdot \text{mol}^{-1}
$$

(10)

This reaction has not been previously reported and is assumed to be the proton dissociations at the amino group and N9.

Scruggs, Acht, and Ross [9] measured the solubility of adenine in methanol at 277.8 K and 309.8 K. Assuming linearity between these temperatures, and the density of

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methanol = 0.79 g·mL⁻¹, we calculate 8.0 mmol Ade (kg MeOH)⁻¹ for the saturated solution. (This is less than the concentration of our final solution in Expt. No. 1012 and the disagreement is probably the result of uncertainty in the purity of the adenine and of the MeOH). Scruggs et al. calculated the enthalpy of solution at 310 K for the saturated solution. Applying our value for ΔCₚ to their value for ΔH, we obtain 17.7 kJ·mol⁻¹ at 298 K which is 21 percent less than our value, 22.5 kJ·mol⁻¹, at 8 mmol·kg⁻¹.

The following ΔCₚ values were determined for reactions of adenine:
in aqueous HCl (1.0 mol·L⁻¹), (107 ± 3) J·mol⁻¹·K⁻¹;
in aqueous NaOH (0.9 mol·L⁻¹), (126 ± 8) J·mol⁻¹·K⁻¹;
in Spectro-grade methanol, (92.9 ± 5.3) J·mol⁻¹·K⁻¹;
and in absolute ethanol, (240 ± 40) J·mol⁻¹·K⁻¹.

7. References
