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## Glossary

| | |
|---|---|
| $P$ | system pressure, mmHg |
| $p_i^0$ | saturation vapor pressure of component $i$ in the absence of salt, mmHg |
| $p_i'$ | corrected vapor pressure of component $i$, mmHg |
| $x_i$ | mole fraction of component $i$ in the liquid phase on a salt-free basis |
| $y_i$ | mole fraction of component $i$ in the vapor phase on a salt-free basis |
| $Z$ | mole fraction of salt in the liquid phase = (mole of salt)/(mole of alcohol + mole of water + mole of salt) |

*Greek Letters*

| | |
|---|---|
| $\alpha$ | relative volatility of methanol with respect to water in the absence of the salt sodium nitrate |
| $\alpha_S$ | relative volatility with the salt present, calculated by using the liquid composition on a salt-free basis |
| $\gamma_i$ | activity coefficient in the liquid phase of component $i$ |
| $\epsilon_i$ | correction factor for the computation of the activity coefficient given by $P/p_i''$ where $p_i''$ is the vapor pressure of pure component $i$ at the temperature at which component $i$ boils in the presence of salt under the respective concentration of salt |

## Literature Cited

(1) Bedrossin, A. A., Cheh, H. Y., *AIChE Symp. Ser.*, **70** (140), 102 (1974).
(2) Furter, W. F., *Adv. Chem. Ser.*, **No. 155**, 26 (1976).
(3) Furter, W. F., *Can. J. Chem. Eng.*, **55**, 229 (1977).
(4) Hala, E., Wichterle, J., Polak, J., Boublik, T., "Vapour-Liquid Equilibrium Data at Normal Pressures", Pergamon Press, London, 1968.
(5) Jaques, D., Furter, W. F., *Ind. Eng. Chem. Fundam.*, **13**, 238 (1974).
(6) Jaques, D., Furter, W. F., *AIChE J.*, **18**, 343 (1972).
(7) Johnson, A. L., Furter, W. F., *Can. J. Chem. Eng.*, **38**, 78 (1960).
(8) Johnson, A. L., Furter, W. F., *Can. J. Chem. Eng.*, **43**, 356 (1965).
(9) Klar, R., Sliwka, A., *J. Phys. Chem.* (*Frankfurt am Main*) **15**, 207 (1958).
(10) Lange, N. A. "Handbook of Chemistry", revised 10th ed., McGraw-Hill, New York, 1967.
(11) Meranda, D., Furter, W. F., *AIChE J.*, **20**, 103 (1974).
(12) Rajamani, R., Srinivasan, D., *Indian Chem. Eng.*, **9**, T 15 (1977).
(13) Rajamani, R., Srinivasan, D., *Indian J. Technol.* (1977).
(14) Ramalho, R. S., Edgett, N. S., *J. Chem. Eng. Data*, **9**, 324 (1964).
(15) Srinivasan, D., Ph.D. Thesis, University of Madras, 1972.
(16) Srinivasan, D., Lakshmanan, C. M., Laddha, G. S., *Indian J. Technol.*, **13**, 431 (1975).
(17) Yoshida, F., Yasunishi, A., Hamada, Y., *Kagaku Kogaku*, **28**, 133 (1964).

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# Thermodynamics of Nucleoside–Solvent Interactions: Inosine and Adenosine in Water and in 1 *m* Ethanol between 25 and 35 °C

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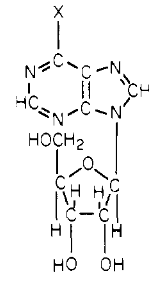
**Enthalpies of solution of inosine and adenosine in water and in 1 *m* ethanol were measured at 25, 30, and 35 °C. Both nucleosides differ only by the functional groups OH and $NH_2$ and have approximately equal enthalpies of transfer from water to aqueous ethanol (0.48 and 0.47 kcal/mol at 25 °C for inosine and adenosine, respectively). The magnitude of the enthalpy of transfer is compared with those for the related purines caffeine and theophylline in the same solvent systems. Heat capacities of solution were obtained from the temperature variation of the enthalpies of solution (29 and 27 cal/(mol K) in water 38 and 18 cal/(mol K) in 1 *m* ethanol for inosine and adenosine, respectively). These values were combined with estimated heat capacities of the solids to yield partial molar heat capacities (96 and 95 cal/(mol K) in water for inosine and adenosine, respectively), showing that solute–solvent interaction in water is insensitive to the interchange of OH and $NH_2$. Heat capacities of transfer of each nucleoside from water to the mixed solvent indicate that the presence of ethanol tends to minimize the leveling effect induced by water on these functional groups. Estimated temperature coefficients of solubility of the nucleosides were calculated from the enthalpies and heat capacities of solution.**

## Introduction

This contribution reports on the enthalpies and heat capacities of solution of inosine and adenosine in water and in 1 *m* ethanol at 25, 30, and 35 °C. The two nucleosides were chosen for the following reasons: Inosine and adenosine are important components of ribonucleic acids, differing only in the functional groups OH and $NH_2$. They are derivatives of purines which include the previously studied caffeine and theophylline (*1–3*). Both nucleosides are sufficiently soluble for calorimetric measurements (*4*) and contain both polar and apolar groups whose interaction with water is important to the conformational stability of nucleic acids (*5*). Ethanol was chosen as a cosolvent because of its well-known but poorly understood effect in altering the solvent properties of water (*6*).



X = OH, inosine
X = $NH_2$, adenosine

Before the behavior of large complicated biochemical molecules in aqueous solutions can be understood, the data of interaction of their component parts in such systems must be determined.

Enthalpies of transfer were obtained from the difference in enthalpies of solution: $\Delta\bar{H} = \Delta H - \Delta H°$. Values of $\Delta C_p$ were

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Table I. Enthalpies of Solution and Transfer of Inosine and Adenosine in Water and 1 *m* Ethanol

Inosine

| system | $t$, °C | runs | $\Delta H°$, kcal/mol |
|---|---|---|---|
| $H_2O$ | 25 | 6 | 6.53 ± 0.04 |
| | 30 | 5 | 6.65 ± 0.04 |
| | 35 | 5 | 6.83 ± 0.04 |

| system | $t$, °C | runs | $\Delta H$, kcal/mol | $\Delta \bar{H}$, kcal/mol |
|---|---|---|---|---|
| 1 *m* EtOH | 25 | 5 | 7.01 ± 0.01 | 0.48 |
| | 30 | 5 | 7.28 ± 0.02 | |
| | 35 | 5 | 7.39 ± 0.02 | |

Adenosine

| system | $t$, °C | runs | $\Delta H°$, kcal/mol |
|---|---|---|---|
| $H_2O$ | 25 | 7 | 7.71 ± 0.02 |
| | 30 | 5 | 7.82 ± 0.03 |
| | 35 | 5 | 7.96 ± 0.02 |

| system | $t$, °C | runs | $\Delta H$, kcal/mol | $\Delta \bar{H}$, kcal/mol |
|---|---|---|---|---|
| 1 *m* EtOH | 25 | 4 | 8.18 ± 0.04 | 0.47 |
| | 30 | 5 | 8.32 ± 0.03 | |
| | 35 | 6 | 8.36 ± 0.05 | |

determined from the temperature variation of the enthalpies of solution, and the partial molar heat capacity was calculated from $\bar{C}_p = \Delta C_p + C_p$. The heat capacities of the solids, $C_p$, were estimated, since such data have been reported for only very few smaller molecules. The partial molar heat capacity, $\bar{C}_p$, may also be obtained calorimetrically from the apparent molar heat capacity, $\varphi C_p$, but such measurements cannot usually be carried out at the lower concentrations possible with the enthalpy of solution method. This precludes studies with sparingly soluble solutes and introduces the possibility of strong solute-solute interactions which may lead to the formation of dimers or higher aggregates in aqueous solutions (*7*).

In general, partial molar heat capacities of electrolytes and nonelectrolytes are sensitive to solute-solvent interactions and in water are enhanced by apolar groups and lowered by polar groups.

## Experimental Section

The calorimeter is similar to that described by Wadsö (*8*), and details of its construction and operation have been described elsewhere (*9*). Inosine and adenosine were obtained from Sigma. Elemental analysis gave the following results: Anal. Calcd for inosine: C, 44.78; H, 4.51; N, 20.89; O, 29.82. Found: C, 44.56; H, 4.71; N, 20.62; O, 29.61. Calcd for adenosine: C, 44.94; H, 4.90; N, 26.21; O, 23.95. Found: C, 45.05; H, 5.06; N, 26.21; O, 23.95. The melting points of both nucleosides were in good agreement with the literature values. The overall range was 0.4–1.8 mmol of nucleoside dissolved in 195 g of water or 193 g of 1 *m* ethanol with the majority of runs below 1.0 mmol per run.

## Results and Discussion

The means of enthalpies of solution with their standard deviations are shown in Table I. No variation in enthalpies over the measured concentration range was observed, and they may thus be considered as infinite dilution values, with no evidence of self-association. Figure 1 shows $\Delta H$ and $\Delta H°$ plotted against temperature, and Table II lists the slopes as $\Delta C_p$ with an estimated overall experimental error of ±8 cal/(mol K). Estimated values of $C_p$ for the solid nucleosides are also shown in Table II, calculated from the sum of the experimental values for adenine (*10*) and glucose (*11*), less Kopp's law contribution values (*12*, *13*) for the excess atoms:

$$C_p(\text{inosine } C_{10}H_{12}N_4O_5) = C_p(\text{adenine } C_5H_5N_5) + C_p(\text{glucose } C_6H_{12}O_6) - C_p(C + 5\,H + N + O) = 34 + 54 - (2 + 12 + 3 + 4) = 67 \text{ cal/(mol K)}$$

Similarly

$$C_p(\text{adenosine } C_{10}H_{13}N_5O_4) = C_p(\text{adenine } C_5H_5N_5) + C_p(\text{glucose } C_6H_{12}O_6) - C_p(C + 4\,H + 2\,O) = 68 \text{ cal/(mol K)}$$

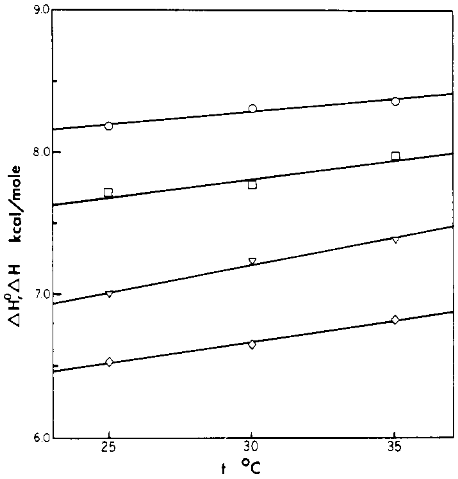

**Figure 1.** Enthalpies of solution of inosine and adenosine: (◇) inosine in $H_2O$; (▽) inosine in 1 *m* ethanol; (□) adenosine in $H_2O$; (○) adenosine in 1 *m* ethanol.

Table II. Heat Capacities of Inosine and Adenosine, cal/(mol K)

| system | solute | $\Delta C_p$ | $C_p$ | $\bar{C}_p$ | $\Delta \bar{C}_p$ |
|---|---|---|---|---|---|
| $H_2O$ | inosine | 29 | 67 | 96 | |
| | adenosine | 27 | 68 | 95 | |
| 1 *m* EtOH | inosine | 37 | | 104 | 8 |
| | adenosine | 18 | | 86 | −9 |

The heat capacities of the solid nucleic acid bases thymine (*14*) and uracil and adenine (*15*) have also been reported recently. It may be noted that the value for adenine, given without experimental details, is within 1 cal/(mol K) of the older one (*10*) used in the above estimation of $C_p$ of inosine and adenosine.

Although their enthalpies of solution differ by more than 1 kcal/mol, the enthalpies of transfer are both nearly the same at 0.5 kcal/mol. The magnitude of $\Delta \bar{H}$ for apolar nonelectrolytes appears to depend partially on the size of the transferred solute. For example, the enthalpies of transfer of the related smaller purines caffeine and theophylline from water to 1 *m* ethanol are 0.88 and 0.65 kcal/mol, respectively (*3*), the addition of one methylene group thus increasing $\Delta \bar{H}$ by 0.2 kcal/mol. In the present study, the polar sugar group appears to lessen the unfavorable interaction difference upon transfer.

The difference between the heat capacities of adenosine and adenine (*15*) in pure water, 95 and 54 cal/(mol K), respectively, allows an estimate of approximately 40 cal/(mol K) for the contribution of the sugar group. Many more systems will have to be investigated to establish the range of validity of such group additivity assumptions in aqueous solutions.

The partial molar heat capacities of both nucleosides in pure water are almost equal, and this is in good agreement with the small differences reported for group contributions of OH and $NH_2$ (2.2 and −0.5 cal/(mol K) for OH and $NH_2$, respectively) (*16*). The heat capacities of transfer differ and show that the addition of ethanol tends to minimize the leveling effect on the above functional groups observed in the presence of pure water.

The high endothermic enthalpies of solution indicate large changes in the temperature coefficients of the solubilities. Since auxiliary data are not available, ideal solution behavior is assumed, and utilizing only enthalpies and heat capacities of solution (*17*), the solubilities at 35 °C for both nucleosides in both solvents are estimated to be ca. 1.5 times those at 25 °C.

## Glossary

| | |
|---|---|
| $\Delta\bar{H}$ | enthalpy of transfer from water to 1 *m* ethanol |
| $\Delta H^\circ$ | enthalpy of solution in pure water |
| $\Delta H$ | enthalpy of solution in 1 *m* ethanol |
| $\Delta C_p$ | heat capacity of solution |
| $C_p$ | heat capacity of solids |
| $\bar{C}_p$ | partial molar heat capacity |
| $\Delta\bar{C}_p$ | heat capacity of transfer |

## Literature Cited

(1) J. H. Stern, J. A. Devore, S. L. Hansen, and O. Yavuz, *J. Phys. Chem.*, **78**, 1922 (1974).
(2) J. H. Stern and L. R. Beeninga, *J. Phys. Chem.*, **79**, 582 (1975).
(3) J. H. Stern and E. Lowe, *J. Chem. Eng. Data*, **23**, 341 (1978).
(4) "The Merck Index", 8th ed., Merck, Rahway, N.J., 1968.
(5) A. L. Lehninger, "Biochemistry", 2nd ed., Worth, New York 1975.
(6) F. Franks and D. J. G. Ives, *Q. Rev., Chem. Soc.*, **20**, 1 (1966).
(7) S. J. Gill, M. Downing, and G. F. Sheats, *Biochemistry*, **6**, 272 (1967).
(8) I. Wadsö, *Acta Chem. Scand.*, **14**, 566 (1960).
(9) S. J. Stoner, M.S. Thesis, California State University, Long Beach, Calif., 1977.
(10) R. D. Stiehler and H. H. Huffman, *J. Am. Chem. Soc.*, **57**, 1741 (1935).
(11) "International Critical Tables", Vol. 5, McGraw-Hill, New York, 1929, p 103.
(12) L. Pauling, "General Chemistry", 2nd ed., W. H. Freeman, San Francisco, 1959, p 638.
(13) G. N. Lewis and M. Randall, "Thermodynamics", revised by K. S. Pitzer and L. Brewer, 2nd ed., McGraw-Hill, New York, 1961, pp 57, 517.
(14) J. Alvarez and R. Biltonen, *Biopolymers*, **12**, 1815 (1973).
(15) M. V. Kilday, *J. Res. Natl. Bur. Stand.* (*U.S.*), **83**, 347, 547 (1978).
(16) N. Nichols, R. Skold, C. Spink, J. Suurkuusk, and I. Wadsö, *J. Chem. Thermodyn.*, **8**, 1081 (1976).
(17) See ref 13, p 227.

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# An Equilibrium Phase Diagram for the Glucose–Cellobiose–Water System at 30.5 °C

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**The solid–liquid equilibrium phase diagram for the ternary system of water–glucose–cellobiose was studied at 30.5 °C. Samples of known composition were equilibrated at 30.5 °C over a period of 7 days. The resultant saturated liquid phase as well as a wet sample of the solid phase were analyzed by high-pressure liquid chromatography. Both graphical analysis as well as optical microscopy were used to identify the solid phases. The compositions of the saturated liquid phase and the identities of the accompanying solid phases are reported. Glucose monohydrate and anhydrous cellobiose were identified as the solid phases. For the three-phase region, the composition of the liquid at the invariant point was calculated to be 50.1 wt % glucose, 5.0 wt % cellobiose, and the remainder water. The two-phase region of solid anhydrous cellobiose and saturated liquid was found to dominate the ternary diagram. This suggests that it is possible to obtain cellobiose in sufficient purity and yield from aqueous solutions obtained by the enzymatic hydrolysis of cellulose.**

## Introduction

Glucose and cellobiose are the major products of the enzymatic saccharification (hydrolysis) of cellulose (*1*). Glucose is presently used extensively in the production of valuable pharmaceuticals. Cellobiose finds important use in bacteriology (*2*). In the synthesis of certain pharmaceuticals, the use of cellobiose as a substrate may prove more efficient than glucose. The limited availability and high cost of cellobiose have inhibited its use up to now. The enzymatic saccharification of cellulose yields cellobiose in significant quantities together with glucose (*1*). A separation scheme needs to be devised to separate the high-value cellobiose from the relatively cheaper glucose. This recovery of cellobiose could provide a new substrate for industry.

Although the binary, ternary and even quaternary systems of various combinations of water, glucose, fructose, and sucrose (*3–12*) have been studied, there is no report in the literature of any system involving cellobiose, one of the major sugars produced by most current hydrolysis processes.

Young (*8*) investigated the D-glucose–water phase diagram and obtained solubility curves for all four solid phases, viz., ice, α-D-glucose, β-D-glucose, and α-D-glucose monohydrate. Taylor (*11*, *12*) has measured the solubilities of cellobiose in water. It was noted that the solid phase in equilibrium with the solution at 20 °C was not hydrated.

Considering ternary and higher order phase diagrams, the work of Kelly is most extensive. Kelly's studies (*3–7*) at 30 °C have shown that glucose occurs most frequently as the monohydrate in the solid phase. Young (*8*) has shown that the transition (in the absence of other solutes) from the monohydrate to the anhydrous glucose solid phase occurs above 54.7 °C (i.e., the monohydrate is the stable solid phase below 54.71 °C). In all of Kelly's studies, no evidence of double compounds or solid solutions of sugars was ever found. Hence this possibility may be eliminated from this study.

## Experimental Section

***Materials.*** The D-glucose and D-cellobiose, as well as the acetonitrile used for liquid chromatography, were of certified ACS purity. Distilled water was used for the preparation of all samples.

***Preparation of Synthetic Samples.*** In accordance with Hill and Ricci's "synthetic complex" method (*13*), it was decided to start the equilibration procedure with samples of known composition of water, glucose, and cellobiose (*14*). Since saturated solutions of sugars together with their precipitated crystals form very viscous slurries which are often difficult to pipet or filter, the synthetic compositions should preferably be chosen so as to give only small quantities of crystals; i.e., they should be close to the solubility curve. On the basis of the results

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