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THERMOCHEMISTRY OF AQUEOUS SOLUTIONS OF NUCLEIC ACID BASES AND THEIR ALKYLATED DERIVATIVES

W. Zielenkiewicz

Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Ul. Kasprzaka 44/52, Poland

Abstract

Enthalpy of solution, ΔH°_{sol}, enthalpy of sublimation, ΔH°_{subl}, apparent partial molar volume and heat capacities, V°_2 and $C^{\circ}_{p,2}$ were determined for aqueous solutions of thirty alkylated derivatives of uracyl and adenine, eight derivatives of cytosine and guanine. Calculated accessible surface areas and molar volumes are presented, too. The values of enthalpy of solution, enthalpy of sublimation can be useful in the studies on the nature of interaction between these compounds and water molecules. Apparent partial molar volume and heat capacity give a new aspect on hydrophob properties of the examined nucleic acid base derivatives.

Keywords: enthalpy of solution, enthalpy of solvation, enthalpy of sublimation, nucleic acid bases, partial molar heat capacities

Introduction

Nucleic acid bases play an important role in organization of structure of nucleic acids. Knowledge of the hydration scheme and hydration energy of particular nucleic acid bases is of great importance in explanation of the effect of an aqueous environment on base pairing and stacking interactions between both purine and pyrimidine [1–3] bases in aqueous solutions and thus spatial organization of polinucleotide chains.

However, little was known experimentally about the hydration schemes, energies and thermodynamics of hydration. We initiated [4–6], a couple of years ago systematic thermodynamic studies of solute–water interactions from experimentally determined values of enthalpies of sublimation and solution as well as partial molar volumes and heat capacities.

The method we have chosen was to screen the functional groups on the skeleton of the base by methyl or other alkyl groups to see the effect of removal of certain polar and apolar atoms of the skeleton from direct interaction with the hydration shell. This was a major reason for choosing alkylated derivatives of nucleic acid bases as objects of our studies. The additional advantage of this ap-

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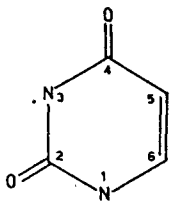
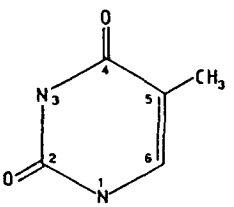
proach is that the methylation of nucleic acid bases plays an important role in the formation of biologically active conformations of nucleic acids leading in some cases to changes [1–3] in specific peculiarities of their three-dimensional structure.

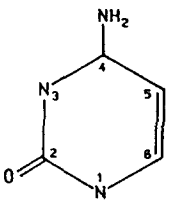
The purpose of this, to certain extent, review article is to collect and discuss in a more general way the experimental data obtained by us. The detailed results of these investigations were described previously [4–22]. Here, the results of thermodynamic investigations for more than 30 alkylated derivatives of uracil and adenine as well 8 derivatives of cytosine and guanine are presented. The structural formula of the basic compounds are as follows of:



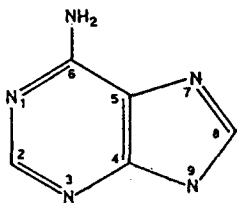
uracil



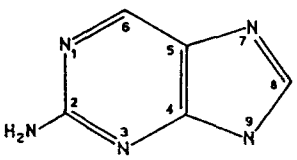
thymine



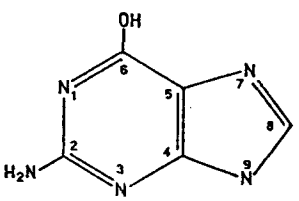
cytosine



adenine
(6-aminopurine)



2-aminopurine



guanine
(2-amino-6-hydroksypurine)

Materials and methods

The examined compounds, differently alkylated nucleic acid bases (Table 1 and Table 2), before usage were thoroughly purified by repeated crystallization and repeated vacuum sublimation. Their identity and purity were checked by melting point determinations, thin layer chromatographic analysis in several solvent systems and, if necessary, by MS and ^3H NMR measurements. In all possible cases the substances investigated were Sigma commercial products of purity better than 99%. Nevertheless, the majority of the compounds used were obtained by individual synthesis, in the laboratory of Prof. dr. M. Dramiński (Military Academy of Medicine, Łódź, Poland), because they are not commercially available.

All the solutions were prepared by weight using degassed and deionized distilled water.

Enthalpies of hydration ΔH°_{hydr} of the compounds studied, which correspond to the hypothetical thermodynamic process of transfer of one mole of solute from the gas phase to liquid water resulting in formation of an infinitely diluted solution were calculated from the experimental heats of solution and sublimation.

The heat of solution of crystalline samples were measured in an isoperibol calorimeter [6] constructed by A. Zielenkiewicz. It consists of a 150 cm^3 measuring steel vessel equipped with a calibration heater (99.7 Ohm), a mechanical stirrer and platinum resistance thermometer (100 Ohm) ensuring determination of temperature with an accuracy of 0.003 deg. Then the calorimeter employed was slightly modified [7] to ensure accurate measurements of the relatively smaller heats of solution of highly alkylated compounds. The 150 cm^3 steel vessel was replaced by the one of 80 cm^3 equipped with the two 150 Ohm thermistors instead of the platinum thermometer used previously. The thermistors were connected to the resistance bridge and IBM PC computer. The values of enthalpies of solution obtained for a series of concentrations were numerically extrapolated back to an indefinitely diluted solution.

The measurements of vapour pressures and successive determinations of the enthalpies of sublimation were carried out by Knudsen's effusion method. The apparatus [12] consists of a steel sublimation cell placed in a water thermostat and connected with a vacuum system of the order 10^{-4} Pa. The examined substance was placed in a duraluminium Knudsen cell of height $h=24$ mm and diameter $\varnothing=20$ mm. The Knudsen cell is closed by a tantalum membrane with an effusion hole. Until vacuum of the order of 10^{-4} Pa is attained and thus the experiment begins, the effusion hole is closed by a steel push with an O-ring whose hand wheel is outside the vacuum system, thus permitting to raise and/or lower the push without disturbing the vacuum. The temperature in the thermostat is kept constant with an accuracy of 0.005 deg by means of a UNIPAN 600 thermoregulator, and it is measured by means of a Systemteknik AB S1220 digital resistance thermometer.

The vapour pressure is calculated according to the equation:

$$p = \frac{m}{taW}\sqrt{\frac{2RT}{M}}$$

where: a – surface of the effusion hole, m – mass of the sublimated substance in time t, T – absolute temperature of the measurements, M – molecular mass of the examined substance, R – gas constant, W – Clausing's coefficient.

The instrument was carefully calibrated using the standard materials. The agreement of the results with those obtained by other methods and instruments

was tested by the measurements for the same compounds using the effusion method elaborated by Colomina *et al.* [8, 24], and quartz–resonator method [6]. The latter method was also applied by us for measurements of the heat of sublimation of several alkylated uracils [6]. Due to low vapour pressure of the compounds studied the measurements were carried out in the large extend of temperatures [6–8, 11, 12, 20–22], always well below the melting temperature of the compound; no thermal decomposition of samples was observed. The enthalpies of hydration, ΔH°_{hydr}, derived from the experimentally determined enthalpies of solution, ΔH°_{sol}, and the enthalpies of sublimation, ΔH°_{subl}, were the basis of the analysis of the interactions of the compounds studied with their liquid environment.

For determination of apparent molar heat capacities $C_{p,\varphi}$ we used direct microcalorimetric measurements of heat capacities per unit volume C^{V}_{p} at constant pressure. To transform heat capacities per unit volume to specific and then apparent molar heat capacities concomitant density measurements were carried out. Differences in densities between solution under study and water were obtained with digital Picker 03 D flow densimeter [25] and Anton Paar DMA 60/602 digital densimeter. The heat capacities per unit volume of the solutions were measured relatively to those of water using a Picker flow microcalorimeter [26, 27] and differential adiabatic scanning microcalorimeters DASM [28] with the cells of 0.47 ml and 1 ml. The C^{V}_{p} values obtained with these two different techniques agree well with each other. The majority of the data presented in this paper was obtained using the Picker microcalorimeter, most useful for the measurements in defined temperature (25°C). The uncertainties in the measurements were respectively 10^{-5} J·K^{-1}·cm^{-3} for C^{V}_{p} and $2\cdot10^{-6}$ g·cm^{-3} for densities. The apparent molar volumes V_{φ} and heat capacities $C_{p,\varphi}$ were calculated from densities and specific heats of the solutions and those of water respectively using the typical expressions [16]. At infinite dilution, the limiting apparent molar quantities V°_{φ} and $C^{\circ}_{p,\varphi}$ are assumed to be identical to the partial molar quantities V°_{2} and $C^{\circ}_{p,2}$, respectively. In many cases, due to the very low concentrations of the compounds studied it was impossible to establish the relation of $C_{p,\varphi}$ *vs.* molality. In these cases it was assumed, that the determined apparent molar volumes and heat capacities are identical to the partial molar quantities V°_{2} and $C^{\circ}_{p,2}$.

Molecular volumes and surface accessible areas S of solutes were calculated on the basis of the crystallographic data for crystalline compounds. The geometry of the bases was generated using the mean values for bond lengths and angles from compiled crystallographic databases [29, 30]. Hydrogen atoms were added assuming C–H distance of 1.09 Å and tetrahedral bond angles. It was assumed that the bases are planar. For $-CH_2-CH_2-\cdots CH_3$ chains the arithmetic mean of 3 different conformations at each carbon atom was taken. Cartesian coordinates were obtained using the EUKLID program (Quantum Chemistry Pro-

gram Exchange, Program N-452 Indiana University). Solvent accessible surface areas were calculated by the Lavery *et al.* method [31]. Molecular volumes were calculated using the algorithm of GEPOL program version 12.0 described by Silla *et al.* [32]. The radius of water molecule was assumed as $\gamma_{HOH} = 1.2$ Å or $\gamma_{HOH} = 1.4$ Å depending on the atom which makes contact with the acceptor atom. Since the compounds considered are highly substituted with alkyl groups, a hydrophobic model of the hydration shell was assumed and the effective solvent – accessible areas were calculated as average values for $r = 1.2$ and 1.4 Å.

Results and discussion

The experimental enthalpies of solution in water at 25°C, ΔH°_{sol}, van't Hoff enthalpies of sublimation, ΔH°_{subl}, and the enthalpies of hydration, ΔH°_{hydr}, are collected in Table 1. The values of partial molar volumes V°_2 and heat capacities $C^{\circ}_{p,2}$, accessible surface areas S and van der Waals volumes V are reported in Table 2.

The data shown in the Table 1 and Table 2 allow the formulation of the following general observations and conclusions.

1) The inspection of the values of the experimental enthalpies of solution, ΔH°_{sol}, at 25°C and sublimation, ΔH°_{subl}, of methylated uracils and adenines reveals that the both enthalpies decrease approximately with the number of methyl groups n_{CH_2}. For example, the enthalpy of solution is large and positive ($\Delta H^{\circ}_{sol} = 33.5$ kJ·mol^{-1}) in the case of the parent adenine and becomes slightly negative for the fully N-methylated derivatives $m_3^{6,6,9}$Ade, ($\Delta H_{sol} = -2.2$ kJ·mol^{-1}) as the internal energy decreases with the stepwise consecutive methyl substitution of the parent compound at the amino and N(9) ring nitrogen atom.

2) Most interestingly, ΔH°_{hydr} of alkylated nucleic acid bases vary not only with the number of CH_2 groups but also with position of their substitution in the ring.

3) The substitution of methyl group at N-ring atom on uracil skeleton brings about a reduction of ΔH°_{hydr} with mean increment close to –2 kJ·mol^{-1}; substitution by CH_3 group either on C(5) or C(6) ring carbon atoms gives an opposite effect, viz. an increase in ΔH°_{hydr} on average by about and 10 kJ·mol^{-1}. This is clear from the comparison of the values of increments of $\delta\Delta H^{\circ}_{hydr}$ for respective pairs of compounds: m^1Ura, Ura; $m_2^{1,3}$Ura, Ura; m^1Thy, Thy; $m_2^{1,3}$ Thy, Thy; and m^5Ura, Ura; m^1Thy, m^1Ura; $m_3^{1,3,6}$Ura, $m_2^{1,3}$Ura; $m^{1,3,5}$Ura, $m_2^{1,3}$Ura. This means, that there exist evident differences of methyl substitution on "polar" and "apolar" side of the uracil skeleton.

In order to explain these various effects of methyl substitution on ΔH°_{hydr} in terms of perturbations caused by the substituents in the hydration scheme of uracil the possible changes in intermolecular interactions between water and po-

Table 1 Enthalpies of solution in water, ΔH°_{sol}, enthalpies of sublimation, ΔH°_{subl}, enthalpies of hydration, ΔH°_{hydr}

Compound		ΔH°_{sol} / kJ·mol^{-1}	ΔH°_{subl} / kJ·mol^{-1}	Ref.	$-\Delta H^{\circ}_{hydr}$ / kJ·mol^{-1}
Uracil	Ura	29.29	120.5	32, 6	91.2
1-methyluracil	m^1Ura	23.5	112.5	6	89.0
Thymine	Thy	24.32	124.4	33, 6	100.1
1,3-dimethyluracil	$m_2^{1,3}$Ura	15.7	101.7	6	86.0
1,6-dimethyluracil	$m_2^{1,6}$Ura	21.7	120.5	–	98.8
1-methylthymine	m^1Thy	22.2	120.1	20, 6	97.9
1,3,6-trimethyluracil	$m_3^{1,3,6}$Ura	12.34	106.7	6	94.4
			111.7	20	
1,3-dimethylthymine	$m_2^{1,3}$Thy	10.2	109.2	6	99.0
1,3,5,6-tetramethyluracil	$m_4^{1,3,5,6}$Ura	10.23	103.9	21	93.7
1,3-dimethyl-6-ethyluracil	$m_2^{1,3}e^{6}$Ura	12.79	96.1	20	83.3
1,6-dimethyl-3-ethyluracil	$m_2^{1,6}e^{3}$Ura	10.15	77.1	21	66.9
1,3-dimethyl-5-ethyluracil	$m_2^{1,3}e^{5}$Ura	8.7	110.0	7	101.3
1,3-dimethyl-5,6-trimethyleneuracil	$m_2^{1,3}(CH_2)_3^{5,6}$Ura	7.6	113.8	7	106.2
1,3-dimethyl-6-propyluracil	$m_2^{1,3}p^{6}$Ura	11.94	109.2	20	97.3
1,6-dimethyl-3-propyluracil	$m_2^{1,6}p^{3}$Ura	12.80	118.5	21	105.7
1,3-dimethyl-5-propyluracil	$m_2^{1,3}p^{5}$Ura	12.4	107.7	7	95.3
1,3-diethylthymine	$e_2^{1,3}$Thy	9.6	95.0	6	85.4
1,3-dimethyl-5,6-tetramethyleneuracil	$m_2^{1,3}(CH_2)_4^{5,6}$Ura	9.7	114.9	7	105.2
1,3-dimethyl-6-butyluracil	$m_2^{1,3}b^{6}$Ura	14.14	90.6	20	76.5
1,6-dimethyl-3-butyluracil	$m_2^{1,6}b^{3}$Ura	10.80	91.6	21	80.8

Table 1 Continued

Compounds		ΔH°_{sol} / kJ·mol^{-1}	ΔH°_{subl} / kJ·mol^{-1}	Ref.	$-\Delta H^{\circ}_{hydr}$ / kJ·mol^{-1}
1,3-dimethyl-5-butyluracil	$m_2^{1,3}b^5$Ura	16.5	101.3	7	84.8
1,3-dimethyl-5,6-pentamethyleneuracil	$m_2^{1,3}(CH_2)_5^{5,6}$Ura	–	111.1	7	–
Adenine	Ade	33.5	127.2	36,10	93.7
6-methyladenine	m^6Ade	24.4	123.4	10	99.0
6,6-dimethyladenine	$m_2^{6,6}$Ade	15.3	115.5	10	100.2
9-methyladenine	m^9Ade	25.1	121.3	10	96.2
8,9-dimethyladenine	$m_2^{8,9}$Ade	25.25	105.8	22, 12	80.5
2,9-dimethyladenine	$m_2^{2,9}$Ade	22.6	126.0	11	103.4
6,9-dimethyladenine	$m_2^{6,9}$Ade	14.9	115.5	10	100.6
6,6,9-trimethyladenine	$m_3^{6,6,9}$Ade	–2.2	101.7	10	103.9
6,8,9-trimethyladenine	$m_3^{6,8,9}$Ade	13.19	98.6	22	85.4
9-methyl-8-ethyladenine	m^9e^8Ade	15.34	127.1	22	118.8
9-methyl-2-ethyladenine	m^9e^2Ade	16.3	130.4	11	114.9
6,9-dimethyl-8-ethyladenine	$m_2^{6,9}e^8$Ade	12.5	94.1	22	81.6
9-methyl-2-propyladenine	m^9p^2Ade	14.2	128.5	11	114.3
9-methyl-2-butyladenine	m^9b^2Ade	12.5	137.7	11	125.2
6,9-dimethyl-8-propyladenine	$m_2^{6,9}p^8$Ade	15.1	129.0	22	113.9
6,9-dimethyl-8-butyladenine	$m_2^{6,9}b^8$Ade	14.5	106.0	22	91.5

lar solute molecule induced by single alkyl substituents were considered in a more general way [7]. Due to the hydrophobic nature, interaction of methylated uracils with water molecules is no doubt of purely van der Waals nature. The presence of an alkyl group may also affect interaction of the diketopyrimidine ring with water by an inductive effect. This effect would increase energy of interaction of base molecule with water, somewhat because the ionization potential of uracil (9.0 eV) drops by about 0.4 eV on either C or N substitution with a methyl group. The effect of an alkyl group can be also envisaged as consisting of the removal of a number of water molecules from their energetically most favorable positions in the first and subsequent layers of the hydration shell surrounding the polar diketopyrimidine ring, accompanied by relaxation of the whole hydration shell into a new dynamic state. This effect must be necessarily connected with a decrease in energy of interaction due to a loss in the energy of polar interactions. According to this model the difference in $\delta\Delta H^{\circ}_{hydr}$ resulting from the substitution with a CH_3- group at an amid ring nitrogen (e.g. on the polar side of the diketopyrimidine ring) and C^5 or C^6 carbon ring substitution can be interpreted as being the net result of changes brought about by N-methylation in the energy of water bringing into the hydration layer; the increase in ΔH°_{hydr} at the C^5 and C^6 substitution on the ring results for the most part from positive contribution of van der Waals interaction of that group with water. The presented ΔH°_{hydr} data show the validity of such interpretation. The conclusion resulting from ΔH°_{hydr} analysis can be only quantitative. The values of ΔH°_{hydr} do not reflect solely energies of interaction between the solute and water molecules in the solvation shell because the transfer of a molecule from the gas phase to water requires energy to form a cavity. So $|\Delta H^{\circ}_{hydr}| = |\Delta H^{\circ}_{int}| - |\Delta H^{\circ}_{cav}|$, where ΔH°_{int} and ΔH°_{cav} corresponding to the enthalpy changes associated with structuring of water around the solute and with cavity formation in pure liquid, respectively. The standardized ΔH_{int} calculations together with those presented previously [6, 7, 10, 11] will be presented elsewhere [23].

4) In N-methylated adenines, variously substituted methyl groups also contribute differently in ΔH°_{hydr}. Substitution by the methyl group on C(2) position gives an increment equal $\delta\Delta H_{hydr}$ ($m^{2,9}$Ade – m^9Ade)=7.2 kJ·mol^{-1}; whereas significantly smaller and different values of increments are observed for single group substitutions at the exocyclic amino nitrogen and N(9) ring nitrogen. This is clear from the values of ΔH°_{hydr} for pairs of compounds m^6Ade-Ade, m^9Ade-Ade and $m^{6,6}$Ade-m^6Ade which correspond to 5.3; 2.5 and 1.2 kJ·mol^{-1}, respectively. The highest increments $\delta\Delta H_{hydr}$ are noted for the following pairs of compounds: $m_2^{8,9}$Ade – m^9Ade, $m_3^{6,8,9}$Ade – $m_2^{6,9}$Ade. They correspond to −16.2 kJ·mol^{-1}, 15.2 kJ·mol^{-1}, respectively. These facts indicate that methyl groups differently reduce the energy of interactions of adenine ring with water.

5) The substitutions of hydrogen atoms of the uracil and adenine rings by ethyl, propyl and butyl in the alkyl side chains cause, in most cases erratic

changes in the values of the enthalpy of hydration. The higher values of the increment of the enthalpy correspond to the odd number of $-CH_2-$groups added, while the lower ones to those with the even n_{CH_2} number. This phenomenon was seen from the results of ΔH°_{hydr} (Table 1) for the series of 6,9-dimethyl-8-alkyladenines [22], 9-methyl-8-alkyladenine [22], 9-methyl-2-alkyladenines [11] as well as in the series of 1,3-dimethyl-6-alkyluracils [20] and 1,6-dimethyl-3-alkyluracils [21]. We were seeking for the reason of this phenomenon in the structure of the solid compounds, because the value of the enthalpy of hydration is influenced, first of all, by the value of the enthalpy of sublimation. X-ray studies show [37], that for one of the series of alkyluracils a good correlation between the values of the enthalpies of sublimation, crystal density and molar volumes exists. It appears, that the calculated crystal packing energies reproduce quite well experimental values of the enthalpy of sublimation. It is worth to be mentioned, that in the series of 5-alkyluracils, a contribution of successive CH_2- groups to ΔH°_{hydr} decreases monotonically.

6) The total enthalpy of hydration ΔH°_{hydr} per unit of water accessible molecular area S (Table 2) is, in the case of thymine (1.5021 $kJ \cdot mol^{-1} \cdot Å^{-2}$) and uracil (1.4662 $kJ \cdot mol^{-1} \cdot Å^{-2}$) some 10 per cent higher than corresponding $\Delta H^{\circ}_{hydr}/S =$ 1.3366 $kJ \cdot mol^{-1} \cdot Å^{-2}$ for adenine. This means, that the diketopirymidine ring is more hydrated, that the 6-aminopurine moiety [10].

7) The results of the determination of partial molar volumes and heat capacities (collected in Table 2) show in general the linear dependencies of partial molar volumes *vs.* the number of $-CH_2-$ groups attached to the skeleton directly and to the alkyl groups thereon. The values of the increments of partial molar values per $-CH_2-$groups are similar to those obtained for other series of hydrophobic compounds – homologous series of hydrocarbons [38], aliphatic amides [39] and various hydrocarbons derivatives bearing polar group [40]. The detail inspection of data V°_2, $C^{\circ}_{p,2}$ obtained is given below.

8) Partial molar volumes of alkylated uracils. In these compounds the increment of partial volume $V^{\circ}_{CH_2}$ for CH_2 group correspond to 16.51 $cm^3 \cdot mol^{-1}$. The influence of the position of the substitution (on carbon or nitrogen) and of the position in the ring of the atom to which the alkyl group is attached could not be clearly evidenced. The slight differences observed between N-alkylated and C-alkylated molecules, can be considered to be of the order of magnitude of the experimental error. The data are in good agreement with previous results obtained by Shahidi *et al.* [16, 41, 42] for $-CH_2-$ substitutions on N or C atoms in the case of amides and amines: V_{CH_2} (N-substituted) −18 $cm^3 \cdot mol^{-1}$, whereas V_{CH_2} (C-substituted) is closer to 16 $cm^3 \cdot mol^{-1}$.

9) Partial molar heat capacity of alkyluracils. The increment in C°_{p2} for CH_2 group for alkyluracils corresponds to 84.62 $J \cdot K^{-1} \cdot mol^{-1}$. Addition of the $-CH_2-$ group on N atoms correspond to an increment of about 70 $J \cdot K^{-1} \cdot mol^{-1}$ in

Table 2 Partial molar volumes V_2^o and heat capacities, $C_{p,2}^o$, accessible surface areas S and molecular van der Waals volumes V

Compound		n_{CH_2}	V_2^o $cm^3 \cdot mol^{-1}$	$C_{p,2}^o$ $J \cdot K^{-1} \cdot mol^{-1}$	Ref.	S	V
Uracil	Ura	0	–	137.0	4	62.2	74.9
1-methyluracil	m^1Ura	1	89.7	~207.1	17	67.0	98.0
Thymine	Thy	1	–	220.0	4	67.3	98.4
1,3-dimethyluracil	$m_2^{1,3}$Ura	2	109.2	~286.4	17	73.5	115.9
1-methylthymine	m^1Thy	2	–	–	–	74.0	117.5
1,3,6-trimethyluracil	$m_3^{1,3,6}$Ura	3	124.2	363.8	14	78.4	135.7
1,3-dimethylthymine	$m_2^{1,3}$Thy	3	–	373.0	4	79.6	136.2
1,3,5,6-tetramethyluracil	$m_4^{1,3,5,6}$Ura	4	139.6	441.9	18	82.8	152.0
1,3-dimethyl-6-ethyluracil	$m_2^{1,3}e^6$Ura	4	140.34	455.2	14	83.9	153.1
1,6-dimethyl-3-ethyluracil	$m_2^{1,6}e^3$Ura	4	141.4	457.3	18	83.9	152.1
1,3-dimethyl-5-ethyluracil	$m_2^{1,3}e^5$Ura	4	–	473.4	4	84.7	154.3
1,3-dimethyl-5,6-trimethyleneuracil	$m_2^{1,3}(CH_2)_3^{5,6}$Ura	5	145.3	441.8	16	86.4	154.5
1,3-dimethyl-6-propyluracil	$m_2^{1,3}p^6$Ura	5	156.4	541.8	14	90.8	172.3
1,6-dimethyl-3-propyluracil	$m_2^{1,6}p^3$Ura	5	157.6	547.0	18	91.6	173.0
1,3-dimethyl-5-propyluracil	$m_2^{1,3}p^5$Ura	5	–	581.9	–	93.2	169.7
1,3-diethylthymine	$e_2^{1,3}$Thy	5	159.6	577.8	16	92.0	172.3
1,3-dimethyl-5,6-tetramethyleneuracil	$m_2^{1,3}(CH_2)_4^{5,6}$Ura	6	157.9	~505	16	90.1	171.0
1,3-dimethyl-6-butyluracil	$m_2^{1,3}b^6$Ura	6	172.42	634.2	–	99.3	182.0
1,6-dimethyl-3-butyluracil	$m_2^{1,6}b^3$Ura	6	173.8	627.0	18	98.7	181.7
1,3-dimethyl-5-butyluracil	$m_2^{1,3}b^5$Ura	6	–	643.0	–	100.3	184.7
1,3-dimethyl-5,6-pentamethyleneuracil	$m_2^{1,3}(CH_2)_5^{5,6}$Ura	7	173.1	~580	16	94.0	186.3

Table 2 Continued

Compounds		n_{CH_2}	V_2° $cm^3 \cdot mol^{-1}$	$C_{p,2}^\circ$ $J \cdot K^{-1} \cdot mol^{-1}$	Ref.	S	V
1,5,N^4-trimethylcytosine	$m_3^{1,5,N^4}Cyt$	3	126.5	406.3	18	81.3	131.9
1,N^4-dimethyl-5-ethylcytosine	$m_2^{1,N^4}e^5Cyt$	4	142.4	515.0	18	87.7	156.0
1,N^4-dimethyl-5-propylcytosine	$m_2^{1,N^4}p^5Cyt$	5	156.7	587.9	18	93.1	182.6
1,N^4-dimethyl-5-butylcytosine	$m_2^{1,N^4}b^5Cyt$	6	172.0	688.0	18	98.8	197.9
Adenine	Ade	0	96.4	228.6	19	–	–
2-aminopurine	N^2Pu	0	91.9	226.1	19	–	–
9-methyladenine	m^9Ade	1	112.0	315.9	19	78.4	159.3
2,2-dimethyl-aminopurine	$m_2^{2,2}N^2Pu$	2	125.4	429.8	19	–	–
8,9-dimethyladenine	$m_2^{8,9}Ade$	2	124.6	548.1	17	–	138.6
2,9-dimethyladenine	$m_2^{2,9}Ade$	2	127.2	557.7	16	–	–
2-ethylguanine	e^9Gua	2	128.7	433.8	19	–	–
2-methylamino-9-methylpurine	$m_2^{2,9}N^2Pu$	2	125.2	455.4	19	–	–
6,8,9-trimethyladenine	$m_3^{6,8,9}Ade$	3	141.1	628.0	16, 17	–	157.5
2-dimethylamino-9-methylpurine	$m_3^{2,2,9}N^2Pu$	3	142.1	559.6	19	–	–
9-methyl-8-ethyladenine	m^9e^8Ade	3	141.5	577.0	17	–	157.1
9-methyl-2-ethyladenine	m^9e^2Ade	3	139.3	~638.0	16	–	157.4
6,9-dimethyl-8-ethyladenine	$m_2^{6,9}e^8Ade$	4	157.4	664.0	16, 17	–	176.6
9-methyl-2-propyladenine	m^9p^2Ade	4	155.2	638.0	16, 17	–	165.8
9-methyl-2-butyladenine	m^9b^2Ade	5	172.7	713.0	17	–	185.6
6,9-dimethyl-8-propyladenine	$m_2^{6,9}p^8Ade$	5	174.2	777.1	17	–	188.9
6,9-dimethyl-8-butyladenine	$m_2^{6,9}b^8Ade$	6	189.1	753.2	17	–	195.3

$C^{\circ}_{p,2}$ while addition to C atoms leads to value closer to 90 $J \cdot K^{-1} \cdot mol^{-1}$, which is in good agreement with the values published by Nichols *et al.* [43, 16]. The values of partial molar heat capacities are about two times higher than the heat capacity for the solid compounds [4]: partial molar heat capacity $C^{\circ}_{p,2}$ for thymine is equal 220 $J \cdot K^{-1} \cdot mol^{-1}$; for solid thymine $C_{p(s)} = 150.4\ J \cdot K^{-1} \cdot mol^{-1}$; for 1-methyluracil $C^{\circ}_{p,2} = 205.0\ J \cdot K^{-1} \cdot mol^{-1}$; $C_{p(s)} = 150\ J \cdot K^{-1} \cdot mol^{-1}$; for 1,3-dimethyluracil $C^{\circ}_{p,2} = 295.0\ J \cdot K^{-1} \cdot mol^{-1}$; $C^{\circ}_{p(s)} = 180\ J \cdot K^{-1} \cdot mol^{-1}$. This shows the hydrophobic nature of the compounds studied.

10) A comparison of the partial molar heat capacities for the following pairs of compounds: $m_3^{1,3,6}$Ura and $m^{1,3,5}$ Ura ($m_2^{1,3}$Thy); $m_2^{1,3}e^6$Ura, and $m_2^{1,3}e^5$Ura; $m_2^{1,6}p^6$Ura and $m_2^{1,3}p^5$Ura; $m_2^{1,2}b^6$Ura and $m_2^{1,3}b^5$Ura shows that values for 5-alkyluracils (thymine and alkylated thymine) are always higher than those observed for 6-alkyluracils. These differences can be attributed [4] to the change in the interaction of a given group (C=O, N–H, C–H or CH_3) with the solvent, caused by adjacent substituent group(s). It can be also noted that there is a difference of about 15 $J \cdot K^{-1} \cdot mol^{-1}$ between partial molar heat capacities for the structural isomers Thy and m^1Ura.

11) The partial molar heat capacities for the following series of compounds: 1,3-dimethyl-6-alkyluracils, 1,3-dimethyl-5-alkyluracils and 1,6-dimethyl-5-alkyluracils indicates [18] that elongation of the alkyl chain independently of the place of substitution gives almost the same increment in $\Delta C^{\circ}_{p,2}$ for one CH_2– group (90 $J \cdot K^{-1} \cdot mol^{-1}$) as that obtained for aliphatic hydrocarbons.

12) Partial molar heat capacities of cyclooligomethylenouracils. In these compounds the variation of increment is not linear [18]. Starting from $m_3^{1,3,6}$Ura (first member of the two series) the increment value is quite small initially (~40 $J \cdot K^{-1} \cdot mol^{-1}$), then increases with the addition of a new $-CH_2-$ segment – approaching the increment values typical for linear chains (~90 $J \cdot K^{-1} \cdot mol^{-1}$) when the ring consists of more then 7 carbon atoms. This observation agrees well with the effects observed in cycloalkanes or other cyclic compounds [43, 18]. As expected, the constraints on the $-CH_2-$ motion due to cyclisation are important for shorter hydrocarbons ring, and heat capacities reflect these energy fluctuations. The same tendencies were also observed on the variations of partial molar volumes V°_2.

13) Partial molar heat capacities and volumes of 5-alkyl-1,N^4-dimethylcytosines. Only 4 compounds: methyl, ethyl, propyl and butyl derivatives of 1,N^4-dimethylcytosine were under study. The linear relations between partial molar values at infinite dilution and the number of $-CH_2-$ groups are $V^{\circ}_2 = 81.5 + 15.1 \cdot n_{CH_2}$; $C^{\circ}_{p,2} = 136.2 + 91.8 \cdot n_{CH_2}$.

14) Partial molar heat capacities of alkylated adenines and aminopurines. For these compounds a linear relationship between $C^{\circ}_{p,2}$ and the number of $-CH_2-$ groups $C^{\circ}_{p,2} = a + b \cdot n_{CH_2}$, where $a = 261 \pm 12.3\ J \cdot K^{-1} \cdot mol^{-1}$ and $b = 95.9 \pm 3.7\ J \cdot K^{-1} \cdot mol^{-1}$ with a product – moment correlation coefficient δ of

0.9624 was found [14]. A more exact correlation of $C_{p,2}=f(n_{CH_2})$ was obtained for these compounds, except the three adenine derivatives: 2,9-dimethyladenine, 6,9-dimethyl-8-butyladenine and 6,8,9-trimethyladenine. In this case, in the above relationship $a=288\pm5.7$ J·K^{-1}·mol^{-1}, $b=105.6\pm1.8$ J·K^{-1}·mol^{-1} and $\delta=0.9938$. Closer inspection of $C_{p,2}$ data of the 6,9-dimethyl-8-alkyladenines and 9-methyl-2-alkyladenines show that are erratic changes in $\Delta C^{\circ}_{p,2}$ with elongation of *n*-alkyl chain. The $\Delta C^{\circ}_{p,2}$ increments calculated for successive pairs of compounds differ considerably one from another and for $m_3^{6,8,9}$Ade and $m_2^{6,9}e^8$Ade, $m_2^{6,9}e^8$Ade and $m_2^{6,9}p^8$Ade, $m_2^{6,9}p^8$Ade and $m_2^{6,9}b^8$Ade correspond to 36.113 and 23.9 J·K^{-1}·mol^{-1}, respectively; for $m_2^{2,9}$Ade and m^9e^2Ade, m^9e^2Ade and m^9p^2Ade, m^9p^2Ade and m^9b^2Ade are equal to 19.3, 61.0 and 75.0 J·K^{-1}·mol^{-1}, respectively. This is probably a result of the different structural features of the solid compounds and then the solutions studied.

For the alkylated adenine derivatives, the $C^{\circ}_{p,2}$ and V°_2 values present a large uncertainty in connection with their low solubility and the concentration dependence of apparent molar quantities was obtained only for $V_{\varphi,2}$.

As it was shown [17] also the evaluation of $C_{\varphi,p}$ *vs.* temperature is quite different depending on the various series of alkyladenines. In the series of 6,9-dimethyl-8-alkyladenines, $C_{p,\varphi}$ values at the given temperature depend strongly on the concentration. In the series of 9-methyl-2-alkyladenines this dependence is not so evident.

The results obtained show that even in the range of very low concentrations (0.004–0.03 *M*) the assumption, that $C_{p,\varphi}$ is identical as $C^{\circ}_{p,2}$, may induce a significant error.

All this indicates that experimental, thermodynamic investigations can be helpful in understanding the nature of solute-water interactions.

* * *

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Zusammenfassung — Für die wäßrigen Lösungen von dreißig alkylierte Uracyl- und Adeninderivaten und acht Cytosin- und Guaninderivaten wurden die Lösungsenthalpie ΔH°_{subl}, die Sublimationsenthalpie ΔH°_{subl}, das scheinbare partielle molare Volumen V°_{2} und die Wärmekapazitäten $C^{\circ}_{p,2}$ bestimmt. Auch die berechneten zugänglichen Oberflächengrößen und molaren Volumen werden dargelegt. Die Werte für die Lösungsenthalpie und die Sublimationsenthalpie können von Nutzen bei der Untersuchung der Art der Wechselwirkung zwischen diesen Molekülen und Wassermolekülen sein. Das scheinbare partielle molare Volumen und die Wärmekapazität liefert einen neuen Aspekt bezüglich hydrofober Eigenschaften der untersuchten Nukleinsäuren-Basenderivate.