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Heats of Hydrolysis of N-Acetyl Imidazole and Butyl Acetamide in Aqueous Solution

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The heats of hydrolysis of N-acetyl imidazole and butyl acetamide in aqueous solution have been determined by combining standard state data with results from heats of solution measurements. The enthalpy changes were found to be -7.28 ± 0.07 and $+ 10.82 \pm 0.06$ kcal/mole, respectively (hypothetical reaction, no ionization of reaction products). The data have been discussed and compared with data for analogous reactions.

Heats of ionization (ionic strength = 0.2) have been determined for acetic acid, $\Delta H_i = + 0.18 \pm 0.05$ kcal/mole; for imidazolium ion, $\Delta H_i = + 8.79 \pm 0.04$ kcal/mole; and for butylammonium ion, $\Delta H_i = + 13.97 \pm 0.05$ kcal/mole.

Enthalpy data for the hydrolysis of N-acetyl imidazole and butyl acetamide, all reactants being in their standard state **, have recently been determined [1,2]. As these compounds can be looked upon as simple biochemical model compounds, the thermochemical standard data have been converted to apply to the biochemically more interesting state *i.e.* dilute aqueous solution. The enthalpy change in going from the pure compound to *ca.* 0.01 M aqueous solution (ionic strength 0.2) have been measured for the actual reaction components. The heats of ionization of butylammonium ion, imidazolium ion and acetic acid have also been measured.

EXPERIMENTAL

Materials. Butylamine, butyl acetamide and acetic acid were described in Ref.[2]. Imidazole (Fluka, *purum*) was purified by recrystallization from benzene followed by sublimation, m.p. 89.4–89.8°C. Titration with standard hydrochloric acid gave an equivalent weight corresponding to 100.0 % purity. N-Acetyl imidazole was prepared and purified as described in Ref.[1] m.p. 103.4–104.0°C.

Solutions. The solution experiments were performed in aqueous solutions of NaCl, HCl, NaOH, imidazole, and acetic acid. NaCl was added to make the ionic strength equal

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** Referring to the hypothetical process of the pure compounds in their condensed state, at 25°C.

to 0.2. Water used was deionized and electrolytes added were of analytical grade. In the case of imidazole, the water was made free from carbon dioxide by boiling. The imidazole solution was acidified with hydrochloric acid to pH 7.1. When handling the imidazole buffer precautions were taken to avoid uptake of carbon dioxide.

Apparatus. Exept for the last two systems recorded in Table 1 an "isothermal-jacket" metal calorimeter (Type D in Ref.[3]) was used. For the systems with solutions of imidazole and acetic acid as calorimetric liquids, a calorimeter of the same type but made from thin-walled (0.9 mm) glass was used. The time to reach thermal equilibrium (*cf.* Ref.[3]) for the glass calorimeter was about 2 min. The calorimeters were charged with 100 ml of liquid and the substance was contained in a sealed glass ampoule. All reactions were very rapid; the reaction period was *ca.* 2 min and the initial and final thermistor resistance values were evaluated graphically.

Calibration. The heat equivalent of the calorimeter including contents was determined by passing a known current for a given time (360 sec) through the heating element. Each system was calibrated by at least four separate experiments. Calibrations were performed after the solution experiments except for some calibrations of the system with N-acetyl imidazole. Variations in heat equivalent in individual experiments were considered insignificant, the mean values being used.

Units of measurements. The results of the calorimetric experiments are expressed in terms of the defined calorie, equal to 4.1840 abs. joules, and refer to the isothermal process at 25°C and to the true mass.

RESULTS

The experimental results are summarized in Table 1. In the table the symbol log R_i/R_f means the expression proportional to the temperature change: R_i and R_f are the corrected thermistor resistance values at the start and the end, respectively, of the main period. ε is the heat equivalent of the calorimetric system expressed in cal/unit of log R_i/R_f. When five or more determinations were performed, the uncertainties are given as standard deviation of the mean. Otherwise, they are the average deviation of the mean.

In the solution experiments with acetyl imidazole the after-period curve was a straight line, but there was some heat evolution due to the hydrolysis of the ester. If the solution process had been instantaneous this would not cause any systematic error, as the R_f-value was evaluated graphically. In the actual case, however, dissolution was complete after *ca.* 1 min and the R_f-value had to be corrected slightly. The correction amounted to *ca.* 1 % of the log R_i/R_f-value. To avoid ionization of the imidazole when it was dissolved in 0.2 M NaCl solution, the pH was raised to 10.5 with sodium hydroxide.

Heats of ionization of butylammonium ion, imidazolium ion and acetic acid. When butylamine was dissolved in the hydrochloric acid solution, *ca.* one tenth of the hydrogen ions were used up and replaced by butylammonium ions. The heat of dilution of hydrochloric acid over the actual concentration range is very small or 0.02 kcal/mole [4]. It can therefore be assumed with confidence that the difference between the measured heats of solution of butylamine in hydrochloric acid and in sodium hydroxide solution represents the heat of ionization of butylammonium ion, so that this is calculated to be $+ 13.97 \pm 0.05$ kcal/mole.

The dissolution of imidazole under conditions (pH 10.5) allowing only an insignificant amount of the compound to be protonized, was found to be accompanied by a positive enthalpy change equal to + 3.06 kcal/mole. When imidazole was dissolved in 0.1 M hydrochloric acid solution, the obtained en-

Table 1. Heats of solution measurements.

Calorimetric liquid	ε	Substance	mmoles	$10^4 \cdot \log R_i/R_f$	$-\Delta H$ kcal/mole
0.2 M NaCl	9052 ± 12	N-Acetyl imidazole	0.885	−5.15	−5.27
			1.381	−8.09	−5.30
			0.660	−3.69	−5.06
			0.939	−5.26	−5.07
			1.420	−8.18	−5.21
				Mean	−5.18±0.05
		Butyl acetamide	1.561	5.98	3.47
			1.311	5.16	3.56
			1.240	4.80	3.50
			1.278	4.93	3.49
				Mean	3.51±0.03
		Imidazole (pH 10.5)	1.360	−4.51	−3.00
			1.162	−3.94	−3.07
			1.444	−4.94	−3.10
			1.201	−4.05	−3.05
				Mean	−3.06±0.03
0.1 M HCl 0.1 M NaCl	9050 ± 5	Imidazole	1.721	10.91	5.74
			1.349	8.53	5.72
			1.970	12.47	5.73
			1.382	8.76	5.74
				Mean	5.73±0.01
		Butylamine	1.388	30.01	19.57
			1.039	22.38	19.49
			1.360	29.34	19.52
			1.103	23.80	19.53
				Mean	19.53±0.02
		Acetic acid	2.255	0.79	0.32
			3.034	1.07	0.32
			1.736	0.65	0.34
				Mean	0.33±0.01
0.1 M NaOH 0.1 M NaCl	9064 ± 8	Butylamine	1.482	9.05	5.54
			1.742	10.67	5.55
			1.380	8.49	5.58
				Mean	5.56±0.02
0.10 M Imidazole 0.15 M NaCl	8840 ± 8	Acetic acid	1.885	19.06	8.94
			1.271	12.95	9.01
			1.309	13.25	8.95
			1.988	19.91	8.85
				Mean	8.94±0.04
0.20 M HOAc 0.18 M NaCl	8860 ± 7	Imidazole	1.661	10.46	5.58
			1.792	11.22	5.55
			2.088	13.08	5.55
			1.965	12.24	5.52
				Mean	5.55±0.02

thalpy change was −5.73 kcal/mole and the heat of ionization of imidazolium ion, at an ionic strength of 0.2, is thus calculated to be + 8.79 ± 0.04 kcal/mole

The heat of ionization of imidazolium ion has been determined previously from studies of the ionization constant as a function of temperature [5]. The value, referring to zero ionic strength, is only + 7.7 kcal/mole and thus differs considerably from that found here.

The heat of solution of acetic acid in the imidazole buffer was determined to be −8.94 kcal/mole. The final pH of the buffer was 6.85 to 6.90. Using 4.61 as the pK_a'-value for acetic acid, it is found that 0.5 to 0.6 % of the acid was undissociated at the end of the experiments. The heat of solution of acetic acid in the buffer under conditions such that acetate ion is formed quantitatively is thus calculated to be −8.98 ± 0.05 kcal/mole. When acetic acid was dissolved in 0.1 M HCl, the enthalpy change was −0.33 kcal/mole. Using the value + 8.79 for the heat of ionization of imidazolium ion, the heat of ionization of acetic acid is calculated to be + 0.14 ± 0.06 kcal/mole.

Within assigned uncertainties the same value, + 0.20 ± 0.03 kcal/mole, is arrived at when comparing results from the measurements of the heat of solution of imidazole in aqueous acetic acid and in aqueous hydrochloric acid.

It has been reported [6,7] that acetic acid to some extent is dimeric even in aqueous solution. Thus, the above calculations do not strictly apply for the reaction $HOAc + H_2O \rightarrow H_3O^+ + OAc^-$ due to the contribution from the process

$$(HOAc)_2(aq) \rightarrow 2\ HOAc(aq) \qquad (1)$$

However, as shown below, this contribution is insignificant in the present case.

When acetic acid was dissolved in hydrochloric acid, the resulting solution was *ca.* 0.02 M with regard to acetic acid. From the equilibrium constant (0.185 mole/l) [6] given for reaction (1), the amount of acetic acid present in the dimeric form at this concentration is calculated to be *ca.* 0.7 %. The heat of dissociation in the gaseous phase is given [8] as + 7.6 kcal/mole of HOAc and as the heat of dissociation in aqueous solution can be expected to be much smaller, the effect will in this case certainly amount to less then 0.05 kcal/mole.

In a 0.2 M acetic acid solution the amount of HOAc present in dimeric form is calculated to be 6.9 %. In the calorimetric experiments, when 2 mmole of imidazole was added, the same amount of acetic acid was ionized and thus removed from the equilibrium mixture of HOAc and $(HOAc)_2$. In the final solution, where [HOAc] + 2 [$(HOAc)_2$] was *ca.* 0.18 M, the amount of acid present in dimeric form is calculated to be 6.2 %. The measured heat effect, therefore, also involves the enthalpy change when *ca.* 0.2_5 mmole of HOAc is formed from the dimer.

There is no correlation between the amount of imidazole added and the heat of solution value, and the obtained heats of ionization for acetic acid are the same for the two sets of measurements within the assigned uncertainties. It can therefore be concluded that the heat of dimerization of acetic acid in water solution is very small — or that the dimerization is not as extensive as is reported. The values obtained for the heat of ionization of acetic acid can therefore be considered as representing the process when only monomer molecules are involved.

The above experiments with acetic acid were not all performed at a strictly constant ionic strength. When imidazole (*ca.* 2 mmoles) was dissolved in the acetic acid solution, the ionic strength was raised from 0.18 to *ca.* 0.20. For the imidazole buffer the ionic strength was calculated to be 0.20 before the addition of acetic acid and 0.21 to 0.22 afterwards. In this case the observed enthalpy change will include heat effects due to the difference in heat of ionization of the imidazolium ions present in the calorimetric liquid, at the two ionic strengths. Since the results of the two measurements were nearly identical, this effect is not significant.

In the following the value $+ 0.18 \pm 0.05$ kcal/mole will be used for the heat of ionization of acetic acid at an ionic strength of 0.2. This differs significantly from the well documented value [9] at zero ionic strength of -0.07 ± 0.05 kcal/mole.

Heat of hydrolysis of N-acetyl imidazole in aqueous solution. The standard heat of hydrolysis of acetyl imidazole was found to be -4.83 ± 0.05 kcal/mole[1]. From this value and from heat of solution data, the enthalpy change for reaction (2) (INH = imidazole)

$$\text{INAc(aq)} + \text{H}_2\text{O} \rightarrow \text{INH(aq)} + \text{HOAc(aq)} \qquad (2)$$

is calculated to be $\Delta H = -7.28 \pm 0.07$ kcal/mole.

Reaction (2) is hypothetical; the real hydrolysis reaction at pH-values of approximately 7 is given by (3)

$$\text{INAc} + \text{H}_2\text{O} \rightarrow \begin{cases} \alpha\ \text{INH} \\ (1-\alpha)\text{INH}_2^+ \end{cases} + \begin{cases} (1-\beta)\text{HOAc} \\ (\beta\ \text{OAc}^- \end{cases} + (\alpha + \beta - 1)\text{H}^+ \qquad (3)$$

The pK_a'-value for acetyl imidazolium ion has been determined [10] to be *ca.* 3.5 and the ester will thus be insignificantly protonized at a neutral pH-value. Using the derived values for the heat of ionization of acetic acid and of imidazolium ion, the heat of hydrolysis according to eqn. (3) will be *ca.* -12.0 kcal/mole at pH 7.00 and an ionic strength of 0.2 (pK_a for imidazolium ion is 6.95 [11]; pK_a' is estimated to be 7.10).

N-Acetyl imidazole and related compounds have recently attracted a considerable interest as possible models for reactive intermediates in enzyme (*e.g.* chymotrypsin) processes [12,13]. The free energy change of reaction (3), $\Delta G'$, has been derived to -13.3 kcal/mole * [14,15] and the acyl-imidazole grouping has therefore been classified as "energy-rich". The present calorimetric results show, that a substantial part of the free energy, which is released when N-acetyl imidazole is hydrolyzed, can be traced back to the enthalpy change in the hypothetical reaction (2), $\Delta H = -7.3$ kcal/mole. The situation here is strikingly different from the case with O- and S-acetates; the latter also being "energy-rich" compounds according to the definition given by Jencks and co-workers [15]. For these acetates the enthalpy changes for the idealized reactions corresponding to eqn. (2) are *ca.* $+ 0.5$ and -0.9 kcal/mole [16], respectively.

* At pH 7 and *ca.* 25°C, $\Delta G'$ = "standard" free energy change at a specified pH. It is based on total concentrations of reactants, including all ionic spieces. The activity for water is taken to unity.

These low ΔH-values are also reflected in lower $-\Delta G'$-values; -4.9 and -7.4 kcal/mole [15] * respectively.

The $\Delta G'$-value includes the release of free energy accompanying neutralization of the acid formed in the hydrolysis process. For acetic acid this quantity is $-$ 3.1 kcal/mole at pH 7 and 25°C. In calculating $\Delta G'$-values the activity of water is taken to be unity, while other activities are taken equal to molar concentrations. With this convention there will be included in $\Delta G'$ an expression for the "driving force of water" in hydrolysis reactions carried out in aqueous media. Based on a molar scale the activity of water is *ca.* 55 and, therefore, this convention will lower the $\Delta G'$-value by 2.4 kcal/mole at 25°C. Thus in $\Delta G'$-data (pH = 7) given for the hydrolysis of O-, S- or N-acetyl compounds *ca.* -5.6 kcal/mole (*i.e.* the major part of the $\Delta G'$-value for O- and S-acetates) is not related to the ester bond breaking.

When considering the total energy released in a certain reaction, the origin of the different contributions to an energy term are of course insignificant. However, when characterizing compounds as "energy-rich" it is important to stress the type of change behind the energy quantity given.

Heat of hydrolysis of butyl acetamide in the gaseous state and in aqueous solution. The standard heat of hydrolysis of butyl acetamide was determined [2] to be $\Delta H^\circ = + 13.20 \pm 0.05$ kcal/mole. This value refers to the hypothetical reaction where pure butyl acetamide and water react to form pure butylamine and acetic acid; all reaction components being in their condensed states. The ΔH°-value includes energy terms caused by intermolecular interactions, in this case very significant due to the extensive formation of hydrogen bonds. Therefore, to obtain the enthalpy changes for the case where there are no interactions between the molecules the values have to be converted to the ideal gaseous state, for which purpose heats of vaporization, ΔH_v, of the reaction components have to be known.

ΔH_v has been determined calorimetrically, at 25°C, for BuNHAc (17.6 ± 0.2 kcal/mole **) and for $BuNH_2$ (8.26 ± 0.04 kcal/mole [17]). For water ΔH_v is 10.52 ± 0.00 kcal/mole [4].

The enthalpy change for the process HOAc(l) → HOAc(g, equilibrium mixture at saturation pressure) has been determined calorimetrically [17] (25°C) to be 5.58 ± 0.05 kcal/mole. Using Weltner's data [8], the enthalpy change for dissociating dimer molecules present in the equilibrium gas mixture will be 6.91 kcal/mole of HOAc. The heat of vaporization, at 25°C, of acetic acid to form monomer vapor thus will be 12.49 ± 0.07 kcal/mole, (the uncertainty is an estimate). From these vaporization data the heat of hydrolysis of butyl acetamide in the gaseous, monomeric state is derived to be $+ 5.8 \pm 0.2$ kcal/mole.

In the hydrolysis of a peptide bond, C—N and O—H bonds are broken, while N—H and C—O bonds are formed. If no other changes influencing the energy of the system are considered, one would expect, from the values of the thermochemical bond energies, Table 2, the hydrolysis reaction to be endothermic by *ca.* 2 kcal/mole. This value is about 4 kcal/mole lower than that actually

* Corrected to 25°C.

** Results from preliminary measurements in this laboratory (E. Morawetz and S. Sunner).

Table 2. Thermochemical bond energies, E [a].

Bond	E, kcal
C—N	70
O—H	110.6
N—H	93.4
C—O	85.5

[a] E-Values are from Ref.[18] except for $E_{C\text{-}N}$ which has been derived from modern heats of combustion data for butylamine and *sec*-butylamine [19].

obtained, which means that in the ideal, gaseous state a peptide bond grouping is *ca.* 4 kcal/mole more resonance * stabilized than a carboxyl group.
If the standard value, $\Delta H^\circ = +13.20$ kcal/mole, is combined with heats of solution data obtained here, ΔH for reaction (4)

$$\mathrm{BuNHAc(aq) + H_2O \rightarrow BuNH_2(aq) + HOAc(aq)} \quad (4)$$

is calculated to be $+10.82 \pm 0.06$ kcal/mole. At pH 7, however, the dominant product species are those given in eqn. (5)

$$\mathrm{BuNHAc(aq) + H_2O \rightarrow BuNH_3^+(aq) + OAc^-(aq)} \quad (5)$$

If heats of ionization of acetic acid and butylammonium ion are taken to $+0.18$ and $+13.97$ kcal/mole, respectively, the enthalpy change for reaction (5) will be -2.97 ± 0.09 kcal/mole. By comparing the data for the reaction in the standard state, for the gaseous state and for reactions (4) and (5) it is obvious that intermolecular forces and ionization energies play a dominant role in the energetics of peptide hydrolysis. While the isothermal reaction is followed by a heat uptake of 5.8 kcal/mole when the unionized reaction components are isolated from each other, the heat uptake is 10.8 kcal/mole when the molecules taking part in the reaction are solvated by water. In going from the unionized state, eqn. (4), to the ionized, eqn. (5), there is a drastic change from $+10.8$ to -3.0 kcal/mole, which mainly reflects the stabilization of the products when the amino group is protonized.

Sturtevant and co-workers have determined heats of hydrolysis for various peptides, involving splitting of lysine-lysine, tyrosine-glycine, glycine-leucine, glycine-phenylalanine, and benzoic acid-tyrosine bonds [20]. Their results, referring to the fully charged state, vary from -1.2 to -2.6 kcal/mole. The value for butyl acetamide is thus slightly more negative. In the comparison it should, however, be noted that the heat of ionization of butylammonium ion is 3—4 kcal/mole higher than those of the ammonium groups of the amino acids in Ref.[20]. Furthermore, heats of ionization of the carboxyl groups of amino acids and peptides are in the order of 0.5 to 1 kcal/mole **, compared to practically zero for acetic acid and benzoic acid [9]. This means that if comparisons are made for the hypothetical case where no charged products are formed, the heats of

* Here, resonance energy of a compound refers to the difference between the experimental heat of formation and that calculated by adding together thermochemical bond energies.
** See *e.g.* Ref.[21].

hydrolysis of the peptides in Ref.[20] are 2—3 kcal/mole less endothermal than that of butyl acetamide.

The experimental material is not extensive enough to draw any definite conclusions regarding the relationship between heats of hydrolysis and structure. Differences in steric interaction between groups in the vicinity of the peptide bond, however, might account for part of the discrepancy. Another factor, intimately coupled to the steric effects, are differences in solvation energies. When gas phase data and data from water solution were compared it was found that the system (BuNHAc + H_2O)aq was stabilized by an amount of *ca.* 5 kcal/mole more than that of ($BuNH_2$ + HOAc)aq. It is thus obvious that the enthalpy terms involved are very substantial. It seems probable that the open structure of butyl acetamide permits a more extensive solvation of the peptide group than those of the amino acid peptides.

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