

## Heats of Hydrolysis of N-Acetylated Imidazole, 1,2,4-Triazole and Tetrazole

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The heats of hydrolysis of N-acetylated imidazole, 1,2,4-triazole and tetrazole have been determined calorimetrically at 25°C. For the idealized hydrolysis reaction



the enthalpy changes were found to be:  $-4.83 \pm 0.05$  kcal/mole for RNH = imidazole,  $-7.29 \pm 0.06$  kcal/mole for RNH = 1,2,4-triazole and  $-10.31 \pm 0.09$  kcal/mole for RNH = tetrazole.

Heats of solution in acetone have been determined for the compounds participating in the hydrolysis reaction. From the heats of solution obtained, heats of hydrolysis referring to the reaction  $\text{RNAc(acetone)} + \text{H}_2\text{O(acetone)} \rightarrow \text{RNH(acetone)} + \text{HOAc(acetone)}$  have been calculated to be:  $-7.52 \pm 0.07$  kcal/mole for R = imidazole,  $-9.03 \pm 0.07$  kcal/mole for R = 1,2,4-triazole and  $-12.35 \pm 0.10$  kcal/mole for R = tetrazole.

Free energy changes in transacetylation from tetrazole  $\rightarrow$  1,2,4-triazole  $\rightarrow$  imidazole, have been briefly discussed.

During recent years a considerable amount of evidence has accumulated, that the imidazole ring of histidine forms an essential part of the active centre of some hydrolytic enzymes\*\*. These hydrolysis processes in which esters or amides are involved, have been considered as two-step reactions. In the first step an imidazole group in the enzyme is believed to be acylated, after which follows a rapid hydrolysis. The kinetics of these reactions as studied on model substances have been extensively investigated. However, very little attention has been paid to the energetic relationships between substrate, enzyme intermediate, and the products. Therefore, in connection with earlier calorimetric work on acetylated compounds<sup>2-5</sup>, it was felt desirable to determine the heat of hydrolysis of N-acetyl imidazole as well as that of N-acetyl 1,2,4-triazole and N-acetyl tetrazole.

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\*\* See, e.g., the reference list given in Ref.<sup>1</sup>

The compounds participating in the idealized hydrolysis reaction V are in condensed states, being solids and liquids. The derived standard heats of hydrolysis, therefore, are not closely comparable. As heats of vaporisation data are not available, heats of solution in acetone, which is believed to be an inert solvent for these compounds have been determined.

Some years ago the kinetics of the hydrolysis of these N-acetyl compounds were studied by Staab <sup>6</sup>, which investigations will be supplemented by some enthalpy data given in this paper.

### EXPERIMENTAL

*Materials.* Imidazole was prepared from D-tartaric acid and hexamethylenetetramine<sup>7</sup>, and the crude product was recrystallized from benzene. After sublimation colourless crystals, melting at 88.7–89.4°C, were obtained. Imidazole was acetylated by acetyl chloride in benzene solution<sup>8</sup>. After removing the solvent in vacuum, the colourless crystalline residue was recrystallised from a benzene-ligroin mixture (1:1) and the product obtained was washed with petrol ether (boiling range 40–60°C) and dried in vacuum. After sublimation the melting point of the ester was 103.0–104.0°C. Potentiometric titration on the acetic acid formed by hydrolysis, gave an equivalent weight corresponding to a purity of 99.7 %.

1,2,4-Triazole was prepared from formamide and hydrazine hydrochloride<sup>8</sup>, and the crude product was recrystallized from ethyl acetate. After sublimation colourless crystals melting at 120.2–120.9°C were obtained.

Acetylation of the triazole by acetyl chloride was performed in benzene solution<sup>8</sup>. After removing the solvent in vacuum, a white crystalline residue melting at about 37°C was obtained. In the further purification use was made of the exceptionally high vapour pressure of N-acetyl 1,2,4-triazole. The crystalline mass was put in a glass bulb fitted with a 30 cm tube. The tube was connected to a vacuum system for *ca* 10 min, after which it was sealed off. The bulb was placed on a sand bath maintained at a temperature slightly below the melting point of the substance, with the tube in a vertical position and after a week, most of the substance had sublimed into the tube, where it formed big colourless crystals. The process was repeated, after which the melting point was 39.7–40.7°C. Potentiometric titration indicated an equivalent weight equal to the theoretical.

Tetrazole was prepared from cyanogen and hydrazoic acid<sup>9</sup> and the crude product was sublimed to give white crystals melting at 153.5–155.0°C. Potentiometric titration indicated the purity to be 99.5 %.

N-Acetyl-tetrazole was prepared from the silver salt of tetrazole by reaction with acetyl chloride in benzene solution<sup>6</sup>. The light yellow product was recrystallised from a benzene-ligroin (8:2) solution, to give colourless needles. After sublimation the substance melted at 58.0–59.0°C. Equivalent weight, obtained by potentiometric titration with sodium hydroxide in aqueous solution, corresponded to a purity of 99.6 %.

Acetone used in the solution experiments was treated with potassium permanganate<sup>10</sup>, dried with Drierite and distilled through a 10-plate column.

The heterocyclic acetates are rapidly hydrolyzed in the air and thus special precautions had to be taken. After the final sublimation, all handling of them, including the filling into ampoules for the calorimetric experiments, was carried out in a "dry air box" fitted with rubber gloves.

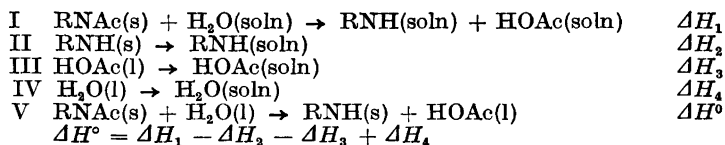
*Apparatus.* The reactions were carried out in a metal "isothermal" calorimeter, which has been described in detail elsewhere (Type D in Ref.<sup>11</sup>).

*Calibration.* The heat equivalent of the calorimeter, including its content, was determined electrically by passing a known current for a given time (300 sec) through the heating element. The calibrations were performed on the system after the reaction had taken place. The result of each calibration experiment was adjusted to give the heat equivalent of a "standard system",  $\epsilon^\circ$ , in which the calorimetric liquid contained the reaction products from a certain amount of substance. The actual heat equivalent values,  $\epsilon$ , were obtained from  $\epsilon^\circ$ , by applying a small correction for the variations in the amount of substance used.

*Calorimetric procedure.* The calorimeter was charged with 100 ml of liquid, either 0.8 N aqueous sodium hydroxide solution or acetone. The heats of hydrolysis measurements were supplemented by determination of heats of solution in order to obtain the standard enthalpy changes of the hydrolysis reactions.

All reactions were very rapid, the reaction period was 5 min, and the initial and final thermistor resistance values could therefore be graphically evaluated.

*Corrections to the standard states:* The idealized, isothermal hydrolysis reaction V is obtained from eqns I–IV, which correspond to the reactions taking place under experimental conditions.



*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. The molecular weights were computed from the 1951 table of international atomic weights<sup>12</sup>.

## RESULTS

The experimental results are summarised in Tables 1–4. In the tables the following symbols have been used:

- $\log R_i/R_f$  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.
- $\epsilon$  the heat equivalent of the actual system in calories per unit of  $\log R_i/R_f$ .

### Heats of hydrolysis

From five calibration experiments the standard heat equivalent (referring to 200 mg of substance) was calculated to be  $9\,071 \pm 5^*$  cal/unit of  $\log R_i/R_f$ . Results from heats of hydrolysis measurements are given in Table 1. The amount of N-acetyl imidazole and -tetrazole participating in the reactions was corrected with regard to the analysis performed.

Results from the heats of solution measurements performed in 0.8 N aqueous solution of sodium hydroxide are given in Table 2.

Table 3 contains a summary of data connected with eqns I–V. The uncertainties given in Table 3 do not include possible systematic errors. As the accuracy of the titrations, by which the purity of the N-acetyl compounds and acetic acid was tested, cannot be considered to be better than  $\pm 0.2\%$ , the true uncertainties are somewhat greater. Taking this into consideration, the standard enthalpy changes will be taken as  $-4.83 \pm 0.05$ ,  $-7.29 \pm 0.06$  and  $-10.31 \pm 0.09$  kcal/mole for the hydrolysis of N-acetylated imidazole, 1,2,4-triazole and tetrazole, respectively.

\* When five or more determinations are performed, the uncertainties are given as the standard deviation of the mean. Otherwise they are the average deviation of the mean.

Table 1.

RNac	mmoles	$\epsilon$	$10^4 \times \log R_i/R_f$	$-\Delta H$ , kcal/mole
N-Acetyl imidazole	2.748	9 075	45.47	15.02
	2.982	9 076	49.29	15.00
	2.318	9 073	38.27	14.98
	2.148	9 073	35.49	14.99
			Mean	$15.00 \pm 0.01$
N-Acetyl 1,2,4-triazole	2.826	9 075	67.65	21.72
	2.742	9 075	65.68	21.74
	3.267	9 076	78.27	21.74
	3.215	9 076	76.86	21.70
			Mean	$21.73 \pm 0.02$
N-Acetyl tetrazole	2.724	9 071	87.52	29.14
	2.151	9 073	69.14	29.16
	2.202	9 073	70.99	29.25
	2.371	9 074	76.19	29.16
			Mean	$29.18 \pm 0.04$

Table 2.

Substance	mmoles	$\epsilon$	$10^4 \times \log R_i/R_f$	$-\Delta H$ , kcal/mole
Imidazole	3.012	9 071	-11.44	-3.45
	2.557	9 070	-9.73	-3.45
	2.654	9 070	-10.06	-3.44
			Mean	$-3.45 \pm 0.01$
1,2,4-Triazole	3.034	9 071	2.81	0.84
	2.909	9 071	2.62	0.82
	2.713	9 071	2.43	0.81
			Mean	$0.82 \pm 0.01$
Tetrazole	2.149	9 069	12.54	5.29
	2.315	9 069	13.38	5.24
	2.822	9 071	16.28	5.23
			Mean	$5.25 \pm 0.02$
Acetic acid	2.988	9 074	44.83	13.61
	3.173	9 071	47.55	13.59
	2.233	9 068	33.80	13.73
	2.650	9 069	39.86	13.64
	3.744	9 072	56.41	13.67
				13.61
			Mean	$13.64 \pm 0.02$
Water	2.733	9 067	0.07	0.02
	3.695	9 067	0.10	0.02
			Mean	$0.02 \pm 0.00$

Table 3.

RNH	$-\Delta H_1$	$-\Delta H_2$	$-\Delta H_3$	$-\Delta H_4$	$-\Delta H^\circ$
Imidazole	15.00	-3.45	13.64	0.02	$4.83 \pm 0.03$
1,2,4-Triazole	21.73	0.82	13.64	0.02	$7.29 \pm 0.03$
Tetrazole	29.18	5.25	13.64	0.02	$10.31 \pm 0.05$

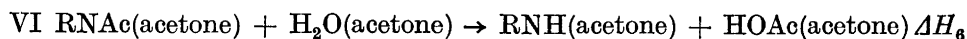
## Heats of hydrolysis in acetone

From three calibration experiments the heat equivalent (variations in the heat capacity of the system were insignificant for the results) was calculated to be  $4\,268 \pm 11$  cal/unit of  $\log R_i/R_f$ . The heats of solution measurements are summarised in Table 4. By combining these heats of solution data with

Table 4

Substance	mmoles	$10^4 \times \log R_i/R_f$	$-\Delta H$ , kcal/mole
Imidazole	2.618	-24.59	-4.01
	1.980	-18.82	-4.06
	1.867	-17.72	-4.05
	Mean		$-4.04 \pm 0.02$
1,2,4-Triazole	1.979	-18.24	-3.93
	2.349	-21.56	-3.92
	Mean		$-3.93 \pm 0.01$
Tetrazole	2.304	-16.28	-3.02
	1.492	-10.64	-3.04
	1.805	-12.89	-3.05
	Mean		$-3.04 \pm 0.01$
N-Acetyl imidazole	2.528	-32.67	-5.51
	0.909	-11.90	-5.59
	3.123	-40.10	-5.48
	Mean		$-5.53 \pm 0.04$
N-Acetyl 1,2,4-triazole	2.398	-25.02	-4.45
	2.634	-27.65	-4.48
	Mean		$-4.47 \pm 0.02$
N-Acetyl tetrazole	1.520	-13.75	-3.86
	1.250	-11.39	-3.89
	Mean		$-3.88 \pm 0.02$
Acetic acid	3.252	1.77	0.23
	1.788	0.66	0.16
	2.833	1.03	0.16
	Mean		$0.18 \pm 0.03$
Water	3.331	- 7.96	-1.02
	3.423	- 8.13	-1.01
	Mean		$-1.02 \pm 0.01$

the given standard heat of hydrolysis, enthalpy changes referring to reaction VI were obtained.



$\Delta H_6$  was calculated to be  $-7.52 \pm 0.07$  kcal/mole,  $-9.03 \pm 0.07$  kcal/mole and  $-12.35 \pm 0.10$  kcal/mole for RNH = imidazole, 1,2,4-triazole and tetrazole, respectively.

## DISCUSSION

From the heats of hydrolysis data referring to reaction VI, the heats of transacetylation, in acetone solution, from N-acetyl-tetrazole to 1,2,4-triazole, and from N-acetyl 1,2,4-triazole to imidazole, are calculated to be  $-3.32 \pm 0.12$  kcal/mole and  $-1.51 \pm 0.10$  kcal/mole, respectively. Considering the symmetry of these transacetylation reactions and the absence of phase changes, the entropy changes must be very small. The standard free energy changes, therefore, should be almost the same as the corresponding enthalpy changes. The given data thus indicate, that from an energetic point of view, the transacetylation reactions are favourable only in the direction N-acetyl-tetrazole  $\rightarrow$  1,2,4-triazole  $\rightarrow$  imidazole. This conclusion is in agreement with the observations made by Staab <sup>6</sup>. Some heats of aminolysis of the N-acetylated compounds investigated here have also been determined. They will be presented in connection with other aminolysis data in a forthcoming paper.

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