# The Heats of Hydrolysis of Aspirin, Thioaspirin, and Their *p*-Analogues

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The heats of hydrolysis of *o*-acetylhydroxybenzoic acid (aspirin), *p*-acetylhydroxybenzoic acid, *o*-acetylmercaptobenzoic acid (thioaspirin) and *p*-acetylmercaptobenzoic acid have been determined calorimetrically at 25°C. For the idealized, isothermal hydrolysis reactions

$$(o,p)HOCOC_6H_4XAc(s) + H_2O(l) \rightarrow$$
$$\rightarrow (o,p)HOCOC_6H_4XH(s) + HOAc \quad (1)$$
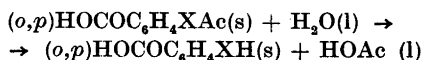
the enthalpy changes were found to be

-6.50 ± 0.07 kcal/mole	for X = O	(*o*)
-4.93 ± 0.08 ,,	,, X = O	(*p*)
-5.85 ± 0.06 ,,	,, X = S	(*o*)
-2.75 ± 0.09 ,,	,, X = S	(*p*)

By heat of solution measurements, the enthalpy changes for the hypothetical hydrolysis reactions in aqueous solution (ionic strength = 0.1)

$$(o,p)^-OCOC_6H_4XAc(aq) + H_2O(aq) \rightarrow (o,p)^-OCOC_6H_4XH(aq) +$$
$$+ OAc^-(aq) + H^+(aq)$$
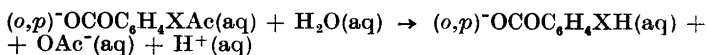
were found to be

-4.43 ± 0.12 kcal/mole	for X = O	(*o*)
-4.95 ± 0.16 ,,	,, X = O	(*p*)
-3.52 ± 0.15 ,,	,, X = S	(*o*)

and for the hypothetical hydrolysis reactions in aqueous solution (ionic strength = 0.1) to the ionized species $^-OCOC_6H_4S^-(aq)$

$+2.20 \pm 0.15$ kcal/mole	(*o*)
$+1.13 \pm 0.15$,,	(*p*)

Heats of ionization (ionic strength = 0.1) have been determined for acetic acid, $\Delta H_i = +0.09 \pm 0.05$ kcal/mole, and for the protonated form of tris(hydroxymethyl)aminomethane (THAM), $\Delta H_i = +11.36 \pm 0.04$ kcal/mole.

This work is part of a systematic study on heats of hydrolysis of *i.a.* O-and S-esters with special reference to their biochemical importance. Earlier, heats of hydrolysis of simple aliphatic O- and S-acetates [1,2,3] as well as of phenylacetate and phenyl thiolacetate [4] have been investigated.

Thioacyl compounds are reactive intermediates in several enzymatic processes; the most studied are the acylderivatives of coenzyme A and their role in acyl transfer processes. Thioacyl compounds are also believed to be reactive intermediates in the enzymatic hydrolysis of proteins by papain and ficin, see *e.g.* [5,6] It has been postulated that the active centers of these enzymes contain a mercaptogroup and a carboxylate ion in close proximity,[7-9] and so thiosalicylic acid can be regarded as a modelsubstance for them.[10] It was therefore felt desirable to determine the heat of hydrolysis of thioaspirin and, as a reference material, of its *p*-isomer. As a comparison, the heats of hydrolysis of the oxygen analogues, aspirin and *p*-acetylhydroxybenzoic acid, have also been determined.

EXPERIMENTAL

Materials

Aspirin, pharmaceutical grade, was purified by recrystallization from alcohol-water,[11] until the equivalent weight, as determined by acidimetric titration after alkaline hydrolysis, was found to be 90.03 Theoretical 90.08. M.p. 131.2° — 131.8°.

p-Acetylhydroxybenzoic acid was prepared from *p*-hydroxybenzoic acid and acetic anhydride according to the method described for aspirin.[11] The substance was purified by recrystallization, first from a solution of equal volumes of water and acetic acid,[11] and then from pure water. The equivalent weight was determined in the same manner as for aspirin and was found to be 90.20. Theoretical 90.08. M.p. 188.7° — 189.4°.

Thioaspirin was prepared from thiosalicylic acid and acetyl chloride.[12] The substance was purified by recrystallization from benzene until the equivalent weight as determined by acidimetric titration in water-ethanol was found to be 196.21. Theoretical 196.23. M.p. 125.5° — 126.0°.

p-Acetylmercaptobenzoic acid was prepared from *p*-mercaptobenzoic acid and acetic anhydride [13] and was purified by recrystallization from a chloroform-benzene solution and then by sublimation. The equivalent weight was determined in the same manner as for thioaspirin and was found to be 196.38. Theoretical 196.23. M.p. 202.8° — 203.6°.

Salicylic acid, Baker Analyzed Reagent, was purified by recrystallization from water until the equivalent weight as determined by acidimetric titration in water-ethanol was found to be 138.16. Theoretical 138.13. M.p. 157.4° — 158.0°

p-Hydroxybenzoic acid, Kebo *purum,* was purified by recrystallization from water until the equivalent weight, as determined in the same manner as for salicylic acid, was found to be 138.22. Theoretical 138.13. M.p. 215.8° — 216.4°.

Thiosalicylic acid exists in two crystalline forms [12] namely a colourless α-form and a yellow β-form.

β-Thiosalicylic acid, Fluka puriss *p.a.,* was purified by recrystallization from water-ethanol. The equivalent weight as determined by acidimetric titration in water-ethanol was found to be 154.29, and as determined by iodometric titration in water-ethanol 154.23. Theoretical 154.19. M.p. 166.3° — 167.3°.

α-Thiosalicylic acid was obtained by sublimating β-thiosalicylic acid in *vacuo.* The equivalent weight was determined in the same manner as for β-thiosalicylic acid: by acidimetric titration it was found to be 154.28, and by iodometric 154.18 Theoretical 154.19. M.p. 166.6° — 167.2°.

p-Mercaptobenzoic acid was prepared from *p*-aminobenzoic acid according to the method described for thiosalicylic acid.[14] The substance was purified by recrystallization from water-ethanol and by sublimation. The equivalent weight was determined in the

same manner as for thiosalicylic acid; by acidimetric titration it was found to be 77.44. Theoretical 77.09. By iodometric titration 154.74. Theoretical 154.19. M.p. 221.6° − − 222.4°.

Tris(hydroxymethyl)aminomethane (THAM), "Sigma 7−9", was recrystallized twice from 96 % ethanol and dried at 80° over night.

Calorimetric measurements

Apparatus. The measurements were performed with an "isothermal-jacket" glass calorimeter.[15],[16]

Calibration. The heat equivalent of the calorimeter, including its content, was determined electrically by passing a known current for a given time (5−7 min) through the heating element. The calibrations were performed on the system after reaction had taken place. The initial and final thermistor resistances (R_i and R_f respectively) were obtained graphically.

Calorimetric procedure. The calorimeter was charged with 100 ml of liquid: The esters were hydrolyzed in a 0.8 N sodium hydroxide solution in water-ethanol (2:3). The hydrolysis experiments were supplemented by heats of solution measurements in the hydrolysis medium to arrive at enthalpy data for the standard state. The heats of solution of the two forms of thiosalicylic acid (see under Materials) were measured in 96 % ethanol. The hydrolysis experiments were further supplemented by heats of solution measurements in a solution of THAM in water, ionic strength 0.1, initial pH 8.05, and in a 0.1 M solution of sodium chloride in water to give data for the processes in water solution.

When the alkaline mixture of water and ethanol, pure ethanol or aqueous sodium chloride were used as calorimeter media the reactions were very rapid; the reaction period varied between 3 and 7 min and the initial and final thermistor resistances could be evaluated graphically. No auto-oxidation of the thiols formed was noticed, as was the case with aliphatic thiols.[1]

When the calorimeter liquid was the THAM-solution the reactions were rather slow except for acetic acid and hydrochloric acid. The reaction period varied between 10 and 30 min. Thermal leakages in these cases were calculated by the Regnault-Pfaundler method.[17] Small corrections were made for differences in stirring energy before and after the ampoules were broken.

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 1961 table of international atomic weights.

IR measurements

The infrared stretching frequencies of the acetyl carbonyl groups of aspirin, *p*-acetylhydroxybenzoic acid, thioaspirin and *p*-acetylmercaptobenzoic acid have been measured in deuterium oxide solution at the Infrared Laboratory, Department of Chemistry, University of Lund. Spectra were obtained with a double-beam Perkin-Elmer model 221 spectrophotometer with sodium chloride prism. A calcium fluoride cell with a pathlength of 0.05 mm was used.

Solutions were prepared immediately before the spectra were taken. The substance and a stoichiometric amount of sodiumbicarbonate (in order to get the carboxyl group ionized) were dissolved in deuterium oxide. It was, however, impossible to get *p*-acetylmercaptobenzoic acid into solution in this way, owing to its low solubility. This substance was therefore titrated with sodium hydroxide, evaporated to dryness, and then dissolved in deuterium oxide.

The peaks were weak, owing to the low concentration of the substances, less than 0.1 M. This was particularly the case for *p*-acetylmercaptobenzoic acid where the uncertainty in the determination of the frequency was estimated to be $\pm$ 5 cm⁻¹.

RESULTS

1. Heats of hydrolysis for the hypothetical process

RXAc(s) + H_2O(l) → RXH(s) + HOAc(l) at 25°C where
R = (o,p) $HOCOC_6H_4$- and X = O or S
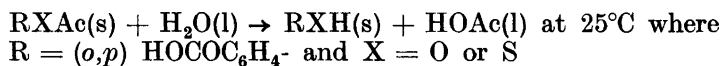
The esters were hydrolyzed in 0.8 N sodium hydroxide solution in water ethanol
(2:3). In order to get the heats of hydrolysis for the reaction above, heats of
solution of the phenols/thiols, acetic acid and water had to be measured in the
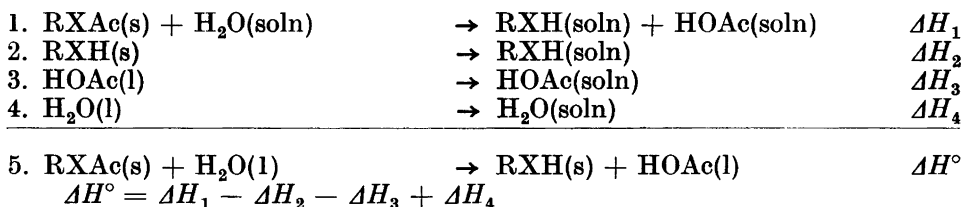same liquid.

The results of the calorimetric measurements are summarized in Tables
1 and 2. When five or more determinations were performed, the uncertainties
are given as the standard deviation of the mean. Otherwise, they are the
average deviation of the mean.

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 end, respec-
tively, of the main period.

ε = the heat equivalent of the actual system in calories per unit of $\log R_i/R_f$.
q = the amount of heat evolved during the experiment.

Corrections to standard states. The ideal isothermal reaction 5 is obtained
from eqns. 1 – 4, corresponding to the reactions taking place under actual
experimental conditions.

1. RXAc(s) + H_2O(soln)	→ RXH(soln) + HOAc(soln)	ΔH_1
2. RXH(s)	→ RXH(soln)	ΔH_2
3. HOAc(l)	→ HOAc(soln)	ΔH_3
4. H_2O(l)	→ H_2O(soln)	ΔH_4

5. RXAc(s) + H_2O(l)　　　　　　→ RXH(s) + HOAc(l)　　　　$\Delta H°$
　　$\Delta H° = \Delta H_1 - \Delta H_2 - \Delta H_3 + \Delta H_4$

Heats of solution in ethanol of the two forms of thiosalicylic acid were
determined. It may be seen from Table 2 that the heat of solution of the α-
modification is 0.42 kcal/mole more endotherm than for the β-modification.
This value is equal to the difference in lattice energy. The heat of solution in the
alkaline water-ethanol solution of α-thiosalicylic acid is thus −10.85 kcal/mole
(−11.27 + 0.42). This latter value has been used in the calculations of the
standard heat of hydrolysis. Thus $\Delta H°$ for thioaspirin refers to the equation

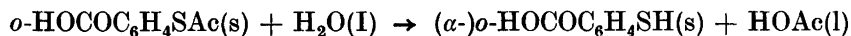o-$HOCOC_6H_4SAc$(s) + H_2O(I) → (α-)o-$HOCOC_6H_4SH$(s) + HOAc(l)

Table 3 contains a summary of the observed data and the heats of hydro-
lysis of the idealized reaction 5. The uncertainties assigned for the $\Delta H°$-values
include possible systematic errors in the analyses and in the ε-values. All
enthalpy values are expressed in kcal/mole.

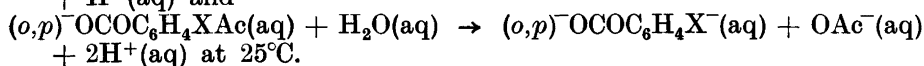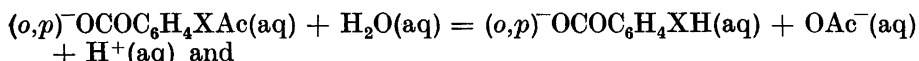Table 1. Heats of hydrolysis and heats of solution in 0.8 N sodium hydroxide solution in water-ethanol (2:3).

Substance	mmoles	$\varepsilon = 6156 \pm 4$	$-\Delta H$ kcal/mole
		$10^4 \times \log R_i/R_f$	
o-HOCOC$_6$H$_4$OAc	2.029	83.94	25.47
	2.393	98.90	25.44
	2.357	96.91	25.31
	2.503	103.25	25.39
	2.524	103.86	25.33
		Mean	25.39 $\pm$ 0.03
p-HOCOC$_6$H$_4$OAc	1.358	67.09	30.42
	1.551	75.90	30.13
	1.618	79.54	30.27
	1.533	75.38	30.28
	1.436	70.71	30.31
	1.346	66.26	30.30
		Mean	30.29 $\pm$ 0.04
o-HOCOC$_6$H$_4$SAc	1.886	85.96	28.05
	1.983	90.43	28.07
	2.028	92.93	28.21
	1.999	91.50	28.18
	2.210	100.88	28.10
		Mean	28.12 $\pm$ 0.03
p-HOCOC$_6$H$_4$SAc	1.816	87.23	29.56
	1.096	91.71	29.62
	1.057	50.96	29.68
	0.990	47.51	29.53
	1.001	48.12	29.60
		Mean	29.60 $\pm$ 0.03
o-HOCOC$_6$H$_4$OH	2.607	31.03	7.33
	2.642	31.90	7.43
	2.419	29.53	7.52
	2.138	25.99	7.48
	1.728	21.31	7.59
		Mean	7.47 $\pm$ 0.04
p-HOCOC$_6$H$_4$OH	2.521	57.27	13.98
	2.518	56.51	13.81
	2.465	56.16	14.02
	1.454	32.82	13.90
	1.546	35.10	13.98
		Mean	13.94 $\pm$ 0 04
β-o-HOCOC$_6$H$_4$SH	1.914	34.96	11.24
	2.120	38.67	11.23
	2.075	38.23	11.34
	2.187	40.02	11.26
	2.000	36.59	11.26
		Mean	11.27 $\pm$ 0.02
p-HOCOC$_6$H$_4$SH	1.256*	31.34	15.36
	1.182	29.86	15.54
	1.075	26.93	15.42
	1.524	38.14	15.40
		Mean	15.43 $\pm$ 0.06

* Corrected to 100 % purity.

Table 2 Heats of solution in 96 % EtOH.

Substance	mmoles	$\varepsilon = 4159 \pm 5$ $10^4 \times \log R_i/R_f$	ΔH kcal/mole
α-o-HOCOC$_6$H$_4$SH	2.071	-24.14	4.85
	1.907	-22.49	4.91
	1.347	-16.19	5.00
	2.006	-23.73	4.92
	2.057	-24.40	4.93
	2.015	-23.61	4.87
		Mean	4.91 ± 0.02
β-o-HOCOC$_6$H$_4$SH	1.966	-21.11	4.47
	2.078	-22.50	4.50
	2.094	-22.61	4.49
	1.953	-21.07	4.49
	2.224	-24.20	4.52
		Mean	4.49 ± 0.01

2. Heats of hydrolysis for the hypothetical reactions:

$(o,p)^-$OCOC$_6$H$_4$XAc(aq) + H$_2$O(aq) = $(o,p)^-$OCOC$_6$H$_4$XH(aq) + OAc$^-$(aq) + H$^+$(aq) and

$(o,p)^-$OCOC$_6$H$_4$XAc(aq) + H$_2$O(aq) $\rightarrow$ $(o,p)^-$OCOC$_6$H$_4$X$^-$(aq) + OAc$^-$(aq) + 2H$^+$(aq) at 25°C.
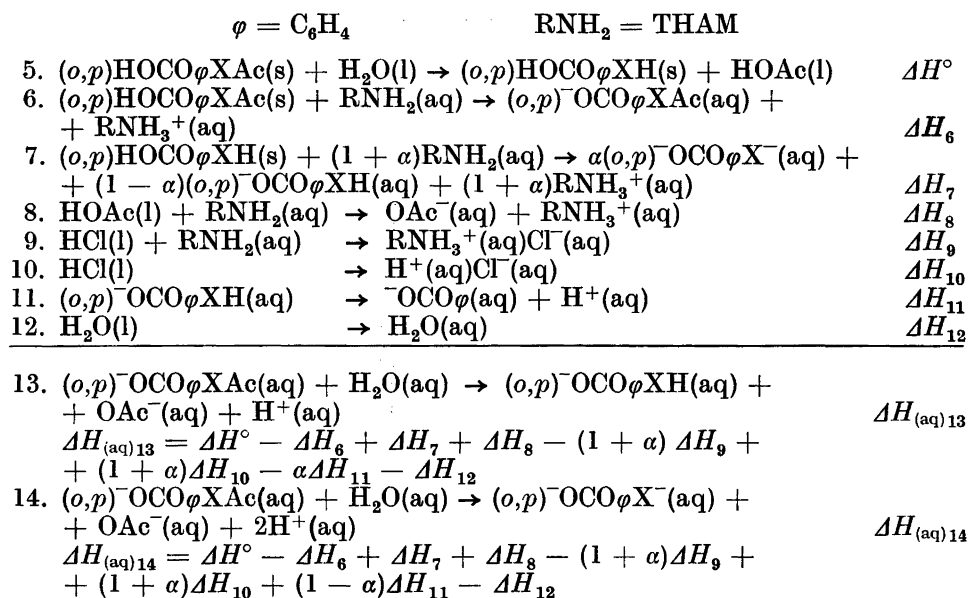
In order to obtain the heats of hydrolysis values for the reactions above, heats of solution in aqueous media had to be measured for all the components listed below in the reaction schemes. As the esters and the corresponding phenols and thiols are only slightly soluble in pure water, a calorimetric liquid had to be chosen where the pH was sufficiently high to allow the carboxylate groups to be ionized and hence the substances to get into solution, but not so high as to cause hydrolysis of the esters. To fullfil these requirements, a buffer solution had to be used. THAM seemed to be the best choice, as the pK of its protonated form is 8.07.[18] The calorimetric measurements are summarized in Tables 4 and 5.

Table 3. Summary of experimental data and values of heats of hydrolysis of the idealized reaction

RXAc(s) + H$_2$O(l) $\rightarrow$ RXH(s) + HOAc(l)

RX-	$-\Delta H_1$	$-\Delta H_2$	$-\Delta H_3$	$-\Delta H_4$	$-\Delta H°$
o-HOCOC$_6$H$_4$O-	25.39	7.47	11.62*	0.20*	6.50 ± 0.07
p-HOCOC$_6$H$_4$O-	30.29	13.94	,,	,,	4.93 ± 0.08
o-HOCOC$_6$H$_4$S-	28.12	10.85	,,	,,	5.85 ± 0.06
p-HOCOC$_6$H$_4$S-	29.60	15.43	,,	,,	2.75 ± 0.09

* Ref.[4]

Enthalpy changes for the hydrolysis reactions in aqueous solution eqns. 13 and 14) are derived according to the reaction scheme below:

$$\varphi = C_6H_4 \qquad\qquad RNH_2 = THAM$$

5. $(o,p)HOCO\varphi XAc(s) + H_2O(l) \rightarrow (o,p)HOCO\varphi XH(s) + HOAc(l)$ $\Delta H°$

6. $(o,p)HOCO\varphi XAc(s) + RNH_2(aq) \rightarrow (o,p)^-OCO\varphi XAc(aq) +$
$+ RNH_3^+(aq)$ ΔH_6

7. $(o,p)HOCO\varphi XH(s) + (1 + \alpha)RNH_2(aq) \rightarrow \alpha(o,p)^-OCO\varphi X^-(aq) +$
$+ (1 - \alpha)(o,p)^-OCO\varphi XH(aq) + (1 + \alpha)RNH_3^+(aq)$ ΔH_7

8. $HOAc(l) + RNH_2(aq) \rightarrow OAc^-(aq) + RNH_3^+(aq)$ ΔH_8

9. $HCl(l) + RNH_2(aq) \rightarrow RNH_3^+(aq)Cl^-(aq)$ ΔH_9

10. $HCl(l) \rightarrow H^+(aq)Cl^-(aq)$ ΔH_{10}

11. $(o,p)^-OCO\varphi XH(aq) \rightarrow {}^-OCO\varphi(aq) + H^+(aq)$ ΔH_{11}

12. $H_2O(l) \rightarrow H_2O(aq)$ ΔH_{12}

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13. $(o,p)^-OCO\varphi XAc(aq) + H_2O(aq) \rightarrow (o,p)^-OCO\varphi XH(aq) +$
$+ OAc^-(aq) + H^+(aq)$ $\Delta H_{(aq)13}$
$\Delta H_{(aq)13} = \Delta H° - \Delta H_6 + \Delta H_7 + \Delta H_8 - (1 + \alpha)\,\Delta H_9 +$
$+ (1 + \alpha)\Delta H_{10} - \alpha\Delta H_{11} - \Delta H_{12}$

14. $(o,p)^-OCO\varphi XAc(aq) + H_2O(aq) \rightarrow (o,p)^-OCO\varphi X^-(aq) +$
$+ OAc^-(aq) + 2H^+(aq)$ $\Delta H_{(aq)14}$
$\Delta H_{(aq)14} = \Delta H° - \Delta H_6 + \Delta H_7 + \Delta H_8 - (1 + \alpha)\Delta H_9 +$
$+ (1 + \alpha)\Delta H_{10} + (1 - \alpha)\Delta H_{11} - \Delta H_{12}$

In Table 6 are summarized data for reactions 5—14. ΔH_{11} is $+8.51 \pm 0.08$ kcal/mole for salicylic acid [28]

    $+ 3.4 \pm 1$ kcal/mole for p-hydroxybenzoic acid [19]
    $+ 5.72 \pm 0.08$ kcal/mole for thiosalicylic acid [20]

ΔH_{11} could not be measured calorimetrically for p-mercaptobenzoic acid [20] but was believed to be very low, around zero. It is therefore not possible to give a reliable value for the heat of hydrolysis for p-acetylmercaptobenzoic acid referring to eqn. 13, but it is estimated bo be around zero. As, however, p-mercaptobenzoic acid is almost completely ionized in the calorimetric measurement (the degree of ionization of $p\text{-}^-OCOC_6H_4SH$ is 0.996) it is possible to calculate the heat of hydrolysis referring to eqn. 14.

ΔH_{12} is taken equal to zero.[21] The uncertainties assigned for the ΔH_{aq}-values include possible systematic errors in the analyses and in the ε-values. All enthalpy values are expressed in kcal/mole.

3. Heats of ionization.

From the heats of solution of aqueous hydrochloric acid, 1.690 mmole/ g in the aqueous THAM solution ($\Delta H = -11.89 \pm 0.04$ kcal/mole) and in 0.1 M sodium chloride ($\Delta H = -0.53 \pm 0.01$ kcal/mole) the heat of ionization of the protonated form of THAM is calculated to be $+ 11.36 \pm 0.04$ kcal/ mole (ionic strength 0.1) This value should be compared with the value obtained calorimetrically at ionic strength 0.013, $+ 10.93 \pm 0.10$ kcal/mole[22]

Table 4. Heats of solution in the aqueous THAM solution, ionic strength 0.1
Initial pH 8.05

Substance	mmoles	$10^4 \times \log R_i/R_f$	$-\Delta H$ kcal/mole	final pH	$\alpha =$ the degree of dissociation of $^-OCOC_6H_4XH$
		$\varepsilon = 8907 \pm 8$			
HCl	0.988	13.33	12.02		
	1.076	14.22	11.77		
	1.047	13.93	11.85		
	1.013	13.56	11.92		
	1.026	13.67	11.87		
			Mean 11.89 ± 0.04		
HOAc	1.501	19.44	11.53		
	1.579	20.56	11.59		
	1.365	17.76	11.59		
	1.402	18.31	11.64		
	1.817	23.78	11.65		
			Mean 11.60 ± 0.02		
o-HOCOC$_4$H$_6$OAc	1.007	6.42	5.68		
	1.063	6.93	5.81		
	0.991	6.22	5.60		
	0.977	6.36	5.80		
			Mean 5.72 ± 0.08		
p-HOCOC$_6$H$_4$OAc	0.991	5.29	4.75		
	1.040	5.62	4.81		
	1.036	5.41	4.65		
			Mean 4.74 ± 0.06		
o-HOCOC$_6$H$_4$SAc	1.013	6.88	6.05		
	0.999	6.79	6.05		
	1.001	7.08	6.30		
	0.967	7.00	6.44		
	0.968	7.00	6.44		
	0.956	6.57	6.12		
			Mean 6.23 ± 0.07		
p-HOCOC$_6$H$_4$SAc	1.002	3.05	2.71		
	0.997	2.86	2.55		
	0.947	2.81	2.65		
			Mean 2.64 ± 0.06		
o-HOCOC$_6$H$_4$OH	1.028	3.93	3.41		
	1.029	3.96	3.42		
	1.045	3.98	3.39		
			Mean 3.41 ± 0.01		
p-HOCOC$_6$H$_4$OH	0.996	5.52	4.94	7.95	0.050[a]
	1.033	5.76	4.97	7.96	0.051
	1.033	5.65	4.87	8.00	0.056
			Mean 4.93 ± 0.04		Mean 0.052 ± 0.002

Table 4, continued.

α-*o*-HOCOC$_6$H$_4$SH	0.941	4.84	4.58	7.88	0.187[b]
	0.944	4.93	4.65	7.86	0.180
	0.999	5.40	4.81	7.85	0.177
	0.861	4.53	4.69	7.88	0.187
			Mean 4.68 ± 0.07		Mean 0.182±0.004
p-HOCOC$_6$H$_4$SH	0.984	10.76	9.73	7.86	0.996[c]
	0.963	10.62	9.82	7.85	0.996
	1.063	11.85	9.93	7.83	0.996
			Mean 9.83 ± 0.07		Mean 0.996±0

[a] pK_2' (ionic strength 0.1) = 9.11.[19]
[b] pK_2 = 8.88,[20] pK_2' (ionic strength 0.1) calculated to be 8.40.
[c] pK_2 = 5.80,[20] pK_2' (ionic strength 0.1) calculated to be 5.32.

Table 5. Heat of solution for aqueous hydrochloric acid, 1.690 mmoles/g in aqueous sodium chloride, ionic strength 0,1

	$\varepsilon = 8907 \pm 8$	
mmoles	$10^4 \times \log R_i/R_f$	$-\Delta H$ kcal/mole
1.045	0.613	0.523
0.943	0.559	0.528
1.101	0.649	0.525
		Mean 0.525 ± 0.002

and the values obtained at zero ionic strength by measuring the dissociation constant at various temperatures; +11.38 kcal/mole [18] and +11.33 kcal/mole.[23]

From the heats of solution of acetic acid in the aqueous THAM solution ($\Delta H = -11.60 \pm 0.02$ kcal/mole) and in a solution where no ionization occurs ($\Delta H = -0.33 \pm 0.01$ kcal/mole)[16] and from the value of the heat of ionization of the protonated form of THAM above, the heat of ionization of acetic acid is calculated to be +0.09 ± 0.05 kcal/mole (ionic strength 0.1). This value is in reasonable agreement with other recent calorimetric measurements; −0.07 ± 0.05 kcal/mole at zero ionic strength [24] and +0.18 ± 0.05 kcal/mole at ionic strength 0.2.[16]

Table 6. Summary of experimental data and values of heats of hydrolysis in aqueous solution.

Sub-ance	$\Delta H°$	ΔH_6	ΔH_7	ΔH_8	ΔH_9	ΔH_{10}	ΔH_{11}	α	$\Delta H_{aq,13}$	ΔH_{aq14}
					R = $^-$OCOC$_6$H$_4$					
OAc	−6.50	−5.72	−3.41	−11.60	−11.89	−0.53	+8.51	0	−4.43 ± 0.12	+4.08 ± 0.14
ROAc	−4.93	−4.74	−4.93	−11.60	−11.89	−0.53	+3.4	0.052	−4.95 ± 0.16	−1.6 ± 1
SAc	−5.85	−6.23	−4.68	−11.60	−11.89	−0.53	+5.72	0.182	−3.52 ± 0.15	+2.20 ± 0.15
RSAc	−2.75	−2.64	−9.83	−11.60	−11.89	−0.53	*ca.* 0	0.996	(0)	+1.13 ± 0.15

Table 7. IR carbonyl frequencies in deuterium oxide.

Substance	IR carbonyl frequency, cm⁻¹
$o\text{-}^-OCOC_6H_4OAc$	1730
$p\text{-}^-OCOC_6H_4OAc$	1730
$o\text{-}^-OCOC_6H_4SAc$	1675
$p\text{-}^-OCOC_6H_4SAc$	1675

4. IR measurements.

In Table 7 are given the infrared stretching frequencies of the acetyl carbonyl groups of aspirin, p-acetylhydroxybenzoic acid, thioaspirin and p-acetylmercaptobenzoic acid.

DISCUSSION

In order to enable a close comparison between different thermochemical data, it is necessary that they refer to comparable aggregation states, preferably the ideal gaseous state where there are no intermolecular interactions. For most of the compounds involved in this study, however, no vaporization data are as yet available. The thermochemical standard state data (eqn. 5) are not internally comparable as lattice energies can be expected to be considerably different for the corresponding reaction components. Nevertheless the ΔH° values are in a general agreement with those for the unsubstituted phenylacetate and phenyl thiolacetate, -6.86 and -2.97 kcal/mole, respectively.[4]

The results for the hydrolyses in aqueous solution (eqns. 13 and 14) are somewhat more favourable for a comparison. The enthalpy values for the hydrolysis reactions as carried out in aqueous solution can formally be looked upon as being composed of two effects: (1) breaking and formation of chemical bonds, and electronic and steric rearrangements, (2) solvation enthalpies. For the two O- and S-esters, respectively, there is no difference in chemical bond rearrangements. The ester carbonyl stretching frequencies in D_2O are equal for the two O-esters and at least very similar for the two S-esters. It thus seems that the resonance stabilization of the two isomeric forms of the O- and S-ester, respectively, are the same.[25]

Salicylic acid is a considerably stronger acid than its p-isomer ($K_{1,o}/K_{1,p}$ *ca.* 30) see, *e.g.* Ref.[26] This has been explained by Branch and Yabroff [26] as caused by an internal hydrogen bond formation in salicylic acid. The hydrogen bond should stabilize the o-hydroxybenzoate ion and thus the ΔH_{aq} value (the hydroxy group unionized) should be more exothermic for aspirin than for p-acetylhydroxybenzoic acid. This is in contrast to the experimental results obtained here, where ΔH_{aq} is found to be about 0.5 kcal/mole more exothermic for the p-isomer. This difference is suggested to be caused by differences in solvation enthalpies.

Scheraga *et al.*[19] calculated the heat of isomerization of the p- to the o-hydroxybenzoate ions to be -5.0 kcal/mole. They called this value the heat of

formation of the hydrogen bond in the o-hydroxybenzoate ion, but as it includes the difference of solvation enthalpies between the two isomeric ions, heat of isomerization seems to be a more adequate name. Using the value of Scheraga *et al.* and the values for the heats of hydrolysis in aqueous solution obtained here (the hydroxy groups unionized), the heat of isomerization for the *p*- and *o*-acetylhydroxybenzoate ions is calculated to be —5 to —6 kcal/mole.

A strong internal hydrogen bond is not expected for thiosalicylic acid, as work on hydrogen bonding of benzenethiol with serveral hydrogen acceptors [27] showed that the benzenethiol hydrogen bonding interaction is weak relative to its oxygen analogue. But if a sufficiently strong intramolecular hydrogen bond exists one would expect the ΔH_{aq} value (the mercapto groups unionized) for thioaspirin to be more exothermic than for *p*-acetylmercaptobenzoic acid, as was expected for the hydroxy esters. As the ΔH_{aq}-value (the mercapto group unionized) for *p*-acetylmercaptobenzoic acid is very uncertain (see under Results) nothing can be discussed in this case.

In the completely ionized thiosalicylic acid, however, no hydrogen bond exists and therefore the less endothermic ΔH_{aq}-value (the mercapto groups ionized) for *p*-acetylmercaptobenzoic acid compared with its *o*-isomer is believed to be caused by differences in solvation enthalpies.

Schonbaum and Bender [10] investigated the catalytic properties showed by thiosalicylic acid on the hydrolysis of *p*-nitrophenylacetate. Their experiments indicated that the catalytic properties were caused by the close orientation of the mercapto and carboxylate groups and it was shown that the S-acetate (thioaspirin) was formed as an intermediate. Smith [7] originally proposed a two-step process for papain catalysis with the formation of a thiol ester as an intermediate. This hypothesis has later been critized by him [6] as being unlikely as the free energy of transacylation substrate → mercapto group should be a highly endergonic reaction step. Work on the hydrolysis of O- and S-esters [3] showed that for comparable compounds there is only a small difference in the ΔH_{aq}-value in concordance with the results obtained here. It is therefore not believed that thermodynamic arguments, at present, discard the tiolester-intermediate hypothesis as changes in the entropy term easily might compensate the minor unfavourable enthalpy change.

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