A Determination of the Standard Free Energy of Hydrolysis of Pyrophosphate by Thermal Measurements.
I. The Heat of Hydrolysis[1]

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Abstract: The heat of hydrolysis of inorganic pyrophosphate has been determined calorimetrically at 25° using a cycle involving measurements of the heats of solution of ortho- and pyrophosphate sodium salts in water and in concentrated perchloric acid. For the hydrolysis at pH 5, where the single ionic species $H_2P_2O_7^{2-}$ and $H_2PO_4^-$ predominate, $\Delta H° = -4.5 \pm 0.5$ kcal/mole. The heat of hydrolysis at approximately neutral pH has also been determined directly using an alkaline phosphatase of *Escherichia coli* which does not require metal ion activators. The value of -3.7 ± 0.2 kcal/mole for the enthalpy change in unbuffered solution at a final pH of 6.9 is in excellent agreement with the value of -3.8 kcal/mole calculated from the indirect determination and the various ionization heats.

Phosphate compounds play a central role in the chemistry of living systems. The hydrolytic fission of phosphate esters and anhydrides, although not utilized directly to maintain the energy requirements of the cell, is nevertheless an extremely useful reference reaction because linear combinations of hydrolysis reactions lead directly to the reactions of interest. Pyrophosphate is the simplest of the phosphate anhydrides, and accurate data for its free energy of hydrolysis over a wide range of pH would be helpful in establishing how esterification influences the thermodynamic reactivity of the anhydride bond. Furthermore, a knowledge of this free energy of hydrolysis, together with the values reported in the literature for the hydrolysis of ATP yielding orthophosphate and for the myokinase reaction (ATP + AMP → 2ADP), would immediately provide a value for the pyrophosphate fission of ATP which is still not known at all precisely, even though the reaction is so important in biosynthesis.

No accurate determination of the standard free energy of hydrolysis of pyrophosphate at 25° has yet been reported; however, a provisional value[2] based on equilibrium measurements gave $\Delta F_{\text{obsd}} = -6.6 \pm 1$ kcal/mole at pH 7.5, 30°, and $\mu = 0.25$. There is a large difference between this value and one of -2.1 kcal/mole estimated a few years ago[3] from a value for ΔH, calculated from the heats of formation of pyrophosphate and orthophosphate,[4,5] Latimer's value of 42 eu for $\bar{S}°(H_3PO_4(aq))$, a value of 62 eu for $\bar{S}°(H_4P_2O_7(aq))$ based on Cobble's empirical equation,[6] and the NBS ionization data for $H_4P_2O_7$. This very striking lack of agreement called for a thorough experimental study to establish firm values for the respective contributions of the enthalpy and entropy changes.

The NBS values[5] for the heats of formation of pyrophosphoric acid and its anions are based on the early work of Giran,[7] where the heat of hydrolysis of pyrophosphoric acid is taken as the difference between the heats of solution of the liquid acid in water and in concentrated sulfuric acid. This procedure ignores the ionization of the strong acid in water and implicitly assumes that the heats of transfer of reactants and products from sulfuric acid to water are either small or compensate each other almost completely. If one accepts the NBS value[5] of $\Delta H = -4.6$ kcal/mole for the reaction

$$H_4P_2O_7(aq) + H_2O(l) \longrightarrow 2H_3PO_4(aq)$$

and calculates ΔH at pH 7 using the most recent heats of ionization of pyro- and orthophosphoric acid determined by Irani, *et al.*,[8] the exothermicity is very small—only 1.6 kcal/mole. This is even less favorable than the value used previously (-6.5 kcal/mole) in arriving at the value of -2.1 kcal/mole for ΔF_{obsd} at pH 7.5, and strongly suggests that the old value for the heat of hydrolysis is open to question.

This view is strengthened by direct measurements of the heat of hydrolysis at neutral pH, which have been made since Giran's determinations. Using inorganic pyrophosphatase to catalyze the reaction, Ohlmeyer and Shatas[9] found $\Delta H = -9.0$ kcal/mole at pH 7.2 and 29°, but did not correct for the heats involved in the change in the ionization state of the buffer and in the differential binding of Mg^{2+} ion to the pyrophosphate and orthophosphate. In a more careful study Ging and Sturtevant[10] attempted to avoid these secondary reactions by swamping the system with orthophosphate, and obtained $\Delta H = -5.8$ kcal/mole at 25°, pH 7.3, and $0.6 < \mu < 1$. Without excess orthophosphate present they found $\Delta H = -7.3 \pm 0.2$ kcal/mole at 25° using 0.05 M veronal buffer pH 7.5.

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[1] This work was presented at the 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March 1966, Abstract N-106.

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[4] W. M. Latimer, "The Oxidation States of the Elements and Their Potentials in Aqueous Solution," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1952.

[5] "Selected Values of Chemical Thermodynamic Properties," National Bureau of Standards Circular 500, U. S. Government Printing Office, Washington, D. C., 1952.

[6] J. W. Cobble, *J. Chem. Phys.*, **21**, 1451 (1953).

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[9] P. Ohlmeyer and R. Shatas, *Arch. Biochem. Biophys.*, **36**, 411 (1953).

[10] N. A. Ging and J. M. Sturtevant, *J. Am. Chem. Soc.*, **75**, 2087 (1953).

Table I

	Reactions	ΔH, kcal/mole
(1)	$Na_4P_2O_7\cdot 10H_2O(c) + 4H^+(HClO_4) \longrightarrow 2H_3PO_4(HClO_4) + 9H_2O(HClO_4) + 4Na^+(HClO_4)$	-52.47 ± 0.24
(2)	$Na_2HPO_4\cdot 7H_2O(c) + 2H^+(HClO_4) \longrightarrow H_3PO_4(HClO_4) + 7H_2O(HClO_4) + 2Na^+(HClO_4)$	-25.55 ± 0.17
(3)	$Na_4P_2O_7\cdot 10H_2O(c) \longrightarrow Na_4P_2O_7(aq) + 10H_2O(l)$	$+10.44 \pm 0.08$
(4)	$Na_4P_2O_7(aq) + 2H^+(aq) \longrightarrow 4Na^+(aq) + H_2P_2O_7^{2-}(aq)$	-0.53 ± 0.05
(5)	$Na_2HPO_4\cdot 7H_2O(c) \longrightarrow Na_2HPO_4(aq) + 7H_2O(l)$	$+11.53 \pm 0.23$
(6)	$H_2PO_4^-(aq) \longrightarrow H^+(aq) + HPO_4^{2-}(aq)$	$+0.80 \pm 0.1$
(7)	$H_2O(l) \longrightarrow H_2O(HClO_4)$	-2.95 ± 0.03

Since there is such serious disagreement between these various values, we have undertaken new determinations, both indirectly using a cycle of reactions somewhat similar to that employed by Giran, and directly using an alkaline phosphatase from *Escherichia coli* that has the great advantage of requiring no specific metal ion activators. These various values will be discussed again later and compared with the results of the present study.

Experimental Section

The 950-ml capacity calorimeter used for all work except the enzymatically catalyzed hydrolysis was patterned after one previously described.[11,12] Using a G-2 Mueller bridge and a Leeds and Northrup 9835-B dc amplifier with a nickel-sensing element, temperature sensitivity was better than 0.0001°. Batteries previously used in the heating circuit were replaced by a Sorensen Model QB28-5 power supply. Voltage variations across a standard 1-ohm resistor in series with the 260 ohms of manganin heating element were generally less than 0.005% during the 1-min heating period. The calorimeter used for the enzyme experiments has a capacity of 100 ml. Its principle of operation is the same as that of a 950-ml calorimeter. A detailed description of the construction and performance of this calorimeter will appear elsewhere.[13] The heat of solution of KCl(c) in water, final concentration 0.05 *M* in KCl, was determined using both calorimeters. Our result of 4.19 ± 0.01 kcal/mole is in agreement with the NBS value.[5] All heats of reactions were measured at 25.0 ± 0.2°.

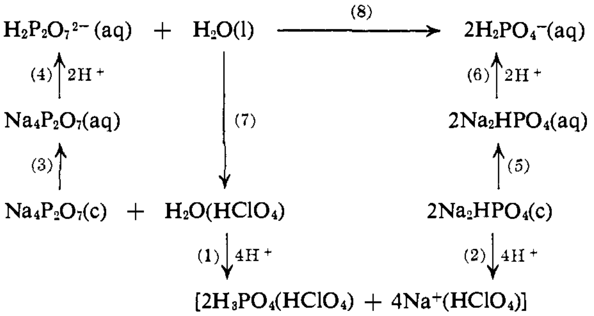
Sodium pyrophosphate decahydrate (Baker Analyzed Reagent), was recrystallized from water. After air drying at room temperature the water content was determined by drying to constant weight at 120°, giving 99.6 ± 0.3% of the theoretical value. Titration with standard acid to the methyl orange end point showed that the material had 99.5% of the expected alkaline strength. A colorimetric determination for orthophosphate in this salt was below the level of detectability (~0.2%). Dibasic sodium phosphate heptahydrate (Baker Analyzed Reagent) was used without further purification. The loss of weight on drying at 105° corresponded to 99.2 ± 0.4% of the theoretical value. The major contaminant appeared to be the anhydrous salt, an inference supported by the alkaline strength which was found to be 101.3 ± 0.4%, slightly higher than the theoretical value. The assay for the perchloric acid (Baker Analyzed Reagent) varied between 70.8 and 70.9%.

Bacterial alkaline phosphatase was obtained from the Sigma Chemical Co. (10 mg of protein/ml in 65% saturated ammonium sulfate). The preparation was centrifuged, washed with 65% saturated ammonium sulfate, centrifuged again, suspended in the same solution at a concentration of 10–40 mg/ml, and stored at 4°.

Results and Calculations

A. The Heat of Hydrolysis of Pyrophosphate. Indirect Determination. At pH 4–5 both pyro- and orthophosphate exist predominantly as the single ionic species $H_2P_2O_7^{2-}$ and $H_2PO_4^-$, respectively. To determine the heat associated with the hydrolysis in this pH range the cycle shown in Scheme I was devised.

Scheme I



Six to ten determinations were made for each reaction in the cycle over the concentration range 2×10^{-3} to 7×10^{-3} *M*. For this range no concentration dependence of ΔH was detectable, and the results listed in Table I are the mean values together with their average deviations.

The use of 70.9% perchloric acid in reaction 1 increases the rate of the hydrolysis. This concentration of acid also reduces the energy equivalent of the calorimeter by 25%, with a corresponding increase in sensitivity. Both these features, particularly the former, reduce the uncertainty in the calorimetric measurements.

The over-all kinetics of reactions 1 and 2 are similar; 50% of the heat was evolved in the first minute and 99% during the initial 10 min. An assay for orthophosphate using the method of Fiske and Subbarow[14] confirmed these results. A more obvious choice of compounds for this study is the corresponding anhydrous ortho- and pyrophosphate salts, which would result in a stoichiometry involving only one $H_2O(HClO_4)$ rather than five as in the present case. However, the small decrease in the uncertainty of ΔH for reaction 7 was found to be far outweighed by the much larger uncertainty arising from the slow dissolution of the anhydrous compounds. The choice of the tetrasodium salt of pyrophosphate and the disodium salt of orthophosphate also avoids the necessity for determining the heat of formation of sodium and hydrogen ions in concentrated perchloric acid.

Reaction 3 was studied by Thomsen[15] at 18° using more concentrated solutions. He reported $\Delta H = 11.7$ kcal/mole. Our result at 25° using dilute solutions is 10.44 kcal/mole and is taken as the standard heat of solution of $Na_4P_2O_7\cdot 10H_2O(c)$.

$$Na_4P_2O_7\cdot 10H_2O(c) \longrightarrow Na_4P_2O_7(aq) + 10\,H_2O(l) \qquad (3)$$

The heat of protonation in reaction 4, $\Delta H = -0.53$ kcal/mole, agrees remarkably well with the value de-

$$Na_4P_2O_7(aq) + 2H^+(aq) \longrightarrow H_2P_2O_7^{2-}(aq) + 4Na^+(aq) \qquad (4)$$

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(11) W. F. O'Hara, C. H. Wu, and L. G. Hepler, *J. Chem. Educ.*, **38**, 512 (1961).

(12) H. S. Hopkins, C. H. Wu, and L. G. Hepler, *J. Phys. Chem.*, **69**, 2244 (1965).

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(15) H. Thomsen, "Thermochemische Untersuchunger," Vol. III, Barth, Leipzig, 1883, p 118.

termined by Irani, *et al.*,[8] who obtained −0.51 kcal/mole for the sum of the third and fourth protonation heats of tetramethylammonium pyrophosphate. The use of this bulky counterion prevents complexing of pyrophosphate which is known to occur with the sodium ion. The agreement between our value and that of Irani suggests that the heat of ion association for the reaction

$$Na^+(aq) + P_2O_7^{-4}(aq) \longrightarrow NaP_2O_7^{3-}(aq)$$

must be small. A small binding heat is further substantiated by the results of our measurements of the heat of solution of $Na_4P_2O_7 \cdot 10H_2O(c)$, final concentrations 1-6 × 10^{-3} *M*, into 0.01 *M* NaOH. We observed ΔH = 10.35 ± 0.08 kcal/mole, close to the value of 10.44 ± 0.08 kcal/mole for reaction 3, *i.e.*, the solution in water.

The heat of solution of the heptahydrate

$$Na_2HPO_4 \cdot 7H_2O(c) \longrightarrow Na_2HPO_4(aq) + 7H_2O(l) \qquad (5)$$

was measured by Thomsen[15] and Pfaundler,[16] who reported 11.60 and 11.38 kcal/mole, respectively. For this heat of solution we obtained 11.53 ± 0.23 kcal/mole. Since no concentration dependence for the heat of solution of this compound was observed, we take the average value ΔH = 11.53 kcal/mole as its standard heat of solution. The uncertainty involved in this measurement was ±0.23 kcal/mole, largely due to the relatively slow dissolution of $Na_2HPO_4 \cdot 7H_2O(c)$.

The heat for the secondary ionization of orthophosphate

$$H_2PO_4^-(aq) \longrightarrow H^+(aq) + HPO_4^{2-}(aq) \qquad (6)$$

was found to be 0.76 kcal/mole at $\mu = 0$, from the temperature variation of the ionization constant.[17] A more accurate potentiometric study[18] reported ΔH = 0.82 kcal/mole. Calorimetric values[8,19] at finite ionic strength for reaction 6 are within 0.05 kcal/mole of these values obtained from potentiometric measurements, and 0.8 has been adopted for the present calculations.

The heat of hydrolysis, ΔH_8, for the reaction

$$H_2P_2O_7^{2-}(aq) + H_2O(l) \longrightarrow 2H_2PO_4^-(aq) \qquad (8)$$

was derived from the results given in Table I as follows. Combining reactions 1 and 3

$$Na_4P_2O_7(aq) + 10H_2O(l) + 4H^+(HClO_4) \longrightarrow 2H_3PO_4(HClO_4) + 9H_2O(HClO_4) + 4Na^+(HClO_4) \qquad (9)$$

$\Delta H_9 = \Delta H_1 - \Delta H_3 = -62.91$ kcal/mole. Similarly with reactions 2 and 5

$$Na_2HPO_4(aq) + 7H_2O(l) + 2H^+(HClO_4) \longrightarrow H_3PO_4(HClO_4) + 7H_2O(HClO_4) + 2Na^+(HClO_4) \qquad (10)$$

$\Delta H_{10} = \Delta H_2 - \Delta H_5 = -37.08$ kcal/mole. Subtracting twice reaction 10 from reaction 9 gives

$$Na_4P_2O_7(aq) + 5H_2O(HClO_4) \longrightarrow 2Na_2HPO_4(aq) + 4H_2O(l) \qquad (11)$$

and $\Delta H_{11} = \Delta H_9 - 2\Delta H_{10} = 11.25$ kcal/mole. Combining eq 11 and 7 gives

$$Na_4P_2O_7(aq) + H_2O(l) \longrightarrow 2Na_2HPO_4(aq) \qquad (12)$$

and $\Delta H_{12} = \Delta H_{11} + 5\Delta H_7 = -3.50$ kcal/mole. Finally, combining reaction 12 with reactions 4 and 6, it follows that $\Delta H_8 = \Delta H_{12} - \Delta H_4 - 2\Delta H_6 = -4.49$ kcal/mole.

Heats of dilution of $H_2P_2O_7^{-2}(aq)$ and $H_2PO_4^-(aq)$ have been measured[8] and found to be small. The heat of hydrolysis thus calculated, $\Delta H_8 = -4.5 \pm 0.5$ kcal/mole, will therefore be adopted as the standard heat of hydrolysis of $H_2P_2O_7^{2-}(aq)$, 0.5 kcal/mole being our estimation of the total maximum uncertainty.

The hydrolysis in alkaline solution, pH 10.5, is approximately represented by reaction 12. However, there are mixed ionic species of ortho- and pyrophosphates at this pH, and ΔH_{12} does not correspond to the actual heat of hydrolysis at any pH in this region. This difficulty is avoided by using reaction 8 as reference, since the primary and secondary ionization constants are separated by more than 4 p*K* units, and as a consequence ΔH_8 is the hydrolysis heat for single ionic species.

B. The Heat of Hydrolysis of Pyrophosphate. Direct Determination. The availability of an alkaline phosphatase from *E. coli* which hydrolyzes inorganic pyrophosphate in the absence of metal ion activators[20] prompted a further study of the direct determination of the hydrolysis heat.

First, the following experiment was carried out to see whether the self-buffering action of the orthophosphate produced makes the use of an additional buffer unnecessary. Tetrasodium pyrophosphate was titrated with HCl to an initial pH of 8.5 so that the predominant ionic species was $HP_2O_7^{3-}$. After hydrolysis the pH was very close to the value for pK_2 for orthophosphate at the operative ionic strength.[21] This is consistent with the release of one proton for each $HP_2O_7^{-3}$, *i.e.*

$$HP_2O_7^{3-}(aq) + H_2O \longrightarrow 2HPO_4^{2-}(aq) + H^+(aq)$$

followed by proton capture by one of the HPO_4^{2-} ions produced to give a final solution where $[HPO_4^{2-}] = [H_2PO_4^-]$.

Kinetic studies using a colorimetric assay for orthophosphate under conditions where pyrophosphate is stable[10] showed that the optimal conditions for calorimetric work at 25° with this unbuffered solution were 5 × 10^{-3} *M* in substrate and 1 mg of alkaline phosphatase/25 μmoles of substrate. Under these conditions 50% of the pyrophosphate is hydrolyzed in 3.5 min and 95% is hydrolyzed in 10 min. The rate of heat output in the calorimeter was found to follow quite closely the kinetics established by chemical assay; 65% saturated ammonium sulfate was used as the supporting electrolyte for the enzyme because in more dilute solution there was an appreciable loss of enzyme activity during the 1-hr equilibration period in the calorimeter.

For the calorimetric measurements the substrate solution was placed in the dewar flask and the enzyme preparation in a small bulb of approximately 2-ml capacity. The results are given in Table II. Column 2 lists the amount of ammonium sulfate used in both the hydrolysis reaction and in a control experiment which was necessary to estimate the heat of dilution of the ammonium sulfate in the pyrophosphate solution.

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[16] L. Pfaundler, *Ber.*, **4**, 773 (1871).

[17] R. C. Phillips, P. George, and R. J. Rutman, *Biochemistry*, **2**, 501 (1963).

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[20] L. A. Heppel, D. R. Harkness, and R. J. Hilmoe, *J. Biol. Chem.*, **137**, 841 (1962).

[21] R. G. Bates and S. F., Acree, *J. Res. Natl. Bur. Std.*, **30**, 129 (1943).

The heat of dilution of the enzyme was neglected since only about 10^{-7} mole was involved. Column 4 gives the heats of dilution of ammonium sulfate in the control experiments, and column 5 the total heat observed for the hydrolysis including q_{control}. The average value for the heat of hydrolysis calculated from these data is -3.7 ± 0.2 kcal/mole for a final ionic strength of 0.07.

Table II. Enzymatic Hydrolysis of Pyrophosphate at 25°

Pyrophosphate, μmoles	$(NH_4)_2SO_4$, μmoles	Final pH	q, cal: Control	q, cal: Obsd	ΔH, kcal/mole
500	520	6.76	−3.10	−1.29	−3.7
500	150	6.93	−1.06	0.68	−3.5
500	120	6.88	−0.87	0.88	−3.5
500	130	6.89	−0.91	1.04	−3.9

The relation between this value and that determined indirectly in section A for the pH range 4–5, *i.e.* -4.5 ± 0.5 kcal/mole, is shown in the following cycle.

$$\begin{tikzcd}
H_2P_2O_7^{2-} + H_2O \arrow[r, "\Delta H\ =\ -4.5"] \arrow[d, "\Delta H\ =\ 0.1"] & 2H_2PO_4^- \text{ (pH 4–5)} \arrow[d, "\Delta H\ =\ 0.8"] \\
HP_2O_7^{3-} \text{(pH 8.5)} + H_2O \arrow[r, "\text{calcd}", "\Delta H\ =\ -3.8"'] & HPO_4^{2-} + H_2PO_4^- \text{ (pH 6.9)}
\end{tikzcd}$$

The two values may be compared by calculating ΔH appropriate to the conditions of the direct determination from the value at pH 4–5 and the known ionization heats. This calculation gives −3.8 kcal/mole, in excellent agreement with the direct determination, and it strongly suggests that all these thermochemical data are *internally* consistent to within at least 0.3 kcal/mole. Furthermore any contribution to ΔH arising from the participation of sodium ion complexes has to be almost identical for the two sets of experimental conditions. With regard to the value at pH 4–5, the contribution is likely to be negligible in view of the protonated phosphate species present and the low concentrations employed. Hence, since the heats of ionization in the above cycle were determined using quaternary ammonium salts, we conclude that sodium ion complex formation also makes a negligible contribution to the value determined directly at pH 6.9, although compensation between the heat of formation of the pyrophosphate complex and the orthophosphate complex may account for this to some extent.

C. Comparison with Other Values in the Literature. The value of −9.0 kcal/mole reported by Ohlmeyer and Shatas[9] for the observed heat of hydrolysis of inorganic pyrophosphates at 29° in pH 7.2 veronal buffer using inorganic pyrophosphatase with Mg^{2+} ion as activator to catalyze the reaction is difficult to reconcile with the present work. The small temperature difference would have a negligible effect, and the presence of Mg^{2+} ions at one-tenth the concentration of pyrophosphate would probably contribute less than 0.5 kcal/mole. A somewhat larger contribution to the observed heat would come from the heat of protonation of the buffer base. Approximately 0.2 mole of H^+ is released per mole of pyrophosphate hydrolyzed at this pH; hence, with a protonation heat of about 6 kcal/mole exothermic, the correction on this account would bring the value to about −7.7 kcal/mole. The most likely explanation for the further discrepancy lies in the experimental technique employed; undoubtedly their measurement of energy changes of less than 1 cal over a period of 1 hr using about 50 ml of solution would have been subject to large uncertainties.

Similar corrections need to be applied to the value of -7.3 ± 0.2 kcal/mole obtained by Ging and Sturtevant[10] at 25° using 0.05 veronal buffer, pH 7.5. They estimated 0.1 mole of alkali per mole of pyrophosphate hydrolyzed in unbuffered solution was needed to restore the final pH to the initial value: this correction would make the observed heat 0.6 kcal/mole less exothermic. The presence of $MgSO_4$ at approximately the same concentration as the pyrophosphate would result in a considerably larger correction. The stability constant for the reaction $Mg^{2+} + P_2O_7^{4-} \rightarrow MgP_2O_7^{2-}$ has been reported[22] as 5×10^5 at 18° in the presence of 0.05 M KNO_3 with $\Delta H = 2.9$ kcal/mole. In studies to be published later, we have found the enthalpy change to be about 3.5 kcal/mole for both the reaction involving $P_2O_7^{4-}$ and $HP_2O_7^{3-}$. On this basis calculations show that at least 70% of the pyrophosphate would be present as the magnesium complex; hence, since the dissociation of the complex is exothermic, the correction involves subtracting about 2.5 kcal/mole, both corrections bringing the heat of hydrolysis down to about −4.2 kcal/mole. However, a precise estimate of these corrections is so dependent on an exact choice of pK_4 for pyrophosphoric acid and the stability constant and on the enthalpy change for the magnesium complex appropriate to the conditions of the hydrolysis experiment, particularly the ionic strength and the nature of the supporting cations, that without parallel determination of these data we believe no better agreement between their value and ours, which required no such corrections, can necessarily be expected.

With regard to Ging and Sturtevant's other calorimetric measurements using a large ratio of orthophosphate to pyrophosphate so as to avoid the problem of these corrections, no comparison can be made between their value of -5.8 ± 0.1 kcal/mole at an ionic strength of 0.6 to 1.0 and our value of -3.7 ± 0.2 kcal/mole at a final ionic strength of 0.07, since the enthalpy change for many aqueous solution reactions is quite dependent upon the ionic strength of the solution.

Discussion

Although part A of this study is similar to that of Giran in that the hydrolysis is carried out in the presence of strong acid, the additional heat measurements of the cycle used here properly allow for the heats of transfer of reactants and products from this medium to an aqueous one and result in a value for the enthalpy change requiring no knowledge of the values for the strong ionization heats of ortho- and pyrophosphate.

In part B it has been shown that for the hydrolysis of compounds such as phosphate anhydrides, where reactants and products have similar pK's, it is possible to measure the enthalpy change directly in otherwise unbuffered solutions, even though hydrogen ion is evolved, by taking advantage of the self-buffering action of the product to hold the pH in the same region. To arrive at the enthalpy change at a constant pH, the observed

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[22] V. P. Vasil'ev, *Russ. J. Phys. Chem.*, 31, 692 (1957).

heat has, of course, to be corrected for the ionization heat of the product. But in the case of phosphate compounds, in general, these ionization heats are quite small, much smaller than those usually encountered with nitrogenous base buffers, so the error introduced by any uncertainty in their ionization heats is of much less consequence.

With the new data now at hand, it is appropriate to reexamine the previous estimate of the standard free energy of hydrolysis of pyrophosphate, which, as indicated in the introduction, was calculated from ΔH_f° for orthophosphate and pyrophosphate,[5] an experimental value of 42 eu for $\bar{S}^\circ(H_3PO_4(aq))$,[4] a value of 62 eu for $\bar{S}^\circ(H_4P_2O_7)$ based on Cobble's empirical equation,[6] and the ionization data for H_3PO_4 and $H_4P_2O_7$.[5] Undoubtedly the true value is much more favorable than this estimate gave one to suppose; *i.e.*, the provisional value based on direct measurements[2] is at least -6.6 kcal/mole at pH 7.5, 30°, and $\mu = 0.25$, compared to the earlier estimate of -2.1 kcal/mole[3] for comparable experimental conditions.

Now the new value of ΔH established by the present experiments accentuates the discrepancy, since, compared to the value used in the previous calculations, the new value is some 2.8 kcal/mole less favorable (*i.e.*, for the pH range 4–5 the old value for ΔH was -7.8 kcal/mole compared to the new value of -4.5 kcal/mole). From this it follows that the value of ΔS used in the previous calculation was much too unfavorable, and this could have arisen from the adoption of a too low value for $\bar{S}^\circ(H_3PO_4(aq))$, a too high value for $\bar{S}^\circ(H_4P_2O_7(aq))$, or some combination of these factors. The best value currently available for $\bar{S}^\circ(H_3PO_4(aq))$ is 37.8 eu,[23] even lower than Latimer's value used in the previous calculation. This accentuates the discrepancy even more, so the value upon which attention becomes focused is that for $\bar{S}^\circ(H_4P_2O_7(aq))$. The recent studies of Riyad, Irani, and Taulli[8] on the ionization of $H_4P_2O_7$ and $H_3P_2O_7^-$ suggest that the increments in $\bar{S}^\circ$ (*i.e.*, the entropies of ionization) may be much larger than the values generally accepted.[5] These revised entropies of ionization would have the desired effect of making ΔS for the hydrolysis reaction more favorable, although of course they in no way provide a check on the entropy value for any individual pyrophosphate species. Assuming that the standard free-energy change for the hydrolysis at pH 4–5 and 25° is the same as the provisional value obtained for pH 7.5 and 30° (the difference in any case would be less than 1 kcal/mole), the new value of -4.5 kcal/mole for ΔH leads to a value of about $+7$ eu for the entropy of hydrolysis for reaction 8. Combined with the well-established entropy of ionization of H_3PO_4 and the new values for the ionization of $H_4P_2O_7$ and $H_3P_2O_7^-$, ΔS for the acidic solution reaction

$$H_4P_2O_7 + H_2O \longrightarrow 2H_3PO_4$$

is found to be about $+9$ eu, from which, taking $\bar{S}^\circ(H_3PO_4(aq)) = +37.8$ eu, it follows that $\bar{S}^\circ(H_4P_2O_7(aq))$ is about 50 eu. Just as $\bar{S}^\circ(H_3PO_4(aq))$ is significantly lower than the value calculated from Cobble's equation, 46 eu, it would thus appear that $\bar{S}^\circ(H_4P_2O_7(aq))$ is lower by an even more significant amount, *i.e.*, 50 eu, compared to the calculated value of 68 eu, which suggests that in relation to many other types of solute in aqueous solution these particular species have some distinctly anomalous entropy property.

The difference is so large for $H_4P_2O_7$, and it has such an important effect on the magnitude of the standard free energy of hydrolysis, that the underlying thermodynamic quantities need very careful checking. We are therefore undertaking a further study of the strong ionizations of pyrophosphoric acid and a determination of the partial molal entropy of $H_2P_2O_7^{-2}(aq)$, based on the measurement of S° for the crystalline sodium salt and a thermodynamic study of its solution in water.

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(23) C. C. Stephenson, *J. Am. Chem. Soc.*, **66**, 1436 (1944).