been reported[13] that will hydrolyze cyclic sugar diesters of phosphoric acid, but to the best of our knowledge the enzyme from *Enterobacter aerogenes* is the first purified diesterase with specificity known to include simple aliphatic diesters of phosphoric acid.

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Enthalpy of Hydrolysis of Simple Phosphate Diesters

Sir:

We wish to report the first determinations of the heats of hydrolysis of simple diesters of phosphoric acid. Such heats have previously been unavailable because most diesters of phosphoric acid are much too resistant to hydrolysis[1] to permit thermochemical measurements; the data are important because of the role such compounds play in biochemistry[2] and in the theory of the hydrolysis of phosphate esters.[3] We have succeeded in making appropriate measurements by utilizing a new phosphodiesterase recently isolated and purified in one of our laboratories.[4]

Although 2′,3′-cyclic nucleotides[5] and salts of ethylene phosphate[6] hydrolyze at moderate rates in strong alkali, salts of trimethylene phosphate[7] and of cyclic AMP,[8] like salts of dimethyl phosphate and of other simple diesters of phosphoric acid,[1] hydrolyze only 10^{-4}–10^{-6} times as fast. Strain has been implicated in the rapid hydrolysis of the five-membered rings; such strain is implicit in the small O–P–O bond angles found for these five-membered rings by X-ray crystallography[9] and explicit in the heats of hydrolysis of methyl ethylene phosphate[10] and of 2′,3′-cyclic nucleotides.[11] Recently, however, Hayaishi, *et al.*,[12] have found that the free energy of hydrolysis of cyclic AMP is −11.9 kcal/mol, a value in excess of the −8.9 kcal/mol for the hydrolysis of ATP.[13] Furthermore, Greengard, *et al.*,[11] found that the heat of hydrolysis of cyclic AMP is −14.1 kcal/mol and that other 3′,5′-cyclic nucleotides show similar enthalpies of hydrolysis; these values are much greater than those averaging around −8 kcal/mol for the 2′,3′-cyclic nucleotides.[11] Nevertheless, X-ray data for 3′,5′-cyclic nucleotides[14] and for trimethylene phosphate[15] yield O–P–O angles similar to those in acyclic phosphates,[16] and give no evidence of strain. No heats of hydrolysis of simple diesters of phosphoric acid or of simple cyclic diesters were previously available for comparison, for the reasons cited above.

We have now obtained the data in Table I. The heat of hydrolysis of sodium ethylene phosphate is about −6.8 ± 0.6 kcal/mol, and so is comparable to those of 2′,3′-cyclic nucleotides; the heats of hydrolysis of sodium diethyl phosphate and of sodium tetramethylene phosphate are smaller by about 4 kcal/mol, and the difference, although only a fraction of the difference in free energies of activation for the hydrolytic processes, is consistent with calculation.[17] The heat of hydrolysis of sodium trimethylene phosphate is about −3.5 kcal/mol. This result is consistent with the kinetic stability of trimethylene phosphate[7] and of cyclic AMP[8] and with calculations of their strain energies.[17,18] On the other hand, the new data for trimethylene phosphate stand in sharp contrast to the thermochemical data for 3′,5′-cyclic nucleotides,[11] which, on hydrolysis, release 7–11 kcal/mol more heat than does trimethylene phosphate. We have at present no explanation for this phenomenon, but believe that the contrast between the behavior of the simple six-membered ring phosphate ester and that of cyclic AMP is of importance.

Barium ethylene phosphate[6] was converted into its sodium salt with sodium sulfate. Trimethylene hydrogen phosphate[7,19] and tetramethylene hydrogen phosphate[7,18,19] were neutralized with sodium hydroxide. Barium diethyl phosphate and barium monoethyl phosphate were analytically pure, and were converted to their sodium salts with sodium sulfate. The buffers used were Tris (Sigma) and piperazine *N*,*N*′-bis(2-ethanesulfonic acid) (Pipes; Calbiochem). p*K* and pH values were measured with a Radiometer TTT1b Titrator. The diesterase was prepared from *Enterobacter aerogenes* grown on dimethyl phosphate as the only source of phosphorus, in accordance with recently developed procedures;[4] the sample used for calorimetry was judged to be at least 98% homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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Table I

Compound	Buffer	ΔH, gross,[a] kcal mol^{-1}	ΔH, protonation of buffer,[b] kcal mol^{-1}	Net ΔH of hydrolysis, kcal mol^{-1}
Sodium ethylene phosphate	Pipes	-9.44 ± 0.04	-2.33 ± 0.3	-7.11 ± 0.3
	Tris	-16.6 ± 0.1	-10.1 ± 0.5	-6.5 ± 0.5
Sodium trimethylene phosphate	Pipes	-5.93 ± 0.13	-2.23 ± 0.3	-3.70 ± 0.3
	Tris	-13.0 ± 0.1	-9.6 ± 0.5	-3.40 ± 0.5
Sodium tetramethylene phosphate	Pipes	-4.69 ± 0.03	-2.07 ± 0.3	-2.62 ± 0.3
Sodium diethyl phosphate	Pipes	-4.81 ± 0.04[c,d]	-2.15 ± 0.3	-2.66 ± 0.3

[a] Gross heat of hydrolysis, measured by flow calorimetry, and corrected for the (minor) heats of viscous flow and dilution. The heat of dilution of Pipes buffer is unusually large and is under further investigation. However, since all solutions used in this work were 0.05 *M* in buffer, no dilution of the buffer is involved in these measurements. [b] Heat of protonation of the basic form of the buffer, calculated for pH 7.32 for Pipes and 7.30 for Tris on the basis of the experimentally determined p*K* values of the products, and heats of ionization. These are 11.34 kcal mol^{-1} for Tris (I. Grenthe, H. Ots, and O. Ginstrup, *Acta Chem. Scand.*, **24**, 1067 (1970)) and 2.59 ± 0.03 kcal mol^{-1} for Pipes, determined in this work [in fair agreement with the value reported by L. Beres and J. M. Sturtevant (*Biochemistry*, **10**, 2120 (1971))]. The major errors in this work arise because of an uncertainty of 3–5% in the protons released around pH 7.3; more refined measurements are planned. [c] Corrected by 0.32 kcal mol^{-1} for the exothermic heat of binding of product to enzyme. [d] Stopped-flow calorimetry.

Measurements of heats of hydrolysis for the cyclic phosphates were made with flow microcalorimeters[20] at several different flow rates, so as to establish that the reactions had proceeded to completion kinetically within the calorimeter. The heat of hydrolysis of diethyl phosphate was measured in the same instruments, used as stopped flow calorimeters. The concentration of diesters used was around 5×10^{-4} *M*, and that of the enzyme (subunit mol wt 29,000) around 2×10^{-5} *M* except for the experiments with diethyl phosphate, where the concentration was about 1×10^{-4} *M*. The kinetics of the hydrolysis of bis(*p*-nitrophenyl) phosphate with the diesterase shows that the enzyme is only slightly inhibited by the products at the concentrations produced in these experiments. However, because of the slow hydrolysis of diethyl phosphate, considerable enzyme (about 20 mol %) had to be used in this case, and therefore the data for diethyl phosphate had to be corrected for the (measured) heat of binding of product to enzyme. This and other standard corrections are shown in Table I. The enzyme does not cause hydrolysis of monoesters under the conditions of the thermochemical experiments, except for monoethyl phosphate, where the extent of hydrolysis is less than 2%. The reactions go to stoichiometric completion, within the experimental error of our techniques, as evidenced (a) by the fact that the heats of hydrolysis of trimethylene phosphate and of ethylene phosphate are about the same whether measured in Tris or Pipes buffer; in view of the corrections for heat of protonation of the buffer, which were calculated on the basis of complete reaction, such would not be so if the reactions were equilibrium limited, and (b) by analyses of product. These analyses were conducted by hydrolyzing the product of the action of the diesterase with alkaline phosphatase, and then analyzing for inorganic phosphate, and by measuring the concentrations of product by titration between appropriate end points.[1]

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Field Dependent Magnetic Susceptibilities of Diethylenetriammonium Chlorocuprate(II), $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$

Sir:

Recent interest in the magnetism of two-dimensional, layer-like, Heisenberg systems and especially the effects of interlayer exchange on the magnetic properties of such systems[1,2] has prompted an investigation of the thermochromic compound $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$. The magnetic susceptibility of this new Heisenberg, two-dimensional compound has revealed behavior which is surprisingly similar to $[C_2H_5NH_3]_2CuCl_4$ despite significant differences in the relative isolation of the corresponding two dimensional layers.

The crystal structure[3,4] of $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$, which is illustrated in Figure 1, in exact analogy with $[C_2H_5NH_3]_2CuCl_4$, consists of a two-dimensional network of square planar tetrachlorocuprate ions. Two additional chlorides from neighboring $[CuCl_4]^{2-}$ ions complete a distorted octahedron about each copper. However, in addition to the protonated amine, the diethylenetriammonium ion, an additional chloride ion lies between these layers along the *b* axis. In $[C_2H_5NH_3]_2CuCl_4$ these adjacent layers are isolated by two layers of ethylammonium groups. The shortest copper–copper separation in $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$ is 5.11 Å, and for the description of the magnetic properties the copper ions can be envisaged as forming a nearly quadratic lattice ($c/a = 1.03$), which

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