The effect of optical activity of the peptides is again exhibited in the stability constants of the metal–peptide complexes. Since the metal complex is formed when the protonated amino group loses its proton, corresponding to pK_2' and a linear form of the peptide, the R groups are *cis* to the peptide bond in the LD(DL)-isomer and *trans* in the LL-(DD)-isomer. The LD(DL)-isomer should then form a more stable complex than the LL(DD)-isomer, provided that the R groups are large enough to inhibit facile complexation with the metal ion. Hence, there will be a greater difference in the stability constants of the diastereoisomeric leucyltyrosines than in the diastereoisomeric alanylalanines complexes with a metal ion. This is borne out by the results given in Table II.

These considerations of the folding–unfolding processes of the dipeptides as a function of *p*H may be extended to predictions of the relative rates of acid or basic hydrolysis of diastereoisomers where attack at the peptide carbonyl is involved. In the *p*H range below pK_1' the dipeptide exists in a linear form, the R group being *trans* to the peptide bond in the LL-isomer and *cis* in the DL-isomer. When the R groups are *trans* they flank to some extent both sides of the peptide bond. This should result in a decreased rate of hydrolysis by hindering attack at the peptide bond. The DL-isomer, however, will be faster in this *p*H range since the R groups are both *cis* to the peptide bond. This is exemplified by the hydrolysis of the LL- and DL-valylvaline in 6 *N* hydrochloric acid at 100° in which the rate of hydrolysis of the DL-isomer was greater than the LL-isomer.[16]

In the *p*H region between pK_1' and pK_2', the peptides exist in the folded form with the R groups *cis* in the LL- and *trans* in the DL-isomer. Thus, the LL-isomer would be expected to hydrolyze faster than the DL in this *p*H range. In the *p*H range beyond pK_2', the behavior of the dipeptides on hydrolysis will be similar to that in the *p*H range below pK_1' with respect to relative rates of diastereoisomers.

TABLE II[a]

Metal ion	log k_1	log k_1k_2
	D-Leucyl-L-tyrosine	
Co^{++}	2.81	5.07
Ni^{++}	3.73	6.66
Zn^{++}	3.39	6.24
	L-Leucyl-L-tyrosine	
Co^{++}	2.42	4.48
Ni^{++}	3.23	5.99
Zn^{++}	2.98	5.66
	L-Alanyl-D-alanine	
Co^{++}	2.83[b]	..
	L-Alanyl-L-alanine	
Co^{++}	2.63[b]	..

[a] Error in log k_1 is ±0.03. [b] Obtained from titration of equimolar mixtures of peptides and $Co(NO_3)_2$, 0.01 *M*.

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(16) J. W. Hinman, E. L. Caron and H. N. Christensen, THIS JOURNAL, **72**, 1620 (1952).

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ity effects are the same as those exhibited by a comparison of the pK_3' of tyrosine with the pK_2' of various aminophenols and the pK_1' of ethylamine with the pK_2' of ethylenediamine.

[CONTRIBUTION NO. 1597 FROM THE STERLING CHEMISTRY LABORATORY OF YALE UNIVERSITY, NEW HAVEN, CONNECTICUT]

The Heats of Ionization of Deoxynucleotides and Related Compounds[1]

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The heats of ionization of the purine group of deoxyadenylic acid, deoxyguanylic acid and related compounds and of the pyrimidine group of deoxycytidylic acid and related compounds have been determined calorimetrically. A parallelism, which extends to bases of other types, between heats of ionization and values of the apparent *pK* is noted.

The results of a recent calorimetric study[2] of the acid denaturation of deoxyribosenucleic acid (DNA) were interpreted[3] on the basis of the assumption that the heats of ionization of the pyrimidine and purine bases are very nearly zero in both native and denatured DNA. Titration data for DNA determined over a range of temperatures[4-6] give conflicting evidence as to the magnitudes of the heats of ionization. It therefore seemed of interest to determine these quantities for the isolated mononucleotides and related compounds, even though it cannot be assumed that these heats are the same as those for the nucleotide units in DNA. The present paper reports values obtained by direct calorimetry, a method which gives results of considerably greater accuracy than that obtainable by application of the van't Hoff equation.

Experimental Procedures and Materials

The twin calorimetric apparatus and method employed have been described[7] previously. In each experiment, the heat evolved when a solution of the base was mixed with an equal volume of a solution containing less than one equivalent of HCl was determined. The amount of HCl bound by the base was calculated from the amount added

(1) This work was aided by grants from the National Science Foundation (G-2855) and the United States Public Health Service (RG-4725).

(2) J. M. Sturtevant and E. P. Geiduschek, THIS JOURNAL, **80**, 2911 (1958).

(3) J. M. Sturtevant, Stuart A. Rice and E. P. Geiduschek, *Discussions Faraday Soc.*, **25**, 138 (1958).

(4) L. F. Cavalieri and B. H. Rosenberg, THIS JOURNAL, **79**, 5352 (1957).

(5) R. A. Cox and A. R. Peacocke, *J. Chem. Soc.*, 4724 (1957).

(6) R. A. Cox and A. R. Peacocke, *Discussions Faraday Soc.*, **25**, 211, 213 (1958).

(7) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **73**, 2454 (1951).

and the initial and final values of the pH of the base solution. The initial solutions of the bases and nucleosides were at approximately pH 7, while those of the nucleotides were adjusted to pH values between 4 and 5. The final pH values of the nucleotide solutions were above 2.5, so that it can be assumed that no significant contributions from the phosphate ionizations are included in the observed heat effects. All solutions were adjusted to an ionic strength of 0.1 M by the addition of NaCl. Initial base concentrations of 2×10^{-3} to 10^{-2} M were employed. HCl solutions were prepared by quantitative dilution of a constant boiling stock solution. The heat of dilution of the HCl solution was negligible in all cases.[8]

The adenine sulfate was an Eastman Kodak "white label" preparation. All the other bases were obtained from the California Foundation for Biochemical Research and were labeled "chromatographically pure." These substances were used without further purification.

The heats of dilution of some of the compounds were determined under the conditions of the ionization experiments. The ionization data were corrected for the heats of dilution, the corrections being of a magnitude comparable to the uncertainty of the ionization heats themselves.

All measurements were performed at 25°.

Results and Discussion

The experimental results are summarized in Table I. For each substance a minimum of four independent determinations of the heat of ionization (the heat change accompanying the dissociation of protons from the conjugate acid) were made.

TABLE I

HEATS OF THE IONIZATION OCCURRING AT THE INDICATED pK' VALUES OF VARIOUS NUCLEOTIDES AND RELATED COMPOUNDS AT 25°

Substance	$\Delta H_{ioniz.}$, cal./mole	Standard error, cal./mole	pK'	$\Phi_H - \Phi_H^\circ$ at 0.005 M, cal./mole[a]
Adenine	3990	200	4.1	−180
Cytosine	4470	180	4.5	(− 30)
Adenosine	3810	60	3.6	(−150)
Deoxyadenosine	3870	80	3.8	−150
Deoxycytidine	4300	50	4.3	− 30
Guanosine	990	250	1.6	−220
Deoxyguanosine	1910	90	2.5	(−220)
5′-Deoxyadenylic acid	2640	120	4.0	(−180)
5′-Deoxycytidylic acid	4280	70	4.4	−240
5′-Deoxyguanylic acid	140	10	2.9	−130

[a] Values in parentheses are assumed values.

The mean values, after correction for heats of dilution, are listed in the second column of the table, and the standard errors of the means are listed in the third column. A conservative estimate of the uncertainties in the heats of ionization is ± (standard error + 50) cal./mole. The data on heats of dilution are summarized in the fifth column, in the form of values of the relative apparent molar heat contents at 0.005 M. At these low concentrations, the heat contents are proportional to concentration. The quantities in parentheses are estimated quantities which were used in correcting the corresponding heats of ionization.

The apparent values of pK listed in the fourth column of the table are average values taken from the literature, except that the value for deoxyadenosine was arrived at by adding 0.2 to the value for adenosine.[9] Insofar as possible, values determined at ionic strengths of about 0.1 M are listed.

(8) J. M. Sturtevant, THIS JOURNAL, **62**, 3265 (1940).

It is interesting to note that for most of the compounds in Table I, the heat of ionization in kcal./mole is approximately equal to the pK'. This generalization applies fairly widely to the ionizations of the acids conjugate to a variety of bases.[10] Since $\Delta F^\circ_{298} \approx 1400\ (pK')$ cal./mole, it follows that $\Delta S^\circ_{298} \approx -1.2\ (pK')$ cal. deg.$^{-1}$ mole^{-1} for these ionizations.

Recent determinations,[11] by means of spectrophotometric observations at 15, 25, 35 and 45°, of the heats of ionization of cytosine and deoxycytidine have given the values $\Delta H_{ioniz.} = 4200 \pm 500$ and 4900 ± 500 cal./mole, respectively. These values are in reasonable agreement with the calorimetric values and thus lend support to the view that the ionization processes in at least these two cases are probably not complex reactions.[12]

A number of interesting points can be made concerning the effect of change of structure on the heat of ionization. In the cytosine series, introduction of the sugar moiety has very little effect on the heat of ionization. The same is true in the adenine series, in which case it appeares that the ribose and deoxyribose moieties produce practically the same effect, as would be expected. However, in the other purine series there is a striking difference between the riboside and the deoxyriboside, which is also manifested in the pK' values. (Guanine could not be measured because of its very low solubility.) Those differences are so large that one suspects that the usual structures for the guanine derivatives do not adequately represent all the intramolecular interactions which actually take place.

Phosphorylation of the deoxyriboside in the 5′-position has practically no effect on the $\Delta H_{ioniz.}$ and the pK' in the case of deoxycytidine, whereas in the cases of both the purine derivatives $\Delta H_{ioniz.}$ is decreased by 1200–1800 cal./mole, with pK' again not undergoing much change.

The data listed in Table I do not, of course, give support to the assumption made in our earlier work[2,3] that the heats of ionization of the bases in native and denatured DNA are negligible. On the other hand it is conceivable that the bases have considerably different heats of ionization in DNA than in isolated nucleosides and nucleotides. Further calorimetric experiments are under way on samples of DNA containing markedly different adenine–guanine ratios from that of the DNA (salmon testes) used in our previous work. Such experiments may serve to clarify the situation with respect to the heats of ionization of the bases in DNA.

Cox and Peacocke,[5,6] on the basis of titration data, concluded that the heats of ionization of adenine, cytosine and guanine in denatured DNA are −1, +2 and +4 kcal./mole, respectively. These quantities are very different from those listed in Table I.

(9) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(10) See, for example, data listed by J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1958, pp. 452, 464.

(11) E. P. Geiduschek, private communication.

(12) For a brief discussion of possible discrepancies between van't Hoff and calorimetric values of ΔH for complex reactions see W. W. Forrest and J. M. Sturtevant, THIS JOURNAL, **82**, 585 (1960).