

turn, would suggest a ready saturation of the surface, which would produce a steady diminution in $k_1 I_2$ as the initial iodine concentration was raised. No such effect is observed in the present reaction and mercury is not thought to present a surface on which strong chemisorption easily takes place.²⁵

It seems likely that aqueous solutions of iodine react with mercury *via* the same mechanism as the isoöctane solutions, but evidence is not conclusive.

Future work will explore the effect of other metals dissolved in the mercury, solvent effects and mechanisms for the reaction of other substrates with mercury. It is hoped that these studies will solve some of the problems raised in the present work.

Experimental

Materials.—Merck reagent grade iodine and Mallinckrodt analytical reagent grade mercuric iodide were used without further purification. Phillips Petroleum Co. spectro grade "isoöctane" was used with no treatment. Distilled water was used in the experiments involving aqueous solutions. Mercury was triply distilled material. Shortly after beginning this study, it was found that mercury which had been used in previous runs could be washed with methanol to remove the surface film. After being washed several times with methanol and then blotted dry with filter paper, the mercury appeared to be clean and give results indistinguishable from those obtained with fresh, unused mercury. This method of purification was then adopted for most of the subsequent work.

Apparatus.—The reaction vessel was a 200-ml. 3-neck round-bottom flask with standard taper 24/40 ground glass joints. The two side necks were stoppered with ground glass stoppers and the center one was fitted with a Tru-bore paddle stirrer. The bottom of the paddle blade, which was used with the flat edge down, was kept 15 mm. above the bottom of the flask. It was discovered that the geometry of the equipment was extremely critical, a variation in paddle height of a few millimeters sometimes leading to a large variation in the observed rate of reaction. A total of four different 200-ml. round-bottom flasks and two different Tru-bore stirrers were used in these studies.

The use of different stirring motors did not lead to any obvious change in rate. A fixed speed stirrer, operating at 200 and 400 r.p.m., was used for most of the work at these stirring speeds. A cone-drive motor, capable of variation in speed from 100 to over 1200 r.p.m., was used for most of the rest of the work. A variable-speed motor, geared down to 10 to 55 r.p.m., was used for the slow stirring rates. The runs with a rate reported as 0.1 r.p.m. were run with no stirring except for three revolutions

of the stirrer just before a sample was to be withdrawn in order to make the solution homogeneous. The samples were taken about every 30 minutes for the very slow reactions.

The reaction vessel was immersed in a water thermostat of conventional design maintained within $\pm 0.2^\circ$ of the nominal temperature.

Optical densities of solutions were measured in a 1-cm. quartz cell with a Beckman model DU spectrophotometer that had a thermostated cell compartment. Continuous ultraviolet spectra were measured with a Beckman model DK-2 recording spectrophotometer. Elementary mercury, mercuric iodide and iodine all have distinctive electronic spectra in isoöctane, as do the latter two in water.

Procedure.—The solution to be studied (150 ml.) was run into the 200-ml. flask which had been placed in a constant-temperature bath. It was stirred for at least 15 minutes while coming to thermal equilibrium. A 3-ml. sample was withdrawn, centrifuged, and the optical density determined at the appropriate wave lengths. The wave lengths used are listed in Table VII. The sample was then returned to the reaction flask. Initial iodine and mercuric iodide concentrations were calculated from the initial optical density measurements. The reaction was begun by stopping the stirrer, adding the weighed mercury sample and, after about 10 seconds, starting the stirrer. Samples were centrifuged to remove any suspended material (mercurous iodide).

TABLE VII

WAVE LENGTHS AND EXTINCTION COEFFICIENTS USED

Compound	$\lambda_{\text{max}}, \text{m}\mu$	ϵ_{max}
I ₂	523	900
HgI ₂	272	4200
Hg	257	5000

Samples withdrawn during the rate-of-solution studies on mercury at higher temperatures were not centrifuged since it was discovered that mercury came out of solution rapidly upon cooling. Subsequently, the sampling and spectrophotometric equipment was preheated at the temperature of the run, the cell compartment was maintained at the same temperature as the reaction, and the measurement was made as quickly as possible. Nevertheless, the rates of solution and reprecipitation at 70° were not very reproducible and are not considered as reliable as those at other temperatures.

Acknowledgments.—We are very much indebted to Drs. Bruckenstein, Reynolds, Lumry and Halpern for helpful suggestions. We are pleased to acknowledge the support provided by the Alfred P. Sloan Foundation through a fellowship to M. M. K.

(25) J. C. P. Migolet, *J. Chem. Phys.*, **21**, 1298 (1953).

[CONTRIBUTION NO. 1726 FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONN.]

The Heat of the Reaction between Polyriboadenylic Acid and Polyribouridylic Acid^{1,2}

BY MARY A. RAWITSCHER, PHILIP D. ROSS³ AND JULIAN M. STURTEVANT

RECEIVED MARCH 6, 1963

Calorimetric measurements of the heat of interaction of polyriboadenylic acid (poly A) and polyribouridylic acid (poly U) in 0.1 M KCl at pH 7 and 10°, 25° and 40° are reported. The heat of reaction divided by the absolute temperature varies linearly with temperature over this range and gives on extrapolation a value of $\Delta H = -7600 \text{ cal. (mole of base pairs)}^{-1}$ at the melting temperature of the poly (A + U) formed. Calorimetric titrations of poly A from neutral to acid pH are interpreted to give tentative values for the heat of the coil-to-helix transformation of poly A, and these data are employed to correct the poly (A + U) heats for the apparent degree of order existing in the conformation of the poly A before the reaction. Extrapolation to the melting temperature of poly (A + U) of these revised values gives for the heat of interaction of random coil poly A with random coil poly U to form the 1:1 helical complex at pH 7 a value of approximately $-8700 \text{ cal. (mole of base pairs)}^{-1}$.

Introduction

The double helical structures of deoxyribonucleic acid (DNA) and of the complexes formed by the interaction of various synthetic polynucleotides are stabilized by forces the origin of which is not fully understood. It is therefore of interest to determine experimentally the energy changes accompanying the forma-

tion of these highly ordered structures from approximately random coil conformations of the constituent polymers. In this paper we report the results of calorimetric measurements of the heat effect accompanying the interaction of polyriboadenylic acid (poly A) with polyribouridylic acid (poly U) to form a complex, poly (A + U), of more highly ordered structure than either of the constituent polymers.⁴ Similar measure-

(1) A preliminary report of this work was presented at the 140th National Meeting of the American Chemical Society, Chicago, Ill., September, 1961.

(2) This work was aided by grants from the U. S. Public Health Service (RG-4725) and the National Science Foundation (G-9625).

(3) National Institutes of Health, Bethesda, Md.

(4) Preliminary calorimetric measurements of this reaction were performed in collaboration with Professor A. Rich of the Massachusetts Institute of Technology.

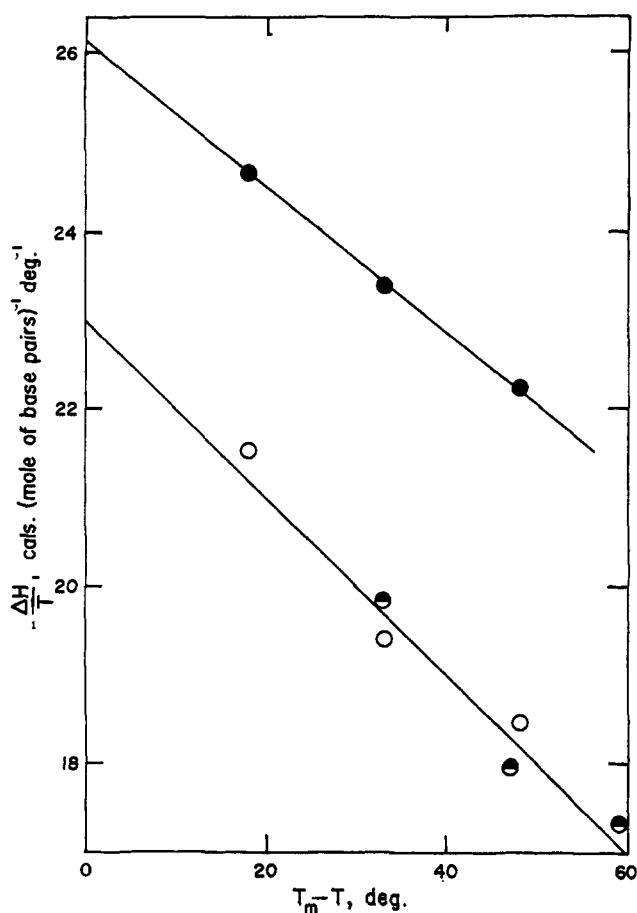


Fig. 1.—The variation with temperature of the heat (ΔH) of the reaction between poly A and poly U at pH 7; T = absolute temperature; T_m = melting temperature of poly (A + U): O, present work, data at 10°, 25° and 40° in 0.1 M KCl, 0.01 M cacodylate buffer; ●, present work corrected for poly A structure (see text); ●, Steiner and Kitzinger, ref. 5, data at 25° in 0.1 M , 0.5 M and 1.0 M KCl.

ments have been reported by Steiner and Kitzinger,⁵ with results in general agreement with those obtained by us. In addition, we report heat data for the conformational change⁶ undergone by poly A in acid solution and suggest the application of these data to correct the data of the poly A-poly U reaction for the extent of organized structure existing in the poly A before reaction.

Experimental

Poly A having a sedimentation coefficient $S_{20,w} = 6.1$ in 0.1 M NaCl was obtained from Miles Chemical Co., Clifton, N. J., and was used without further purification. Poly U, prepared by enzymic polymerization,⁷ had a sedimentation coefficient $S_{20,w} = 7.2$ in 0.1 M NaCl. Polymer solutions were adjusted to pH 7.0 and contained 0.1 M KCl; the concentrations, in monomer molarities, were determined (after dilution, if necessary) spectrophotometrically using $\epsilon_{259}^{259} = 1.010 \times 10^4$ and 0.943×10^4 $M^{-1} \text{cm}^{-1}$ for poly A and poly U, respectively. A Cary model 14 spectrophotometer was used for absorption measurements; the instrument was fitted with a thermostated cell holder, and actual solution temperatures were measured with a thermocouple. A Beckman model 76 meter was employed for pH measurements. Analytical grade reagents and glass-distilled water were used throughout.

In calorimetric experiments on the interaction of poly A and poly U, equal volumes of polymer solutions of approximately

equal concentrations were mixed in the apparatus described by Buzzell and Sturtevant.⁸ These solutions contained 0.01 M sodium cacodylate buffer in addition to 0.1 M KCl. The heat in calories per mole of base pairs formed was calculated on the basis of the polymer present in limiting amount. In the calorimetric experiments with poly A alone, solutions of the polymer were mixed with equal volumes of various concentrations of HCl, the ionic strength being held constant at 0.10 M by addition of KCl. In both types of experiments polymer concentrations of approximately 3.5×10^{-4} M (after mixing) were employed. It was found that the extinction coefficient (259 $\mu\mu$) of poly A at this concentration (1-mm. cell) agrees with that observed in much more dilute solutions at pH 7.0 and between pH 5.5 and 4.0. There was obvious aggregation below pH 4.

Titration curves were determined for poly A at 25° and 10° using a thermostated magnetically stirred titration cell. Titrant (0.1 M HCl or 0.1 M KOH) was added from a Gilmont micro-pipet, and the solutions were saturated with pre-purified nitrogen.

Results and Discussion

Interaction of Poly A and Poly U.—The results of the final series of experiments are summarized in Table I. The value for ΔH at each temperature is the mean of four determinations, and the uncertainty intervals given include the standard error of each set of measurements and an estimate of 2% for calibration uncertainties.

TABLE I

THE HEAT OF INTERACTION OF POLYRIBOADENYLIC ACID AND POLYRIBOURIDYLIC ACID IN 0.1 M KCl, 0.01 M CACODYLATE BUFFER AT pH 7.0

Temperature, °C.	10	25	40
$-\Delta H$, cal. (mole of base pairs) ⁻¹	5220 ± 110	5780 ± 130	6740 ± 270
$-\Delta H$ (corrected) ^a	6290 ± 190	6970 ± 170	7720 ± 290

^a Corrected for the degree of order in the conformation of poly A before reaction; see the discussion in the next section.

The heat evolution in the reaction of poly A with poly U increases with temperature (Table I). Within experimental error, the function $\Delta H/T$, where T is the absolute temperature, varies linearly with temperature. This is shown in Fig. 1 (open circles). Extrapolation to the "melting temperature," $T_m = 331^\circ \text{K}$, of poly (A + U)^{9,10} gives $\Delta H_{331} = -7600$ cal. (mole of base pairs)⁻¹. Although the transition from helix to coil is rather broad for poly (A + U), we may assume that the free energy is close to zero at T_m and calculate a value of -23 cal. deg.⁻¹ (mole of base pairs)⁻¹ for the entropy change at this temperature.

Steiner and Kitzinger⁵ determined the heat of the reaction of poly A with poly U in 0.1, 0.5 and 1.0 M KCl at 25°. Their results are plotted in Fig. 1 (half-filled circles), using 72° and 84° for the melting temperatures^{9,11} in 0.5 and 1.0 M KCl, and are seen to lie on approximately the same line as the data reported in this paper. This result suggests that for poly (A + U), the heat of the transition at T_m is independent of the value of T_m , although the observed heat of reaction is dependent upon the conditions employed.

It is known¹² that poly A and poly U can react to form a complex containing A and U in the ratio 1:2. However this reaction is very slow compared to the formation of the 1:1 complex, and since our calorimetric experiments were of short duration, and no Mg^{++} was present, it may be assumed that no significant amounts of the 1:2 complex were formed. Furthermore, Miles¹³

(8) A. Buzzell and J. M. Sturtevant, *J. Am. Chem. Soc.*, **73**, 2454 (1951).

(9) P. D. Ross and J. M. Sturtevant, *ibid.*, **84**, 4503 (1962).

(10) The melting temperature was determined at a polymer concentration approximately one-tenth that used in the calorimetric experiments. It is assumed that this quantity is independent of concentration.

(11) The value for 1.0 M KCl is estimated from the values observed⁹ at lower ionic strengths.

(12) G. Felsenfeld, D. R. Davies and A. Rich, *J. Am. Chem. Soc.*, **79**, 2023 (1957).

(13) H. T. Miles, *Biochim. Biophys. Acta*, **30**, 324 (1958); **45**, 196 (1960).

(5) R. F. Steiner and C. Kitzinger, Sixth Annual Meeting of the Biophysical Society, Washington, D. C., February, 1962.

(6) R. F. Beers and R. F. Steiner, *Nature*, **179**, 1076 (1957); J. R. Fresco and P. Doty, *J. Am. Chem. Soc.*, **79**, 3928 (1957); R. F. Steiner and R. F. Beers, *Biochim. Biophys. Acta*, **32**, 166 (1959); J. R. Fresco and E. Klemperer, *Ann. N. Y. Acad. Sci.*, **81**, 730 (1959).

(7) R. F. Steiner and R. F. Beers, "The Polynucleotides," Elsevier Publishing Co., Amsterdam, 1961, pp. 374-378.

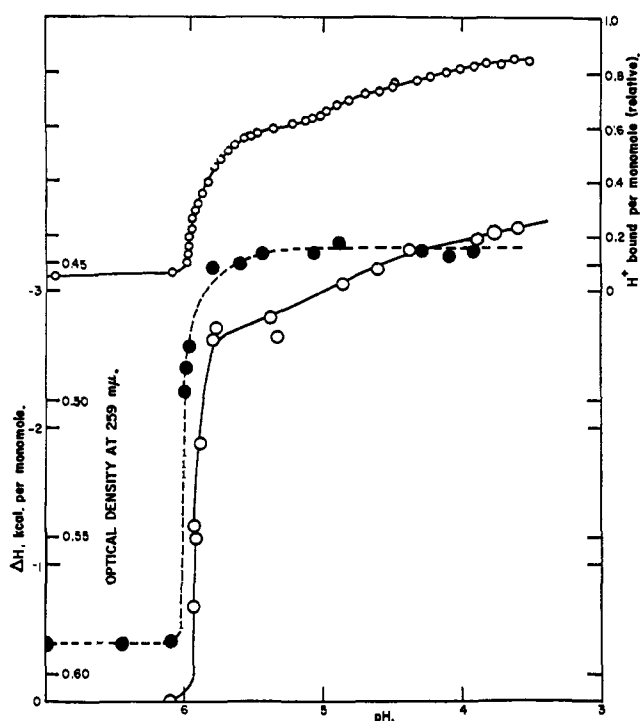


Fig. 2.—The conformational transition of poly A at acid values of pH in 0.1 M KCl at 25°: O, cal. per mole of nucleotide liberated when HCl is added to lower the pH from 7 to the value indicated; o, moles of hydrogen ion bound per mole (relative); ●, optical density (1-cm. cell) at 259 m μ .

has found pronounced differences between the infrared spectra of the 1:1 and 1:2 complexes, and has shown¹⁴ that no detectable change in the spectrum of the 1:1 complex dissolved in 0.1 M NaCl at pH 7, 25°, takes place in a period of 6 weeks. This technique should be able easily to detect the formation of the 1:2 complex to the extent of 10% under these conditions.

The heat of the reaction of poly A with poly U varies rapidly with temperature, the variation corresponding to a change in apparent heat capacity in the reaction given by $\Delta C_p = -(23.0 - 0.103T_m) - 0.206T$ cal. deg.⁻¹ (mole of base pairs)⁻¹; the value at the melting temperature is -57 cal. deg.⁻¹ (mole of base pairs)⁻¹.

Transition of Poly A.—Poly A undergoes a conformational change⁶ when it is exposed to values of pH lower than about 6, as indicated by a decrease in absorption at 259 m μ and changes in optical rotation, light scattering molecular weight and hydrodynamic properties. X-Ray diffraction studies of the fiber at low pH indicate that the acid form of poly A is a two stranded helix.¹⁵

The fact that the optical rotation and the ultraviolet absorption of poly A at pH 7 gradually increases as the temperature is raised suggests that at this pH and 25° the polymer has a partially ordered structure. If this is the case, a correction for the heat effect attendant on disordering the poly A structure should be applied to the data in Table I if it is desired to have them pertain to the formation of a double helix from two randomly coiled polymers. Calorimetric measurements of the poly A transition at 10° and 25° were made in an effort to evaluate such corrections. The measurements were paralleled by determinations of the binding of hydrogen ions by poly A and of its absorption at 259 m μ as functions of the pH.

The results obtained at 25° are summarized in Fig.

(14) H. T. Miles, private communication.

(15) A. Rich, D. R. Davies, F. H. C. Crick and J. D. Watson, *J. Mol. Biol.*, **3**, 71 (1961).

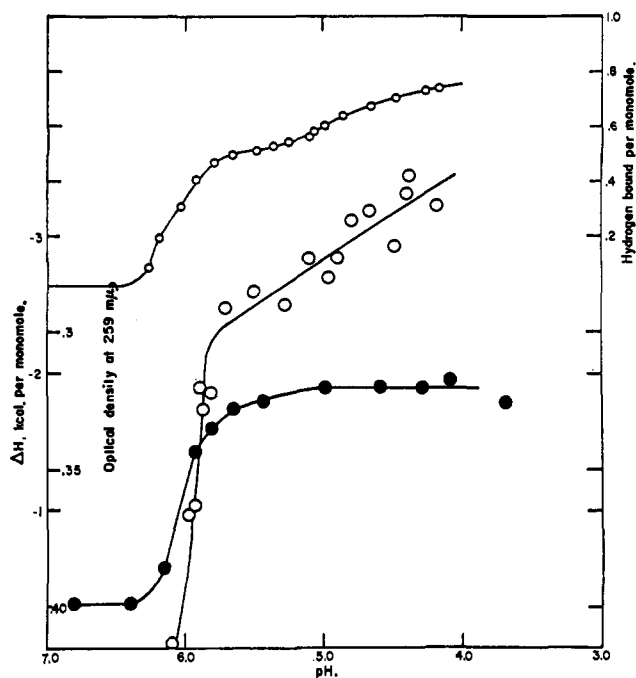


Fig. 3.—The conformational transition of poly A at acid values of pH in 0.1 M KCl at 10°: O, cal. per mole of nucleotide liberated when HCl is added to lower the pH from 7 to the value indicated; o, moles of hydrogen ion bound per mole (relative); ●, optical density (1-cm. cell) at 259 m μ .

2. The spectrophotometric observations indicate a sharp transition occurring close to pH 6. The heat data also show a marked change in apparent heat content at this pH, but a continuing heat evolution at lower values of pH where no further optical density change takes place. We have tentatively attributed this low pH heat evolution to the binding of protons by the polymer. In the region of pH between 5.0 and 3.7 a total of 450 cal. mole⁻¹ is liberated and 0.190 mole of hydrogen ion is bound per mole of adenine. From these figures one obtains for the heat of ionization 2400 cal. per mole of adenine residues, in reasonable agreement with the value 2640 reported earlier¹⁶ for 5'-deoxyriboadenylic acid. In the pH range 7.0 to 5.0 where the conformational transition takes place, 3000 cal. mole⁻¹ are liberated and 0.605 hydrogen ion is bound. If we assume the heat of ionization to be independent of the number of H⁺ bound in the present system, we obtain -1550 cal. (monomer mole)⁻¹ as the heat of the poly A transition at 25°.

The result that an apparent heat of ionization is obtained for helical poly A contrasts with earlier calorimetric observations¹⁷ on the acid denaturation of DNA which led to the conclusion that the heats of ionization of the bases are close to zero in native DNA.

The extinction coefficients of poly A under various conditions are listed in Table II. If it is assumed that at low pH and low temperature the polymer is in a completely helical conformation, and that at high pH and high temperature it is in a random coil conformation, and that under intermediate conditions there is equilibrium between these extremes with the hypochromism varying linearly with the position of the equilibrium, it appears that in the experiments summarized in Fig. 2 a transition from 43.5% helical order in neutral poly A to 100% helix in the acid form was observed. On this

(16) M. Rawitscher and J. M. Sturtevant, *J. Am. Chem. Soc.*, **82**, 3739 (1960).

(17) J. M. Sturtevant and E. P. Geiduschek, *ibid.*, **80**, 2911 (1958); J. M. Sturtevant, S. A. Rice and E. P. Geiduschek, *Discussions Faraday Soc.*, **25**, 138 (1958).

basis we obtain for the heat of the complete transition -2740 ± 200 cal. (monomer mole) $^{-1}$. Similarly a value of -1800 ± 250 cal. (monomer mole) $^{-1}$ is obtained from the data summarized in Fig. 3 for the heat of the transition at 10°

TABLE II
EXTINCTION COEFFICIENT OF POLY A IN 0.1 M KCl

Temp., °C.	pH	$\epsilon_{252} \times 10^{-4}$, $M^{-1} \text{ cm.}^{-1}$	Helix, % ^a
10	4.00	0.760	100 (assumed)
25	4.00	.760	100 (assumed)
10	7.00	.944	58.5
25	7.00	1.010	43.5
40	7.00	1.085	26.5
80	7.00	1.202	0 (assumed)

^a NOTE ADDED IN PROOF.—A correction has not been applied for the effect of protonation in lowering the extinction coefficient of the monomer and presumably also of the polymer. When this effect is included, the estimated values of the helical content are raised about 3% and the calculated heats are unchanged within the experimental errors cited above.

The above interpretation of the poly A data is weakened by the fact that there is less experimental justification for the assumption that poly A exists in only one ordered conformation than there is in the case of the 1:1 poly (A + U) complex.

The heat of the poly A transition can be employed in conjunction with the apparent percentage of helical structure given in Table II to evaluate on a tentative basis corrected heats for the formation of helical poly (A + U) from two random coil polymers. These corrected heats are given in the third row of Table I. The correction at 40° was calculated using the value -3680 cal. (monomer mole) $^{-1}$ for the poly A transition, derived on the assumption of a constant change in apparent heat capacity in the transition of -60 cal. deg. $^{-1}$ (monomer mole) $^{-1}$. The uncertainty limits given in Table I include the experimental uncertainty in the corrections applied. The corrected heats are plotted in Fig. 1 (filled circles), and extrapolate to a value of approximately -8700 cal. (mole of base pairs) $^{-1}$ at the melting temperature of poly (A + U).

The data presented in this paper for poly (A + U) would lead to values of 3 to 4 kcal. per hydrogen bond if all of the observed heat changes accompanying polynucleotide helix formation were attributed to hydrogen bonding between the base pairs. Values of 1–2 kcal. are usually estimated for hydrogen bonds of the types occurring in polynucleotides in aqueous solution. This work, therefore, lends support to the view that forces other than those due to hydrogen bonds are involved in the stabilization of polynucleotide helices.

In earlier work¹⁷ the heats of the acid denaturation of salmon testes DNA in 0.1 M NaCl at 5° , 25° and 40° have been measured calorimetrically. At 25° it was found that 4880 cal. (mole of base pairs) $^{-1}$ was absorbed when the pH was lowered from 7 to 2.8 or lower; that is, when the native helical conformation was changed to that of random coils. If we assume, with DeVoe and Tinoco,¹⁸ that the heats of ionization of the bases in denatured DNA are the same as found¹⁶ for the 5'-deoxymononucleotides, and take the base pairs in the DNA to be 59.2% adenine-thymine pairs, we may compute the heat for the hypothetical process DNA (denatured, pH 7) = DNA (native, pH 7) to be -8260 cal. (mole of base pairs) $^{-1}$. This value is slightly lower than the value -8700 found for the coil to helix transition in poly (A + U) at its melting temperature but considerably higher than the value -5780 cal. found for the interaction of poly A and poly U at 25° .

The data presented in this paper together with the data of Steiner and Kitzinger⁵ suggest that for poly (A + U) the heat of transition at T_m is independent of the value of T_m . For the same to hold with respect to the heats of denaturation of DNA it would be necessary for the ionizations of the bases in denatured DNA to be accompanied by a decrease in heat capacity of approximately 100 cal. deg. $^{-1}$ mole $^{-1}$. Lewin and Tann¹⁹ found $\Delta C_p = -110$ cal. deg. $^{-1}$ mole $^{-1}$ for adenine ionization. From our data on poly A we estimate $\Delta C_p = -80$ cal. deg. $^{-1}$ mole $^{-1}$ for the ionization of adenine residues in helical poly A.

(18) H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 500 (1962).

(19) S. Lewin and N. W. Tann, *J. Chem. Soc.*, 1466 (1962).

[CONTRIBUTION FROM THE MELLON INSTITUTE, PITTSBURGH, PENNSYLVANIA]

Reactions of Alkali Metal Derivatives of Metal Carbonyls. II. Reactions between Acid Chloride Derivatives and the Sodium Derivative of Cyclopentadienyliron Dicarboxyl¹

BY R. B. KING

RECEIVED DECEMBER 21, 1962

The iron complexes $C_5H_5Fe(CO)_2CO(CH_2)_nCOFe(CO)_2C_5H_5$ ($n = 3$ and 4), $C_5H_5Fe(CO)_2(CH_2)_3COFe(CO)_2C_5H_5$ and $C_5H_5Fe(CO)_2CO(CF_2)_3COFe(CO)_2C_5H_5$ have been prepared as stable orange crystalline solids by reactions between $NaFe(CO)_2C_5H_5$ and the appropriate acid chloride derivative. Reaction between the carbonyl chloride derivatives R_2NCOCl ($R = CH_3$ and C_2H_5) and $NaFe(CO)_2C_5H_5$ yields the relatively unstable orange volatile crystalline solids $R_2NCOFe(CO)_2C_5H_5$ ($R = CH_3$ and C_2H_5). Reaction between $[C_5H_5Fe(CO)_2]_2$ and thiolbenzoic acid yields the thiolbenzoate $C_6H_5C(O)SFe(CO)_2C_5H_5$ as a stable orange crystalline solid.

Although several compounds of general formula $RFe(CO)_2C_5H_5$ ($R = CH_3$, C_2H_5 , etc.) have been prepared by the reaction between alkyl halides and the sodium salt $NaFe(CO)_2C_5H_5$ ² and several compounds of general formula $C_5H_5Fe(CO)_2(CH_2)_nFe(CO)_2C_5H_5$ by the reactions between certain polymethylene dibromides and $NaFe(CO)_2C_5H_5$,¹ the reactions between acid chlorides and $NaFe(CO)_2C_5H_5$ have been little investigated. The only reported example of such a

reaction is the reaction between $NaFe(CO)_2C_5H_5$ and acetyl chloride to give the acetyl derivative $CH_3COFe(CO)_2C_5H_5$.³ The experimental details of this reaction, however, do not yet seem to have been described. This paucity of acyl derivatives of general formula $RCOFe(CO)_2C_5H_5$ is in contrast to the existence of numerous acyl derivatives of manganese of general formula $RCOMn(CO)_5$.^{3,4}

(1) For part I of this series see R. B. King, *Inorg. Chem.*, **2**, 531 (1963).

(2) G. Wilkinson and T. S. Piper, *J. Inorg. Nucl. Chem.*, **3**, 104 (1956).

(3) T. H. Coffield, J. Kozikowski and R. D. Closson, Chemical Society, Special Publication 13, Abstracts of Papers submitted at the International Conference on Coordination Chemistry, London, April 6–11, 1959, p. 126.