two rate constants which can be determined separately, k_4 and k_5, both are apparently decreased by all the salts employed. With calcium and strontium chlorides the large decrease in rates in the neutral and alkaline *p*H regions can be correlated with a decrease in the concentration of the labile form of the protein. With other salts the effect is a function of ionic strength and cannot be explained in terms of specific ion binding. Similarly, changes in the value of K_4 alone could not account for the decrease in experimental rates in the alkaline region. In the acid region, however, where the effect of calcium and strontium chlorides is qualitatively the same as that of the other salts, it would appear that the increases in rates noted result in decreases in one or more of the constants K_1, K_2 and K_3. The data in Table I indicate that a change in the value of K_2 is most likely. In the absence of salt these three constants must have values well above 10^{-3} M, and probably as in the case of ricin[14] are too acid to be dissociation constants of the carboxyl groups. The nature of these very acid groups is unknown.

The prototropic group of chymotrypsinogen which could have a pK of 7.1 in urea solution is most likely one of the imidazolium side chains[15] or the α-ammonium group of the terminal cystine residue.[16] Since an imidazole side chain has been postulated to be an essential part of the active site of chymotrypsin,[17] and since calcium chloride increases the activity and reduces the autolysis of this enzyme,[4,8,9] the possibility arises that the calcium binding reported here occurs at the group in the precursor which becomes a part of the active center of the enzyme. Further experiments are planned in order to investigate this possibility.

In a study of the urea denaturation of ovalbumin, Simpson and Kauzmann[18] found, for example, that calcium and magnesium ions accelerated the rate of change of the optical rotation at *p*H 7.6, while anions such as sulfate were found to be inhibitory. This pattern is quite different from the results of the present study, where no salt tested increased the rate of denaturation at neutral *p*H. This difference may represent a fundamental difference in key structural features of the two proteins or may mean that the two methods measure quite different phases of the over-all process of denaturation.

Initially the experiments described here were attempted using 6.6 M urea solution. At this concentration the reactions are considerably slower, and the extent of spectral change was found to be a function of *p*H. This was interpreted as an indication that the reaction measured was reversible and that equilibrium values of the absorbance change were obtained rather than final values. Because the equilibrium is attained only slowly and the *p*H of unbuffered urea solutions drifts slowly toward *p*H 7.5,[18] it was found necessary to use stronger solutions of urea so that reactions were complete within 15 to 20 minutes. In some cases, for example when the high concentrations of calcium chloride were used, this was not possible because of the very slow rates even in 7.9 M urea. However, the kinetics and the results of long time experiments indicate that in these cases the reactions still go to completion, so that the effect of salt is not that of shifting the equilibrium point. In the future it is hoped that the difficulties involved in the study of these equilibrium reactions can be overcome, so that equilibrium and rate constants of reverse reactions can be determined.

Acknowledgment.—The author wishes to acknowledge the support and helpful suggestions of Dr. B. H. J. Hofstee during the course of this work.

(15) P. E. Wilcox, E. Cohen and W. Tan, *J. Biol. Chem.*, **228**, 999 (1957).

(16) F. R. Bettelheim, *ibid.*, **212**, 235 (1955).

(17) L. W. Cunningham, *Science*, **125**, 1145 (1957).

(18) R. B. Simpson and W. Kauzmann, THIS JOURNAL, **75**, 5139 (1953).

PALO ALTO, CALIFORNIA

[CONTRIBUTION NO. 1531 FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

The Heat of Denaturation of Ferrihemoglobin in Acid Solution[1]

BY W. W. FORREST AND J. M. STURTEVANT

RECEIVED FEBRUARY 19, 1959

The reversible acid denaturation of horse ferrihemoglobin in formate buffer has been subjected to calorimetric study. At 25° in formate buffer a heat absorption of 10.0 ± 0.3 kcal./mole (mol. wt. 68,000) is observed, the kinetics of heat absorption agreeing fairly well with the kinetics of denaturation reported by Steinhardt and co-workers.[2] At 15°, a heat evolution of 76.0 ± 1.6 kcal./mole is observed; at this temperature the calorimetric kinetics deviate considerably from the denaturation kinetics given by Steinhardt, *et al.* The large influence of temperature on the heat of the reaction is indication of the complexity of the reaction. The results obtained are discussed in connection with the nature of the reaction.

The acid denaturation of horse ferrihemoglobin has recently received detailed study by Steinhardt[2] and his co-workers. The reaction is reversible (although accompanied by a considerably slower irreversible denaturation), and it takes place at a measurable rate, which increases with decreasing *p*H, when the *p*H of a ferrihemoglobin solution is taken below about *p*H 4.5 (at 25°). The reaction is accompanied by a loss of solubility at the isoelectric point, a large decrease in absorp-

(1) This research was aided by grants from the National Science Foundation (G179) and the United States Public Health Service (RG3996).

(2) (a) J. Steinhardt and E. M. Zaiser, THIS JOURNAL, **75**, 1599 (1953); (b) E. M. Zaiser and J. Steinhardt, *ibid.*, **76**, 1788 (1954); (c) E. M. Zaiser and J. Steinhardt, *ibid.*, **76**, 2866 (1954); (d) J. Steinhardt and E. M. Zaiser, *Advances in Protein Chem.*, **10**, 151 (1955); (e) J. Steinhardt, E. M. Zaiser and S. Beychok, THIS JOURNAL, **80**, 4634 (1958); (f) S. Beychok and J. Steinhardt, *ibid.*, **81**, 5679 (1959).

tion at 406 mμ, and a striking increase in the number of groups titratable in the acid range of *p*H values. It has also been shown by Tanford[3] that the reaction is accompanied by a marked increase in intrinsic viscosity. Steinhardt,[2] *et al.*, have studied the kinetics and equilibrium of the reaction, utilizing solubility, spectrophotometric and titration data. The denaturation process has appeared to follow first-order kinetics but in recent work[2e] the regeneration process has been found to be of a more complex kinetic type.

Beychok and Steinhardt[2f] have concluded that approximately 36 acid binding groups become available to titration during denaturation and that 22 of these groups are in some way "masked" in the native protein so as to be inaccessible to protons. Tanford[3] has shown that a degree of expansion and dissociation (see below) of the molecule consistent with the observed viscosity increase could decrease electrostatic interactions sufficiently to account for a considerable portion of the uptake of protons observed by Steinhardt, *et al.*; assumptions regarding "masking" are unnecessary with respect to these protons.

Field and O'Brien[4] have concluded on the basis of sedimentation and diffusion measurements that human hemoglobin undergoes reversible dissociation into half-molecules at low *p*H, the extent of dissociation increasing with dilution and with decreasing *p*H. It is thus possible that the observations of Steinhardt and co-workers, as well as those reported here, are complicated by dissociation, which may well be an integral part of the denaturation reaction. All heat values are reported here on the basis of a molecular weight of 68,000.[5]

In view of the complexity of this interesting reaction, it seemed useful to measure the enthalpy changes accompanying it.

Experimental

Ferrihemoglobin was obtained by the oxidation with potassium ferricyanide of recrystallized horse oxyhemoglobin prepared by the method of Keilin and Hartree.[6] Stock solutions of ferrihemoglobin were deionized by passing them through an ion-exchange column.[7] The ion-free preparations were found to be quite stable and could be stored in the cold room for some months without the formation of appreciable insoluble material. The kinetics of denaturation of solutions prepared from deionized material was indistinguishable from that of solutions prepared from dialyzed protein.

The protein content of ferrihemoglobin solutions was determined by Kjeldahl nitrogen analysis, taking the nitrogen content[5] to be 16.8%, and was checked by determination of the absorption of suitably diluted samples at 500 mμ.[8] The "initial" absorption of 0.030% solutions at 406 mμ in formate buffers of 0.02 *M* ionic strength in the *p*H range 3.5–4.0 gave a mean extinction coefficient of 159 ± 2 (per mmole Fe per l., protein mol. wt. = 68,000) in good agreement with the value 162 reported by Zaiser and Steinhardt.[2b]

All calorimetric and spectrophotometric experiments were carried out in formate buffers 0.02 *M* in sodium formate, with no other added salt present. The *p*H was determined at the temperature of each experiment by means of a glass electrode standardized with a buffer 0.1 *M* in acetic acid and 0.1 *M* in sodium acetate, the *p*H of which was taken to be 4.66 at 15° and 4.65 at 25°.[9]

The calorimetric apparatus and method have been previously described.[10] In each calorimetric experiment equal volumes of a solution of ferrihemoglobin in formate buffer at *p*H 4.8 and of a formate buffer of lower *p*H were mixed. In preliminary runs at 25° a rather large heat absorption lasting for several hours was observed on mixing the reactant solutions. This endothermic reaction appeared to be heterogeneous since its rate was temporarily increased by stirring the contents of the calorimeter. It was found that this reaction, which presumably involved buffer species, was completely eliminated by saturating the reactant solutions with N_2 and excluding O_2 from the system.

In the process of reducing the *p*H of the ferrihemoglobin solution from 4.8 to the *p*H of denaturation, an instantaneous heat effect which was several times as large as the slow heat effect associated with the denaturation took place. The extrapolation procedure[10] employed in the calculations in principle gives the heat of the slow process independent of the heat of mixing. However, the large heat of mixing rendered difficult the accurate determination of the heat of the slow reaction, and limited both the range of ferrihemoglobin concentrations which could be employed and the *p*H range which could be covered.

Results

Heat of Ionization of Formic Acid.—Since hydrogen ions are taken up by the protein during the denaturation, there is a contribution to the observed heat of denaturation arising from the liberation of these protons by the buffer acid. We have determined the heat of ionization of formic acid under the conditions of the denaturation experiments in order to be able to apply appropriate buffer corrections. A solution of formate buffer of *p*H 4.0, 0.04 *M* in sodium formate, was mixed in the calorimeter with an equal volume of dilute HCl. The resulting heat effects, after correction for the heats of dilution of the HCl,[11] are summarized in Fig. 1. The slopes of the lines give the heats of ionization, which turn out to be −7 cal./mole at 25° and +341 cal./mole at 15°. From these figures, $\Delta C_p = -34.8$ cal. mole^{-1} deg.$^{-1}$. Electromotive force measurements[12] lead to −23 cal./mole for the heat of ionization at 25° and −41.7 cal. mole^{-1} deg.$^{-1}$ for ΔC_p, both at zero ionic strength.

The Heat and Rate of Denaturation at 25°.—The data obtained at 25° are summarized in Table I. The first column gives the final *p*H, the second column the calories absorbed per liter and the third column the heat of reaction in kcal./mole. The heat absorption proceeded according to apparent first-order kinetics, with the rate constants listed in the last column of Table I. The individual points in each run fell on a semilogarithmic plot with an average deviation usually amounting to less than 1% of the total change, up to 80–90% completion.

The logarithms of the rate constants are plotted against the *p*H in Fig. 2. They adhere reasonably well to a straight line relation of slope −2.4. The rates determined by Steinhardt and Zaiser[2] by spectrophotometry at 406 mμ follow the rela-

(3) C. Tanford, THIS JOURNAL, **79**, 3931 (1957).

(4) E. O. Field and J. T. P. O'Brien, *Biochem. J.*, **60**, 656 (1955).

(5) H. Neurath and K. Bailey, Eds., "The Proteins," Vol. 1, Academic Press, Inc., New York, N. Y., 1953, p. 215.

(6) D. Keilin and E. F. Hartree, *Proc. Roy. Soc.* (*London*), **B117**, 1 (1935).

(7) H. M. Dintzis, Ph.D. Thesis, Harvard University, 1952.

(8) B. L. Horecker, *J. Biol. Chem.*, **148**, 173 (1943).

(9) R. G. Bates, *Chem. Revs.*, **42**, 1 (1948).

(10) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **69**, 607 (1947).

(11) J. M. Sturtevant, *ibid.*, **62**, 3265 (1940).

(12) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold Publishing Co., New York, N. Y., 1958, p. 667.

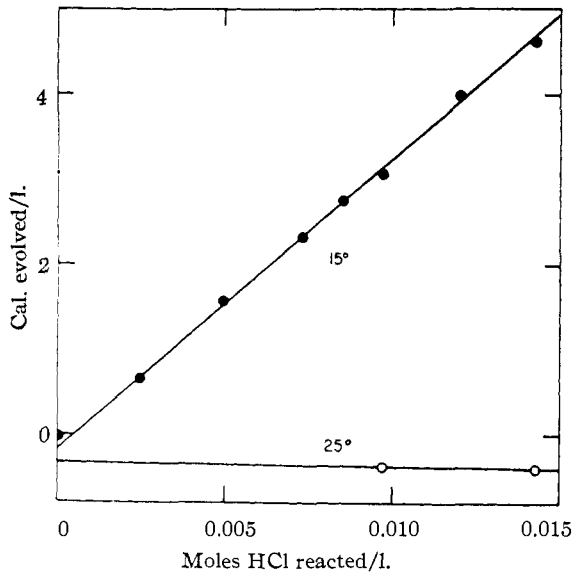


Fig. 1.—The heat evolved on adding HCl to formate buffer at 15 and 25°. Final ionic strength was 0.02 *M*.

tion indicated by the dashed line, which has a slope of −2.5. We have performed a few spectrophotometric rate determinations, using a Cary recording spectrophotometer, with the results shown in Fig. 2. There is also given in Fig. 2 a single rate constant determined by the *p*H-stat method.[13]

TABLE I

THE ACID DENATURATION OF HORSE FERRIHEMOGLOBIN IN FORMATE BUFFER (0.02 *M* SODIUM FORMATE) AT 25°

*p*H	Cal. absd. per l.	Δ*H*, kcal./mole[a]	*k*, min.$^{-1}$
3.79	1.26	11.3	0.029
3.78	1.22	10.9	.046
3.77	1.09	9.8	.045
3.75	0.97	8.7	.075
3.73	0.94	8.4	.050
3.67	0.92	8.2	.068
3.67	0.97	8.7	.080
3.65	1.01	9.0	.098
3.65	0.95	8.5	.14
3.65	1.06	9.4	.14
3.64	1.16	10.4	.080
3.64	1.07	9.6	.071
3.61	1.26	11.5	.13
3.60	1.25	11.2	.12
3.59	1.24	11.0	.13
3.53	1.32	11.7	.18
3.52	0.85	9.6	.20
3.50	1.18	10.6	.21
3.50	1.43	12.7	.19
3.49	1.19	10.6	.19
3.43	1.16	10.4	.29
3.40	0.93	8.3	.29
3.40	1.03	9.2	.35
		Mean 10.0	
		St. error of mean 0.3	

[a] Mol. wt. 68,000.

(13) G. W. Schwert, H. Neurath, S. Kaufman and J. E. Snoke, *J. Biol. Chem.*, **172**, 221 (1948).

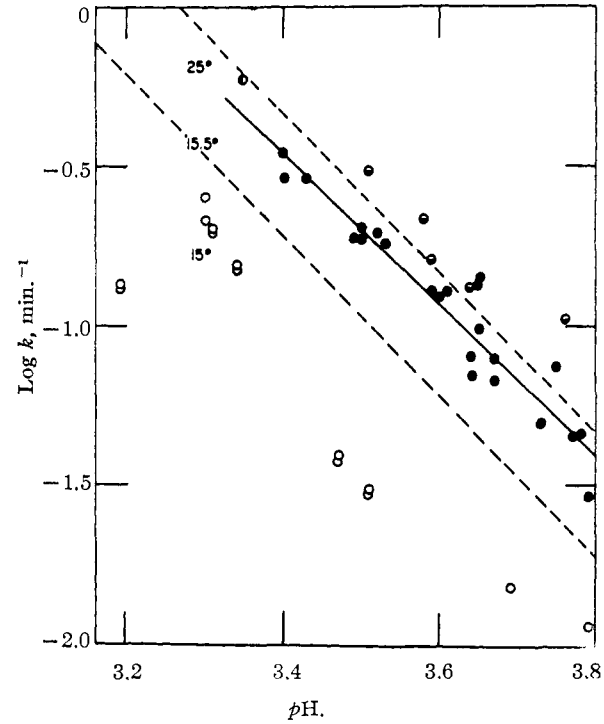


Fig. 2.—Rate of denaturation of horse ferrihemoglobin as a function of *p*H in formate buffer (0.02 *M* sodium formate). Methods of observation: ○, calorimetric at 15°, 0.61 and 1.17% protein; ●, calorimetric at 25°, 0.76% protein; ◐, *p*H-stat at 25°, 0.61% protein; ◒, spectrophotometric at 25°, 0.03% protein. Dashed lines, data of Zaiser and Steinhardt, ref. 2.

The calorimetric rates are sufficiently close to those determined by other methods so that it seems legitimate to assume that the denaturation reaction is the process actually observed in the calorimeters.

According to the equilibrium data of Steinhardt and Zaiser,[2b] based primarily on spectrophotometric data in formate buffers at a protein concentration considerably lower than that used in the calorimetric experiments, the reaction should have been incomplete at the higher *p*H values listed in Table I. Within the rather large uncertainty of the individual heat values, there is no indication of such incompleteness in the calorimetric data.

The Heat and Rate of Denaturation at 15°.—The data observed at 15° are given in Table II, listed in the same manner as in Table I. As is the case at 25°, the heat changes follow apparent first-order kinetics with good accuracy. The logarithms of the calorimetrically observed first-order rate constants are plotted against the *p*H in Fig. 2. They do not appear to vary linearly with *p*H as do the 25° data and they deviate rather widely from the spectrophotometric rates at 15.5° reported by Steinhardt and Zaiser. In spite of these differences in rates (perhaps partly attributable to the widely different concentrations used in the two types of experiments), we shall assume that the calorimetric observations pertain to essentially the same denaturation process as studied by Steinhardt and Zaiser.

There is some indication in the heat data in Table II of incomplete reaction at *p*H 3.79 and 3.69. If the ΔH values for these two *p*H's are corrected according to the equilibrium data given by Steinhardt and Zaiser, they agree very well with the mean ΔH for 15°. These two values were omitted in computing the mean value given in Table II.

TABLE II

THE ACID DENATURATION OF HORSE FERRIHEMOGLOBIN IN FORMATE BUFFER (0.02 *M* SODIUM FORMATE) AT 15°

*p*H	Cal. evolv. per l.	$-\Delta H$, kcal./mole[a]	k, min.$^{-1}$
3.79	4.70	52.6	0.012
3.69	5.65	63.2	.015
3.51	7.23	80.9	.031
3.51	7.32	81.8	.030
3.47	7.72	86.3	.038
3.47	6.90	77.2	.039
3.34	12.3	71.7	.15
3.34	11.7	68.2	.16
3.31	13.0	76.0	.20
3.31	13.9	81.4	.20
3.30	11.9	69.4	.22
3.30	12.6	73.9	.25
3.19	12.0	70.2	.14
3.19	12.8	74.8	.13
	Mean	76.0 (excl. *p*H 3.79 and 3.69)	
	St. error of mean	1.6	

[a] Mol. wt. 68,000.

Discussion

The Change in Heat Capacity.—The denaturation was found to be strongly exothermic at 15° and weakly endothermic at 25°. If it is assumed that 36 protons are bound per mole of protein during the reaction, the heat at 15° resulting from separating them from the formic acid buffer would be + 12,500 cal. per mole of protein, while that at 25° would be negligible. We thus obtain for the corrected heat of the denaturation reaction −88,500 cal./mole at 15° and +10,000 cal./mole at 25°. Comparison of these two figures indicates that for the denaturation process at 20°, ΔC_p = +9850 cal. mole^{-1} deg.$^{-1}$. The only previously reported[14] ΔC_p approaching this magnitude is +8000 cal. mole^{-1} deg.$^{-1}$ for the increase in apparent heat capacity of bovine serum albumin in solution when the *p*H is dropped from 5 to 3. The similarity of these figures, together with the various experimental indications[3,15] that both processes are accompanied by molecular expansion, suggests that these processes may be fundamentally somewhat similar, in spite of the fact that the reaction is much slower in the case of ferrihemoglobin than in the case of serum albumin, and that the variation of equilibrium with *p*H is different in the two cases.

The Nature of the Acid-binding Groups.—Beychok and Steinhard[2f] concluded that of the approximately 36 groups which become available to titration during the denaturation, 22 are "masked" in the native protein and are probably imidazole groups of histidine residues. It is of interest to consider the calorimetric data in connection with the nature of the acid-binding groups.

The heats of ionization of carboxylic acids are usually not far from zero,[16] and those of imidazolinium groups lie in the range + 7000 to + 9000 cal./mole.[16] If it is assumed that ionization heats are not significantly altered by environmental effects within the protein molecule, it would be expected that the ionization processes accompanying the denaturation would result in a contribution of the order of − 175,000 cal./mole to the total heat of denaturation at both 15 and 25°. This figure is about twice as large as the observed heat of denaturation at 15° and many times as large as, and of opposite sign to, the heat of denaturation at 25°.

It is, of course, possible that a large heat evolution due to ionization reactions is partially balanced at 15°, and overbalanced at 25°, by a large heat absorption due to molecular expansion, solvent penetration and other effects accompanying the denaturation reaction. However, the addition of protons to bovine serum albumin[14] below *p*H 5 takes place with a net heat effect which is consistent with the view that the protons are all added to carboxylate ions, and that the configurational changes which take place as a result of the addition of protons do not produce large heat effects. In view of the similarity of the configurational changes in bovine serum albumin and in ferrihemoglobin, briefly mentioned above, it is concluded that the present data for ferrihemoglobin are more reasonably interpreted on the basis of protons adding predominantly to carboxylate ions rather than to imidazole groups.

Apparent Heats of Reaction Deduced from Equilibrium Measurements.—Steinhardt and Zaiser[2c,e] estimated from the temperature coefficient of the spectrophotometrically observed equilibrium constants that the *apparent* heat of reaction is practically zero between 15.5 and 25°, and about + 32,000 cal./mole between 0.2 and 15.5°. Their discovery[2e] of the complicated kinetics of the regeneration reaction renders the interpretation of these values uncertain, but even so there appears to be sharp disagreement with the calorimetric observations,[17] which indicate a much more negative heat at 15 than at 25° rather than a more positive one. A situation of this sort can conceivably arise where different observational methods are employed. As a simple example consider the case of two successive first-order reversible reactions

$$A \underset{\Delta H_1}{\overset{K_1}{\rightleftharpoons}} B \underset{\Delta H_2}{\overset{K_2}{\rightleftharpoons}} C \qquad (1)$$

(14) P. Bro and J. M. Sturtevant, THIS JOURNAL, **80**, 1789 (1958).

(15) C. Tanford, J. G. Buzzell, D. G. Rands and S. A. Swanson, *ibid.*, **77**, 6421 (1955).

(16) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1958, pp. 452 and 464.

(17) In this connection it should be noted that in macromolecular systems there is no general necessity for agreement between van't Hoff and calorimetric heats of reaction per mole, quite apart from the usually negligible difference to be expected because the van't Hoff isochore gives the standard heat of reaction, since the size of the "mole" appearing in the van't Hoff value is unknown. This has been illustrated in the denaturation of deoxyribose nucleic acid (J. M. Sturtevant, S. A. Rice and E. P. Geiduschek, *Disc. Faraday Soc.*, **25**, 138 (1958)). This point of course has no bearing on the difference in sign of ΔH observed in the present case.

Suppose equilibrium measurements are based on observations of a property to which only B contributes and the presence of C is unsuspected. The apparent equilibrium constant is then

$$K_{app} = \frac{b_e}{a_0 - b_e} = \frac{K_1}{1 + K_1K_2} \quad (2)$$

where a_0 is the total concentration and b_e is the equilibrium concentration of B. Differentiation with respect to temperature gives

$$\Delta H_{app} = \frac{\Delta H_1 - K_1K_2\Delta H_2}{1 + K_1K_2} \quad (3)$$

A calorimetric experiment would give for the observed heat of reaction per mole of A originally present

$$\Delta H_{obs} = \frac{K_1(1 + K_2)\Delta H_1 + K_1K_2\Delta H_2}{1 + K_1 + K_1K_2} \quad (4)$$

If the calorimetric experiment were performed under conditions where A is completely reacted ($K_1 >> 1$)

$$\Delta H_{obs} = \Delta H_1 + \frac{K_2}{1 + K_2}\Delta H_2 \quad (5)$$

It is obvious that with arbitrary values of the various quantities, equations 3 and 4 can give very different results. For example, if $\Delta H_1 = K_1K_2\Delta H_2$, $\Delta H_{app} = 0$ and $\Delta H_{obs} = \Delta H_1$. Similarly, if only C is observed

$$K_{app} = \frac{c_e}{a_0 - c_e} = \frac{K_1K_2}{1 + K_1} \quad (6)$$

and

$$\Delta H_{app} = \frac{\Delta H_1}{1 + K_1} + \Delta H_2 \quad (7)$$

It is evident, in view of these considerations, that it is not necessarily surprising to find apparent reaction heats deduced from indirect equilibrium measurements which do not agree with heats determined calorimetrically.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

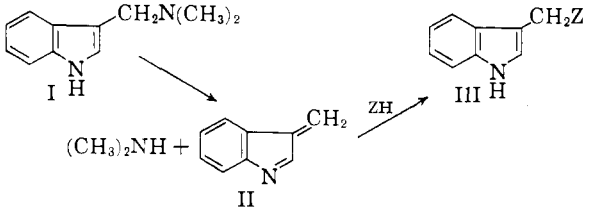
Synthesis and Reactions of α-Cyanogramine[1]

BY PHILIP N. JAMES[2] AND H. R. SNYDER

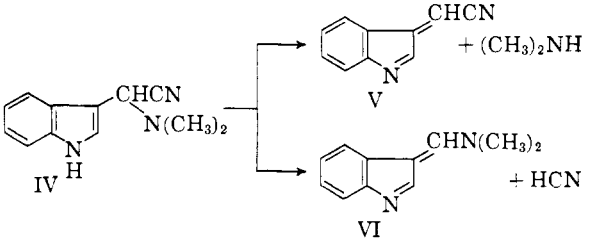
RECEIVED JUNE 26, 1959

The synthesis of α-cyanogramine from indole-3-aldehyde by the Strecker reaction is reported. This compound does not undergo the simple alkylation reactions characteristic of gramine, but does exchange its dimethylamino group with piperidine and does release dimethylamine on pyrolysis. It is easily converted to the parent aldehyde by hydrolysis, reduced to gramine by lithium aluminum hydride, converted to 3-dimethylaminomethylene-3H-pseudoindole by strong, non-aqueous bases, and converted to a yellow crystalline solid, which is not a simple alkylation product, by diethyl nitromalonate. Its reaction with methyl iodide is complex, tetramethylammonium iodide being the only product isolated. It forms an unstable monopicrate which decomposes to dimethylamine picrate on attempted recrystallization. The significance of these reactions in relation to a proposed mechanism for carbon–carbon alkylations with gramine is discussed.

There is now ample evidence for the elimination–addition mechanism of reactions in which Mannich bases of the gramine (3-dimethylaminomethylindole, I) type serve as alkylating agents.[3] The reactive intermediate appears to be 3-methylene-3H-pseudoindole (II), which is formed in the presence of a reagent such as an active methylene compound that immediately adds to it to form the alkylation product III.



α-Cyanogramine (IV) would appear to offer an interesting alternative to the loss of dimethylamine, for a 3H-pseudoindole could arise by the elimination of either the secondary amine or hydrogen cyanide. The present work was undertaken to search for evidence of the two different eliminations.



α-Cyanogramine was prepared from indole-3-aldehyde by a modification of the Strecker reaction.[4] It proved to be much less stable than gramine, decomposing not far above 100° and undergoing hydrolysis to indole-3-aldehyde even in neutral solution. Its sensitivity precludes its use in many reactions which find valuable synthetic applications with gramine.[5] The thermal decomposition occurred with the loss of dimethylamine (70% yield, isolated as the phenylthiourea) and the formation of a red-brown gum which resisted all attempts at separation into pure sub-

(1) Abstracted in part from a Thesis submitted by Philip N. James to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1957.

(2) National Science Foundation Fellow, 1955–1957.

(3) J. D. Albright and H. R. Snyder, THIS JOURNAL, **81**, 2239 (1959).

(4) For a discussion of methods of synthesis of α-aminonitriles, see V. Migrdichian, "The Chemistry of Organic Cyanogen Compounds," A.C.S. Monograph Series No. 105, Reinhold Publishing Corp., New York, N. Y., 1947, Ch. 10.

(5) See J. H. Brewster and E. L. Eliel, "Organic Reactions," Vol. VII, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 99.