[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF ILLINOIS]

The Formation of Hydrazine by Interaction of Ammonia and N-Chlorosuccinimide in Alkaline Solutions

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RECEIVED SEPTEMBER 26, 1955

N-Chlorosuccinimide (NCS) reacts with aqueous ammonia in solutions made strongly alkaline by the addition of sodium hydroxide to form hydrazine. Yields of hydrazine rise with increase in the mole ratio of NH_3/NCS and go through a maximum at a mole ratio NaOH/NCS = approximately 2.5 for solutions containing NH_3/NCS in a 100:1 mole ratio. The addition of small quantities of gelatin also improves hydrazine yields. Other N-chloro derivatives such as N-chlorophthalimide and 1,3-dichloro-5,5-dimethylhydantoin (commercially available as "Halane") also have been found to give hydrazine when allowed to react with aqueous ammonia under similar conditions. These reactions entail the intermediate formation of chloramine which then reacts with excess ammonia under appropriate conditions to give hydrazine.

Introduction

Many investigators have studied the reactions of various organic halogen compounds containing so-called "positive halogen" with hydrazine and ammonia. Macbeth and co-workers[1] have shown that hydrazine can be oxidized quantitatively to nitrogen by the bromo derivatives of malonic ester and by N-halo compounds such as the chloramides, N-chlorosuccinimide and N-chlorosulfonamides. Under the same conditions ammonia is oxidized only by a limited number of such halogen compounds. Only N-chloro- and N-bromosuccinimide and the N-dichlorosulfonamides exert an oxidizing action on ammonia. Significant is the observation that the volume of nitrogen gas evolved in such ammonia oxidations is always 1 to 4% lower than the theoretically calculated amount.

Audrieth and co-workers[2] have recently studied the reaction between *t*-butyl hypochlorite and ammonia. The addition of strong base in the form of sodium hydroxide was found to inhibit oxidation of ammonia to nitrogen and to result in the formation of hydrazine. Preliminary observations in this Laboratory had shown that N-chlorosuccinimide (NCS) acts upon ammonia in alkaline solutions to give appreciable quantities of hydrazine, especially if a large excess of ammonia is employed. This interesting observation has now been studied in some detail and has been extended to other N-halo compounds.

Experimental

Materials.—The N-chlorosuccinimide was obtained from the Arapahoe Chemical Company and was recrystallized before use. Schaar granular USP gelatin was employed. Other N-halo compounds were prepared from the corresponding imide derivatives. In each case the content of active halogen was checked analytically by reaction with potassium iodide and subsequent titration with thiosulfate. The active halogen content was found to vary between 95–97% of theory. This factor was taken into consideration when given weights of substances were employed in carrying out a particular reaction.

Analytical Methods.—The direct iodate method in strong hydrochloric acid solution was employed for the determination of hydrazine.[3] Brilliant Scarlet 3-R[4] was used as indicator.

A method was developed for the determination of hydrazine in the presence of active halogen based upon the observation that thiosulfate decomposes active halogen compounds in alkaline solution but has no effect upon hydrazine. The following procedure was used to determine semi-quantitatively the rate of reaction between NCS and ammonia.

An aliquot of the reaction mixture was removed from the solution and immediately introduced into an excess of thiosulfate solution. The resulting solution was acidified with hydrochloric acid in the cold. The excess thiosulfate was then decomposed by boiling with concentrated HCl. In order to obtain reproducible values, it was found necessary to boil the reaction solution for at least two hours with concentrated hydrochloric acid and to replenish from time to time the acid and water lost by evaporation.

Experimental Procedure.—Test solutions were prepared containing one mole of ammonia, 0.1 g. of gelatin (added as a freshly prepared 1% solution), 0.01 mole of NCS and 0.03 mole of sodium hydroxide added as a 6 *M* solution. The concentration of one of the components was varied for each series of experiments. The concentrations of the other components were held constant. The respective solutions of ammonia, gelatin and sodium hydroxide were mixed, cooled and diluted to 100 ml. The solid halogen compound was then added in one single increment, and the solution was shaken thoroughly to effect complete solution. Twenty five ml. quantities were removed for analysis. All reactions were carried out at room temperature except for the one series of experiments in which determination of the rate of the reaction was made at 0°.

The formation of hydrazine was verified by acidifying the reaction mixture with acetic acid and adding salicylaldehyde to precipitate salicylalazine. In two experiments using the quantities specified above, there were isolated 1.32 and 1.37 g. of salicylalazine, m.p. 214–215°, corresponding to 55 and 57% yields (iodate analysis, 58% yields).

Effect of the Hydroxide Ion Concentration upon the Yield of Hydrazine.—In the absence of sodium hydroxide yields of hydrazine varied between 5 and 8%. Yields were found to rise with increasing concentrations of added sodium hydroxide and to reach a maximum of about 60% in solutions containing a mole ratio of NaOH/NCS $\cong$ 2.5. Further additions of alkali were found to affect hydrazine yields adversely. Analytical results for a typical run using the standard quantities of reagents are depicted graphically in curve A, Fig. 1.

When potassium carbonate was used as added base, a much slower increase in hydrazine yields was observed. Even for a ratio of about 20 moles K_2CO_3 per mole of NCS, the yield amounted to only 21%. The data for a typical experimental series are presented in curve B, Fig. 1.

The Influence of the Ammonia/NCS Ratio.—Variation in the mole ratio of ammonia to NCS from 20/1 to 200/1 showed that the hydrazine yield increases rapidly at lower mole ratios, but more slowly when a ratio of 100/1 (ammonia/NCS) is exceeded (see Fig. 2).

Influence of Gelatin.—For the standard test solution in the absence of gelatin, yields between 3 and 5% of hydrazine are obtainable. If the quantity of gelatin is gradually increased, there is observed an initial marked increase in the yield of hydrazine, after which the increase is slower and the yield becomes fairly constant when more than 10 g. of gelatin per mole NCS is present. On the basis of these findings, the standard test solution was set up to correspond to 10 g. of gelatin per mole of NCS.

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(1) A. K. Macbeth and co-workers, *J. Chem. Soc.*, **119**, 1356 (1921); **121**, 904, 2169, 2527 (1922).

(2) L. F. Audrieth, E. Colton and M. M. Jones, THIS JOURNAL, **76**, 1428 (1954).

(3) R. A. Penneman and L. F. Audrieth, *Anal. Chem.*, **20**, 1058 (1948).

(4) Brilliant Scarlet 3-R was found to be an excellent indicator for active halogen. This particular dye is immediately decolorized in the cold in hydrochloric acid solution by any compound containing active halogen.

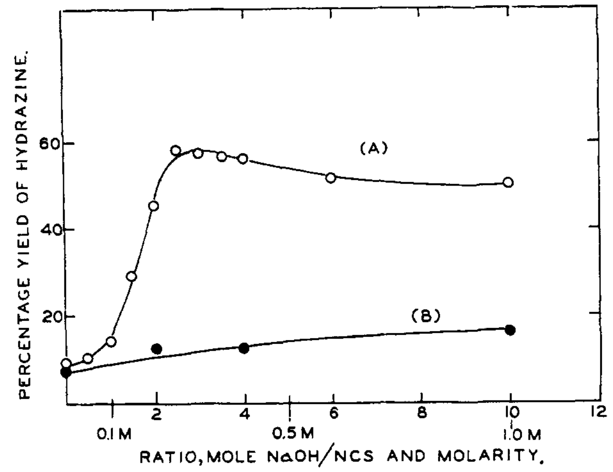

Fig. 1.—Effect of NaOH (A) and K_2CO_3 (B) on the yield of hydrazine obtainable from N-chlorosuccinimide and aqueous ammonia; mole ratio NH_3/NCS = 100; gelatin content, 0.1 g. per 100 ml. of reaction mixture. The mole ratio NaOH/NCS and the molarity of NaOH are both given on the horizontal axis.

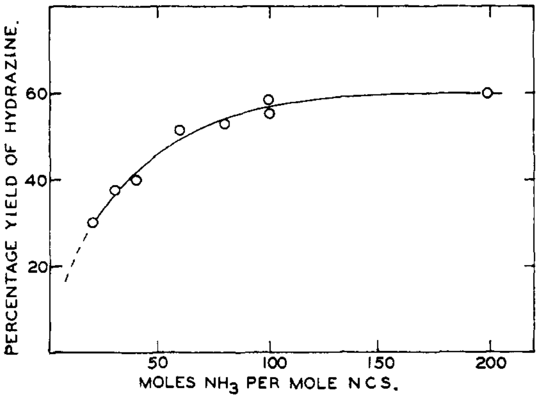

Fig. 2.—Effect of mole ratio of NH_3 to NCS on the yield of hydrazine. Test solutions contained 0.01 mole NCS, 0.03 mole NaOH and 0.1 g. of gelatin per 100 ml.

Reaction Rates.—Two series of experiments were carried out using fourfold quantities of the test solutions described above to determine semiquantitatively the hydrazine yield as a function of time at 0 and at 25°. At 0°, the reaction is not complete even after a five-hour period (see Fig. 3).

Reaction of Other N-Halo Compounds with Ammonia in Alkaline Solutions.—Both N-chlorophthalimide and "Halane" (1,3-dichloro-5,5-dimethylhydantoin) were found to give hydrazine by reaction with ammonia in alkaline solution. Here again, yields were found to rise upon the addition of strong base (sodium hydroxide), although neither one of these substances was found to be as effective in producing hydrazine by reaction with ammonia as was N-chlorosuccinimide. N-Bromoacetamide and N-bromosuccinimide were also allowed to react with alkaline solutions of ammonia, but no hydrazine was obtained in either instance. Vigorous reaction was found to occur with the evolution of nitrogen.

Discussion

Chloramine has been shown to be the active intermediate in the formation of hydrazine by the interaction of ammonia with either sodium hypochlorite (Raschig synthesis) or *t*-butyl hypochlorite.[2] The initial step in the interaction of NCS and other N-chloro derivatives with ammonia also involves formation of chloramine. We have been able to extract chloramine from the cold aqueous reaction mixture by means of ether and thus verify its formation as the reactive intermediate. Subsequent conversion of the chloramine to give maximum yields of hydrazine will take place effectively only if the reaction is then carried out under conditions more strongly alkaline than those afforded by the presence of ammonia *per se*. The presence of gelatin as a metal deactivator is also desirable if substantial yields of hydrazine are to be achieved by the interaction of NCS and ammonia in alkaline solution.

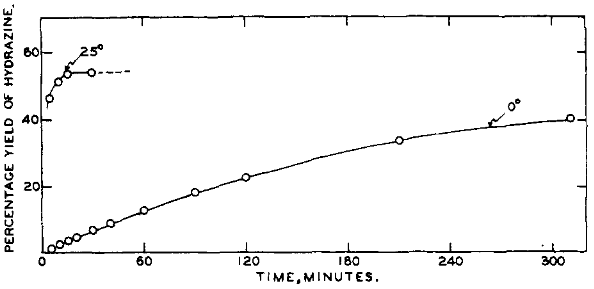

Fig. 3.—Effect of temperature on the ammonia-N-chlorosuccinimide reaction; initial concentrations $[NH_3]$ = 10 *M*; [NCS] = 0.1 *M*; [NaOH] = 0.3 *M*. Test solutions contained 1 g. of gelatin per liter.

Our experimental results again demonstrate conclusively that yields of hydrazine depend markedly on the $[OH^-]$ concentration. The study of the NCS–ammonia reaction, in which the N-chloro compound serves merely as a source of chloramine, has enabled us to confirm our earlier observations with respect to the mechanism of the Raschig synthesis.[2]

Since the stability of aqueous chloramine decreases as solutions are made more strongly alkaline,[5] we undertook in the present study to determine the effect of $[OH^-]$ greater than those used in any of our earlier studies.[2,6] It is interesting to note that the yields of hydrazine rise to a maximum but begin to decrease in solutions containing added sodium hydroxide in concentrations exceeding approximately 0.25 *M*. We are inclined to consider this effect to be due to the tendency for chloramine to undergo decomposition (self oxidation–reduction) to an even greater extent as the hydroxide ion concentration is increased.[5] Such a chloramine decomposition reaction thus becomes increasingly competitive, as the $[OH^-]$ is raised beyond a certain point, with the reaction leading to hydrazine formation. For the more strongly alkaline solutions consideration must also be given to the fact that increase in $[OH^-]$ concentration favors formation of hypochlorite based upon the equilibrium

$$NH_2Cl + OH^- \rightleftharpoons NH_3 + OCl^-$$ [7]

It thus becomes obvious that *p*H control is very essential in all reactions involving formation of hy-

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(5) L. F. Audrieth and R. A. Rowe, This Journal, **77**, 4726 (1955).

(6) M. M. Jones, L. F. Audrieth and E. Colton, *ibid.*, **77**, 2701 (1955).

(7) R. E. Corbett, W. S. Metcalf and F. G. Soper, *J. Chem. Soc.*, 1927 (1953).

drazine in aqueous solution from ammonia and chloramine. Our data suggest that a pH range of 13.2–13.4 should be maintained if maximum yields of hydrazine are to be obtained.

Acknowledgments.—Our thanks are extended to Mrs. Marlies Zimmer, Mr. R. W. Whitney and Mr. Paul C. Moews for their assistance with the analytical determinations. This investigation was sponsored by the Office of Ordnance Research as one phase of a fundamental study of the synthesis of hydrazine, Contract No. DA-11-022-ORD-828.

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[CONTRIBUTION FROM GIBBS LABORATORY, DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

The Mild Acidic Degradation of Desoxyribose Nucleic Acid

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RECEIVED JULY 13, 1955

The molecular weight and size determined by light scattering and the intrinsic viscosity and the rate of production of dialyzable fragments have been studied as a function of time for the sodium salt of desoxyribose nucleic acid (DNA) prepared from calf thymus. The analysis of the data is based upon the statistics of degradation of single chains and double chains. It is found that following the immediate contraction of the DNA molecule upon lowering the pH to 2.6 by dialysis a gradual degradation of three types of bonding gets underway. The phosphoester bonds break at the rate of about one bond per weight average DNA molecule per 10 hours. The purines are liberated at about 200 times this rate. Substantial evidence indicates that during the early part of the degradation the two original polynucleotide strands making up the native molecule are held together at a few places by what are thought to be residual hydrogen bonds, clusters of hydrogen bonds or regions of extensive chain entanglement. These, however, also decay with the result that the degradation after about 50 hours is simply that of the random scission of single polynucleotide chains which are constantly losing purine groups.

With the growing acceptance of the Watson–Crick model as representative of the main configurational features of desoxyribose nucleic acid (DNA) and with increasing agreement on the weight, shape and size of this substance in solution, it has become of interest to examine the response of DNA to chemical and physical agents which will disrupt it to a limited extent. In such a study the motive lies in finding out if the two-stranded model can account for the changes induced or whether such evidence demonstrates the presence of other structural features. In addition genetic considerations suggest that the two strands should be separable, a fact which invites the attempt to bring this about and provide, if this is possible, undegraded polynucleotide chains. Finally, there is the possibility that any increase in our knowledge of its molecular behavior may help sharpen the picture of the biological functions and the process of duplication of DNA that is gradually evolving. This present study is concerned with the effects of dilute acid on DNA. The effects of base, heat, denaturing agents and desoxyribonuclease have also been examined in this Laboratory and will be reported shortly.

It is useful to classify what is already known concerning the response of DNA to acid into two parts; the region of mild acidity near pH 3 where degradation is not evident and the region of strong acidity where considerable chemical degradation is known to occur. The most remarkable feature of the mildly acid region is the anomaly in the titration curve discovered by Gulland, Jordan and Taylor[2] wherein the first titration from neutral solution to either extreme of the pH produces an irreversible change in the titration curve thereafter. The latter titration curve is explicable in terms of the pK's of the same purine and pyrimidine groups that in the Watson–Crick model are involved in hydrogen bonding. The reluctance of native DNA to bind protons, as the initial titration curves show, is properly ascribed to the blocking of the titratable groups due to their involvement in hydrogen bonding.

Coincident with the breakdown of the native, hydrogen-bonded structure by lowering of the pH there is a precipitous fall in the specific viscosity.[3] For some time this drop in viscosity was interpreted as a disaggregation or depolymerization. However, by a light scattering investigation[4] it was possible to show that there was no change in molecular weight upon lowering the pH to 2.6; there was, however, a marked decrease in the molecular size consistent with the drop in viscosity. Thus a gradual increase in acidity of DNA in saline solution causes no change until near pH 3. Then simultaneously there occurs a breakdown of the hydrogen bonded structure, the acceptance of protons by groups that normally titrated at higher pH and a contraction of the DNA molecules that corresponds to at least a fifty-fold decrease in the volume they occupy in solution.

Under more strongly acid conditions purines (adenine and guanine) are liberated and internucleotide bonds are broken. Chargaff and co-workers[5] have shown that at pH 1.6 and 37° about 24 hours are required for the removal of all the purines. By this time the DNA molecules have been fragmented into several hundred pieces. This degraded product is known as apurinic acid. Exhaustive acidic hydrolysis yields, of course, the pyrimidines, sugars and phosphoric acid.

Thus it is clear that mildly acidic conditions result in changes in macromolecular configuration with no depolymerization (although with perhaps

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[1] Atomic Energy Commission Fellow 1952–1954.

[2] J. M. Gulland, D. O. Jordan and H. F. W. Taylor, *J. Chem. Soc.*, 1131 (1947).

[3] J. M. Creeth, J. M. Gulland and D. O. Jordan, *ibid.*, 1141 (1947). Our own measurements of this effect show that when the pH is lowered by the addition of buffer having a pH of 2.5 the viscosity begins to fall at pH 3.1 and reaches 10% of its original value at 2.9.

[4] M. E. Reichmann, B. H. Bunce and P. Doty, *J. Polymer Sci.*, **10**, 109 (1953).

[5] C. Tamm, M. E. Hodes and E. Chargaff, *J. Biol. Chem.*, **195**, 49 (1952).