anhydride gives a very small yield of the anticipated iodobenzoyl-*o*-benzoic acid, but large quantities of benzoyl-*o*-benzoic acid and iodine. These acids are easily converted to the corresponding anthraquinones by being heated with sulfuric acid. It has likewise been shown that the methyl thio ether of mercapto-benzoyl-*o*-benzoic acid may be synthesized by this method. A detailed procedure for the analysis of organic compounds for fluorine has been worked out.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

A STUDY OF THE CHEMICAL BEHAVIOR OF ALPHA- AND BETA-HYDROFORMAMINE CYANIDES

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The substances, α- and β-hydroformamine cyanides, which melt at 129° and 86°, respectively, are isomeric compounds that result by the interaction of formaldehyde, ammonium chloride and potassium cyanide. These substances, which have hitherto been registered in chemical literature by the name *methylene aminoacetonitrile*, possess the empirical formula $C_9H_{12}N_6$, but are of unknown constitution.[2] In order to acquire a knowledge of the molecular structure of these two isomeric compounds, we have begun a comparative investigation of their chemical behavior. This research has already opened up several new chemical problems of both theoretical and practical interest. Furthermore, in a preliminary pharmacological research these substances have been found to possess interesting physiological properties which have also led to a renewed interest in the constitution of these compounds. In this paper, therefore, new data will be presented which reveal characteristic differences in chemical behavior between these isomers, and from which we are able to deduce fundamental conclusions with respect to their chemical constitution.

α-Hydroformamine cyanide behaves like a saturated compound and in no reaction thus far applied has definite evidence been obtained that it contains the unsaturated ethylene linkages which are characteristic of Schiff bases. The results obtained by Delepine[3] with hydrocyanic acid and, also, the fact that no reduction has been observed to take place with production of methylamino groups are experimental results quite in accord with the behavior of a saturated compound.

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[1] Constructed from a dissertation presented by H. W. Rinehart in 1923 to the Faculty of the Graduate School of Yale University in Candidacy for the degree of Doctor of Philosophy.

[2] Johnson and Rinehart, THIS JOURNAL, **46**, 768 (1924).

[3] Delepine, *Bull. soc. chim.*, [3] **29**, 1200 (1903). Bailey and Lochte, THIS JOURNAL, **39**, 2443 (1917).

The most striking differences in chemical structure have been revealed by a careful study of the chemical behavior of these two isomers when subjected to hydrolysis by acids and alkali. Jay and Curtius[4] investigated the behavior of α-hydroformamine cyanide towards hydrogen chloride in alcoholic solution and observed the smooth formation of the hydrochloride of ethyl amino-acetate. As intermediate products of this change they were able to prove the successive formation of amino-acetonitrile $NH_2.CH_2CN.HCl$ and its imido ether derivative,[5] $NH_2.CH_2C(:NH)OC_2H_5.HCl$. When he repeated this work, Klages[6] claims to have obtained a secondary product of hydrolysis to which he assigned the empirical formula $C_{17}H_{40}O_{13}N_4.4HCl$. Its constitution has never been established. This investigator also states that when the isomer melting at 86° (β-hydroformamine cyanide) is hydrolyzed by hydrochloric acid, formaldehyde and a hydrochloride of unknown constitution melting at 165° are produced.

In the present investigation comparative hydrolysis experiments have been carried out on both the α- and β-hydroformamine cyanides. Hydrolysis by means of hydrogen chloride in alcoholic solution was the first reaction which was applied to both isomers. In the case of the α-isomer, only formaldehyde, the hydrochloride of ethyl amino-acetate and ammonia were identified, as Jay and Curtius and Klages had already shown.[4,6] From the β-modification under the same conditions were obtained formaldehyde, ammonium chloride and a solid which behaved in every way like the hydrochloride of an amine. On account of its instability it has not yet been possible to establish its constitution. The most interesting observation is the fact that no ethyl amino-acetate could be identified as a product of hydrolysis of β-hydroformamine cyanide. In the light of these interesting results we at once turned our attention to a quantitative study of the distribution of nitrogen when each isomer is subjected to hydrolysis in neutral, acid and alkaline solution.

α- and β-Hydroformamine cyanides undergo characteristic changes when subjected to alkaline hydrolysis in aqueous solution and ammonia is set free in both cases. The α-isomer produces ammonia equivalent exactly to one-half of the nitrogen in its molecule, while the remainder is held in stable combination. β-Hydroformamine cyanide, on the other hand, readily gives up two-thirds of its nitrogen as ammonia, leaving one-third behind in combined state. These results are further positive evidence, therefore, of quite different molecular structures for the two compounds. Moreover, in the case of β-hydroformamine cyanide we have confirmation of the molecular formula $C_9H_{12}N_6$, as a result of this quanti-

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[4] Jay and Curtius, *Ber.*, **27**, 59 (1894).

[5] Jay and Curtius, *Ber.*, **31**, 2490 (1898).

[6] Klages, *J. prakt. Chem.*, **65**, 192 (1902).

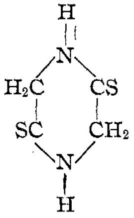
tative distribution of nitrogen; for the only compounds from which we can obtain exactly two-thirds of the nitrogen are those which contain three, or a multiple of three, nitrogen atoms in the molecule. Obviously, neither $C_3H_4N_2$ nor $C_6H_8N_4$ fulfils this requirement.

α- and β-Hydroformamine cyanides are hydrolyzed less easily by heating them with sulfuric acid than with alkali. While ammonia is produced, in neither case was the amount obtained as great as when the two isomers were digested with alkali. Also, as the concentration of the acid used for hydrolysis was increased, the molecule of the β-isomer was more susceptible to a hydrolytic change, as measured by the amount of ammonia formed. The stability of α-hydroformamine cyanide, on the other hand, was but slightly affected by changes of acid concentration.

By digestion in aqueous solution, α- and β-hydroformamine cyanides are slowly decomposed with formation of ammonia, but the rate at which ammonia is evolved is different for the two isomers. Under exactly similar conditions the α-isomer after two hours' digestion with water yields about 25% of its nitrogen as ammonia, while the β-isomer, on the other hand, yields only 1 to 2%. In the case of the α-isomer ammonia is evolved at first very rapidly, and the rate of production falls off rapidly as the digestion is continued. While the total amount of ammonia formed by prolonged digestion could finally be raised to about 40% of the nitrogen present in the molecule, it was observed that over one-half was produced during the first half-hour of boiling and about four-fifths during the first hour. After three hours of boiling, relatively little ammonia was generated. In contrast with this behavior, the β-isomer under similar treatment broke down slowly and during seven hours of boiling evolved ammonia at a nearly constant rate. The total amount obtained corresponded to about 17% of the total nitrogen present in the molecule. In the light of these results it is quite apparent that the two isomers are to be represented by different constitutional formulas. Furthermore, methods of purification of either isomer involving crystallization from hot water are to be avoided.[6] In all of the above methods of hydrolysis a strong odor of formaldehyde is easily detected in the case of both the α- and β-isomers. The formation of this aldehyde and ammonia by hydrolysis of both isomers is the only point of similarity in chemical behavior between the two forms which has thus far been observed.

The chemical behavior of the α- and β-isomers on hydrolysis under the conditions described above is quite in accord with what might be expected if we assume the presence of free cyanide radicals in the molecules of both isomers. The questions at once arise, do these two substances contain the same number of such radicals, and what chemical means can be devised to reveal the presence of free cyanide groups without introducing conditions which might lead to alteration of the internal molecular constitution. The reaction which we have applied to throw light on this question is that involving the addition of hydrogen sulfide to the cyanide group to obtain thio-acid amide combinations. The behavior of this reagent towards nitriles of amino acids has already been investigated by Johnson and Burnham.[7] $R.CN + H_2S \longrightarrow R.CS.NH_2$.

By this method of attack still further confirmatory evidence has been obtained that the two isomers under investigation are to be represented by decidedly different molecular constitutions. From the α-hydroformamine cyanide by addition of hydrogen sulfide a product was obtained which, analyzed for sulfur and nitrogen, gave values in close agreement with the formula $C_9H_{12}N_6.H_2S$. Evidently one cyanide radical entered into combination and the compound may be represented constitutionally as follows: $C_8H_{12}N_5.CS.NH_2$. Here again we have confirmation of the trimolecular formula for α-hydroformamine cyanide, as well as evidence that the reagents used in the thio-amide formation had not caused its depolymerization. Such depolymerizing changes could be effected, however, if the treatment with hydrogen sulfide was continued over a long period of time. Prolonged treatment with hydrogen sulfide led to the formation of secondary products of reaction resembling in properties those obtained by Johnson and Burnham in their work on amino-acetonitrile. The formation of dithiopiperazine (I) is quite in accord with what might be predicted if the α-isomer breaks down to form amino-acetonitrile as an intermediate product.



I

Toward β-hydroformamine cyanide, on the other hand, hydrogen sulfide exhibited a pronounced chemical affinity, as contrasted with the slow action on the α-isomer. The analytical determinations obtained for sulfur and nitrogen revealed an atomic ratio corresponding to the empirical formula $C_9H_{12}N_6.2H_2S$. In other words, here we have experimental evidence that two free cyanide radicals are functioning in the molecule of β-hydroformamine cyanide, and the primary product of addition may be expressed structurally as follows: $C_7H_{12}N_4.(CSNH_2)_2$. Prolonged treatment with hydrogen sulfide leads to a deep-seated destruction of the molecule with formation of new products of decomposition. The structure of these substances will be established by further investigation.

[7] Johnson and Burnham, *J. Biol. Chem.*, **9**, 449 (1911).

Physicochemical data in support of the conclusion that α- and β-hydroformamine cyanides are to be represented as highly saturated compounds have been contributed by Professor Emma Carr of Mount Holyoke College. She has very kindly examined the absorption spectra of these isomers and finds that the acetone solutions of both isomers are extremely transparent. Even in molar concentrations no measurable increase in the absorption of either solution over that of the acetone itself was detected. This spectrographic evidence, therefore, strongly supports the conclusion that no ethylene linkages occur free in either isomer.

Experimental Part

Hydrolysis Experiments with α- and β-Hydroformamine Cyanides.— In our first trials to determine chemical behavior and to measure the rate of hydrolysis of α- and β-hydroformamine cyanides by digestion with sulfuric acid of different strengths, we operated uniformly as follows: One-half g. of the respective isomer was digested for two hours with a constant volume of sulfuric acid of known strength. Evaporation was avoided by conducting the digestion in a Kjeldahl flask attached to a reflux condenser. After the digestion was stopped, the acidity of the solution was destroyed by adding an excess of sodium hydroxide solution of known strength. The ammonia was then removed by distillation and quantitatively determined by neutralization with standard acid. The results in duplicate of five hydrolysis experiments are recorded in Table I.

TABLE I

HYDROLYSIS EXPERIMENTS

Sodium hydroxide used to expel ammonia

Concn. of H_2SO_4 %	α-Isomer % Total nitrogen as NH_3	β-Isomer % Total nitrogen as NH_3
40	50.2 ; 50.1	67.1 ; 66.4
30	50.3 ; 50.4	67.4 ; 67.5
20	53.0 ; 50.1	66.2 ; 65.6
10	50.5 ; 51.3	65.9 ; 65.7
5	51.9	66.3 ; 65.7
0[a]	51.4	65.9

[a] In this experiment the digestion was conducted with 125 cc. of water to which was added 10 cc. of 40% sodium hydroxide solution.

While the purpose of this series of hydrolysis experiments was to show the effect of various strengths of sulfuric acid on the course of the hydrolysis, the constancy of the analytical values obtained indicated that the final result was influenced by the action of alkali used to expel ammonia. Accordingly, the last experiment was conducted without addition of acid, and the ammonia quantitatively determined. It is quite apparent from the results obtained that the alkali had been responsible in all six experiments for the constant amount of ammonia evolved in each case.

In order to avoid the hydrolytic influence of sodium hydroxide and to obtain data regarding the true influence of change in sulfuric acid concentration, a series of hydrolysis experiments was run in which magnesium hydroxide was used to expel free ammonia. The results obtained in these experiments are recorded in Table II. In each case the amount of substance used for hydrolysis was 0.5 g.

TABLE II

HYDROLYSIS EXPERIMENTS

Magnesium oxide used to expel ammonia

Expt.	Concn. of H_2SO_4 %	α-Isomer % Total nitrogen	β-Isomer % Total nitrogen
1	60	48.86 ; 24.15	26.38 ; 35.9
2	50	39.20 ; 31.74	46.30 ; 54.9
3	40	38.48 ; 40.83; 43.90 ; 42.31; 43.65	47.87 ; 44.27; 59.0 ; 60.30; 61.6
4	30	39.49 ; 44.31; 39.77	63.69 ; 63.22; 63.34 ; 64.49
5	20	39.28 ; 39.27; 40.40	50.17 ; 49.15 ; 56.54
6	10	34.89 ; 32.67; 26.26	36.23 ; 38.45; 37.20
7	5	14.31 ; 17.23; 22.28	24.88 ; 20.84; 26.85
8	0	29.31 ; 23.69; 26.34	1.95 ; 3.46; 5.11

It is quite apparent from the results of Expt. 8 in Table II that digestion with magnesium hydroxide brings about a pronounced hydrolysis in the case of α-hydroformamine cyanide, while the β-isomer, on the other hand, undergoes very slight hydrolytic action in its presence. In order to determine whether the magnesium hydroxide is an influencing factor here, hydrolysis experiments were conducted in which each isomer was digested with pure water for two hours, and the ammonia generated removed by distillation. The results of these experiments are recorded in Table III.

TABLE III

HYDROLYSIS IN AQUEOUS SOLUTION

Percentage of total ammonia, α isomer................	24.2	26.85
β isomer................	1.3	1.48

It is quite apparent from the results of this series of experiments that magnesium hydroxide exerts no real hydrolytic influence on the α- and β-isomers and that the results obtained in Expt. 8 of Table II are due entirely to the hydrolytic action of water alone.

Rate of Hydrolysis with Water.—In order to determine the rate at which each of the two isomers is hydrolyzed by boiling with water, the following experiment was carried out: one-half g. of the respective isomer was placed in a Kjeldahl flask with 150 cc. of water. The flask was connected with a condenser arranged for distillation, and the water distillate run into standard hydrochloric acid (0.1023 *N*). By means of a dropping funnel water was allowed to flow into the flask at about the same rate that it distilled through the condenser. At regular intervals the receiving beaker was changed for another containing always a known volume of standard acid. By titration of the excess of standard acid with standard alkali the ammonia collected in each time interval was determined. The results obtained are recorded in Table IV.

TABLE IV

RATE OF HYDROLYSIS IN AQUEOUS SOLUTION

Time interval, minutes		30	30	60	60	60	60	60	60	420
Cc. of HCl neutralized by NH_3	α-isomer	31.29	13.80	5.55	3.68	1.20	1.00	0.80	0.60	57.92
	β-isomer,	1.24	1.60	4.30	5.50	4.86	3.77	1.53	1.85	24.65

The α-isomer undergoes quite rapid decomposition by the action of boiling water, and in seven hours of boiling 40.3% of the total nitrogen is evolved as ammonia. The β-isomer, on the other hand, undergoes very slow change by boiling with water, since after seven hours' digestion only 17.1% of its nitrogen is liberated as ammonia. A graphical representation of these interesting results is shown in Fig. 1.

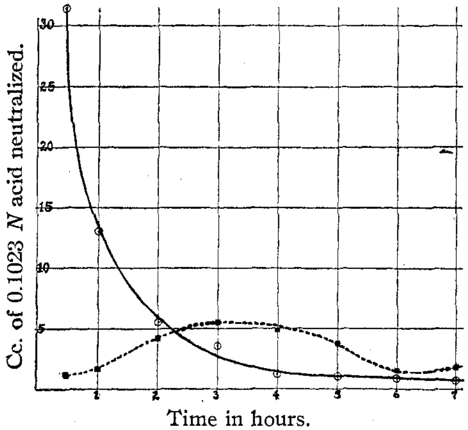


Fig. 1.—Rate of hydrolyses. ———— α-Hydroformamine cyanide. - - - - - - - β-Hydroformamine cyanide.

Hydrolysis of α- and β-Hydroformamine Cyanides with Hydrochloric Acid in Alcoholic Solution.—The α-isomer undergoes smooth conversion to the hydrochloride of ethyl amino-acetate and, as is well known, serves as a practical and economical source of this amino-acid ester.

The β-isomer, when warmed in an alcoholic solution of hydrochloric acid, is decomposed with formation of ammonium chloride, but the hydrochloride of ethyl amino-acetate has not been identified among the hydrolysis products. Among these latter is a brown solid which gave off hydrogen chloride when exposed to air, but thus far it has not been possible to establish the constitution of this solid. We are continuing the study of the changes which take place when the β-isomer is decomposed by an alcoholic solution of hydrogen chloride.

The Action of Hydrogen Sulfide on α-Hydroformamine Cyanide.—Ten g. of the isomer melting at 129° was finely pulverized and suspended in a mixture of 100 cc. of 95% alcohol and 50 cc. of concd. ammonia. A flask was used for the reaction and was equipped with a tube stirrer through which hydrogen sulfide could be conducted into the alcohol during the stirring operation. After treatment for one hour with a rapid current of hydrogen sulfide, the α-isomer partially dissolved and the solution became warm, but no addition product containing hydrogen sulfide separated. On standing overnight a light colored product had separated. This was collected on a filter, washed with alcohol and water and then dried at 30–35° (weight, 10.82 g.). From this, by extraction with hot alcohol, a pink, crystalline solid was obtained, which was purified by recrystallization from alcohol, using Norite for decolorization. By this procedure 5.20 g. of a white, crystalline substance was obtained. This melted between 125° and 140° with decomposition to yield a red solid when the melting point was determined by the ordinary method; but when the acid bath was heated separately and the capillary tube containing the substance thrust into it for only a few seconds at a time, the solid melted to a clear, colorless liquid at 152–153°. It is insoluble in water, ether, acetone, chloroform, carbon disulfide and benzene, and soluble in alcohol to the extent of about 0.5 g. per 100 cc. Dil. alkali, as well as heat, decomposes it. Because of this latter effect, recrystallization from alcohol has not yielded an entirely pure product. The analyses, in spite of this, show that a monothio-amide has been formed.

Analyses. Calc. for $C_8H_{12}N_5.CSNH_2$: S, 13.47; N, 35.29. Found: S (Carius), 12.81, 12.46; N (Kjeldahl), 35.06, 34.77.

After the extraction of the reaction product above with hot alcohol, 3.20 g. of a fluffy, gray powder remained, which corresponded in properties in every way with the dithiopiperazine that Johnson and Burnham have described in a previous paper.[7] When hydrogen sulfide was passed for 6–8 hours into ammoniacal alcohol containing the α-isomer, the product obtained was almost entirely a substance having the properties of dithiopiperazine. This is evidence that the α-isomer is slowly broken down under the influence of ammonia and hydrogen sulfide with intermediate formation of aminoacetonitrile or its thio-amide derivative. When hydrogen sulfide was passed in for more than 8 hours, the red solid containing sulfur which Johnson and Burnham describe is obtained.

Although physical properties of the above sulfur compound agree well with Johnson and Burnham's description of dithiopiperazine, the analytical values obtained agree more closely with the calculated values for a true thiopolypeptide derivative, namely, $NH_2.CH_2.CSNH.CH_2.CSNH_2$.

Analyses. Calcd. for $C_4H_6N_2S_2$: S, 43.84; N, 28.79; calcd. for $C_4H_9N_3S_2$: S, 39.26; N, 25.76. Found: S (Carius), 40.92, 41.24; N (Kjeldahl), 25.24, 25.82.

Action of Hydrogen Sulfide on β-Hydroformamine Cyanide.—Five g. of β-hydroformamine cyanide was finely ground in a mortar and suspended in a mixture of 100 cc. of 95% alcohol and 25 cc. of concd. aqueous ammonia. Hydrogen sulfide was passed into the solution for 3 hours, as described for the previous experiment. There was evidence of immediate reaction, in that the solution turned orange in color and showed a slight heat of reaction. The β-isomer dissolved and a colorless crystalline solid separated which, when washed with water and dried at room temperature, weighed 3.64 g. This was insoluble in all organic solvents tried (ether, alcohol, acetone, chloroform, carbon disulfide and benzene) and also in dilute mineral acids, but dissolved with decomposition in 10% alkali. When heated in a capillary tube the addition product began to turn yellow at 120°, became dark red at 140°, blackened and finally melted with evolution of hydrogen sulfide at 148° to form a clear, dark red liquid. Because of decomposition by heat and of insolubility, purification for analysis was impossible. The values, in spite of this, agree very closely with those calculated for the dithio acid amide compound.

Analyses. Calc. for $C_7H_{12}N_4.(CSNH_2)_2$: S, 23.53; N, 30.88. Found: S (Carius), 23.72, 23.51; N (Kjeldahl), 29.93, 30.63.

If the hydrogen sulfide treatment is applied to the β-isomer in warm solution, a product is obtained which is probably due to a secondary reaction. Three g. of the β-isomer was suspended in a mixture of 125 cc. of 95% alcohol and 10 cc. of concd. aqueous ammonia. The solution was refluxed on a water-bath while hydrogen sulfide was passed in for 6 hours. Three g. of an amorphous, yellow-brown solid was obtained which had a sharp, mercaptan-like odor.

When this yellow-brown solid was allowed to stand in contact with ammonium hydrosulfide in alcoholic solution (its mother liquor) and the alcohol was allowed to evaporate spontaneously at room temperature, a black, amorphous solid was obtained which has not yet been identified.

Summary

1. α- and β-Hydroformamine cyanides differ in their chemical behavior when hydrolyzed in acid, alkaline and neutral solution. A quantitative determination of the distribution of nitrogen proves that they are to be represented by quite different molecular structures.

2. α-Hydroformamine cyanide undergoes hydrolysis in alcoholic solution with hydrochloric acid, giving the hydrochloride of glycine ester. From the β-isomer this compound is not obtained, but instead ammonium chloride and a nitrogen derivative of unknown constitution are formed in the decomposition.

3. The two isomers differ decidedly in the rate at which they are broken down by hydrolysis. The α-compounds decompose rapidly under such conditions, while the β-form is only gradually decomposed by long digestion.

4. The two isomers (α and β) interact with hydrogen sulfide to form, respectively, the addition compounds, $C_8H_{12}N_5(CSNH_2)$ and $C_7H_{12}N_4(CSNH_2)_2$. Prolonged treatment of the α-isomer with hydrogen sulfide leads to the formation of dithiopiperazine or thiopolypeptide combinations.

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