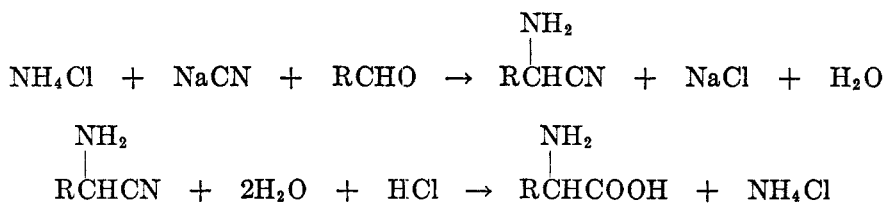


CARBOXYMETHYLATION OF AMINES. I. PREPARATION OF ETHYLENEDIAMINE TETRAACETIC ACID

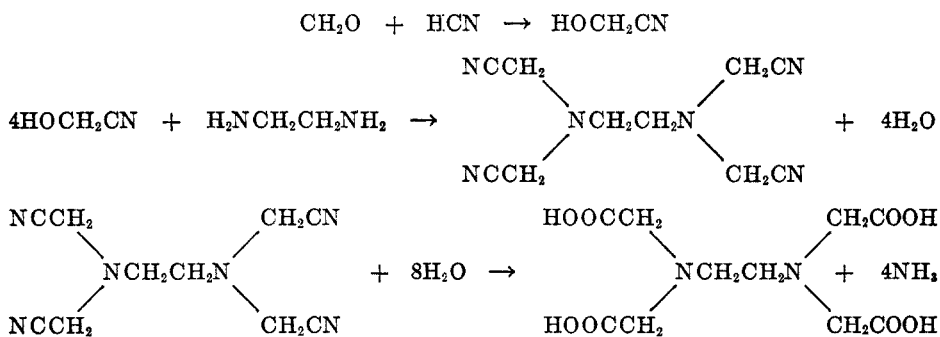
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The reactions of cyanides and aldehydes with ammonia and with amines have been rather extensively investigated. The most well-known of these reactions is the Strecker Synthesis (1) which was first used by Strecker in 1850 but which has since been modified by other workers. A general reaction for the modified type of Strecker Synthesis is:



An example of this type of reaction has been described in detail by Kendall and McKenzie (2). One of the chief disadvantages of this method of preparation is the evolution of free hydrogen cyanide, which takes place on the addition of hydrochloric acid. This type of reaction has recently been extended to amines by Ulrich and Ploetz (3) who described the reaction of hydrogen cyanide and formaldehyde with ethylenediamine and other polyamines. The reaction may be illustrated schematically by the following equations:

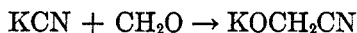


Although the yields of intermediate nitriles are fairly high, the chief disadvantages of this synthesis for the preparation of alpha amino acids are the problem of handling large quantities of hydrogen cyanide gas and the difficulties in hydrolyzing the nitriles that are formed in the reaction.

In 1933, F. C. Bersworth (4), working with Dr. William H. Warren at Clark University, devised a method for producing alpha amino acids by treating solutions of aliphatic amines with alkali cyanides and an aliphatic aldehyde. This

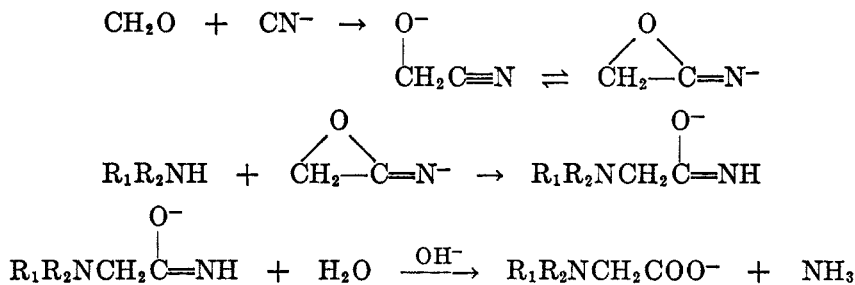
type of reaction is quite different, both in the conditions employed and in the results obtained, from the Strecker type of synthesis given above and has been subjected to considerable amount of investigation in the laboratories of Clark University. The commercial procedure for carrying on this reaction has been described by Bersworth (5). This is the first of a series of publications dealing with these reactions, which shall be called carboxymethylation of amines. It is the purpose of this paper to describe in some detail the optimum conditions and by-products obtained for the reaction of ethylenediamine with sodium cyanide and formaldehyde. There is also some consideration of the probable reaction mechanism but that is not the primary purpose of this report.

Some suggestion as to the course of the reaction may be obtained from the preliminary work done by Kohn (6) who first studied the reaction between potassium cyanide and formaldehyde and postulated the formation of a salt of glyconitrile according to the following reaction:



He tried to isolate the pure nitrile from the reaction of calcium cyanide with formaldehyde but was unsuccessful. Mutschen (7) showed that the nitrile is not appreciably decomposed at room temperature for fifty hours in solution and remains unchanged up to 100 hours in the presence of excess alkali. The pure glyconitrile was isolated by Polstorf and Meyer (8).

We have found that the nitrile decomposes rapidly at temperatures above 30°. Also, we have found that the reaction of an alkalinized solution of glyconitrile with an amine produces a good yield of the amino acid. It seems probable, therefore, that it is an intermediate in the reaction. From information available at present, the following tentative course for a general carboxymethylation reaction is suggested.

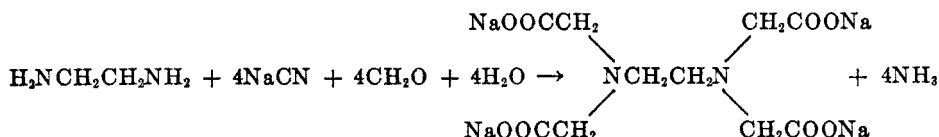


where R_1 and R_2 represent hydrogen or alkyl groups. The evolution of the ammonia begins immediately and continues throughout the course of the reaction. It is therefore probable that the reaction does not involve the formation of the substituted alpha aminonitrile intermediate, as in the case of the reaction described by Ulrich and Ploetz (3), which takes place in neutral and acid solution. The possibility that the alkaline reaction takes place through the hydrolysis of glyconitrile to form a salt of glycollic acid was ruled out by Smith (9) who showed that alkaline solutions of glycollic acid do not react with ethylenediamine. In fact everything about the carboxymethylation reaction indicates that it is

radically different from the Strecker type of synthesis. One of the most striking characteristics of the new reaction is the fact that strongly alkaline conditions are necessary. High alkalinity seems to have two effects, both of which favor the reaction: first, the ammonia is driven off more rapidly and side reactions are thus prevented; and secondly, the presence of a strong base has a catalytic effect on the reaction (probably because it favors formation of the alcoholate ion of glyconitrile as is shown in the mechanism proposed above).

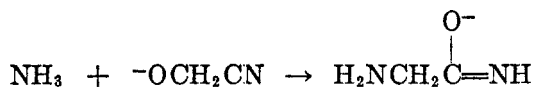
The usual method of producing ethylenediamine tetraacetic acid is the reaction of chloroacetic acid or its salts with ethylenediamine. This usually produces a fair yield of the desired material but many by-products are formed.

Since the reaction of ethylenediamine with sodium cyanide and formaldehyde to form ethylenediamine tetraacetic acid has been carefully investigated, it is given here as a first example of carboxymethylation. The over-all reaction may be represented by the following equation:



The reaction was first attempted by adding four consecutive molar amounts of sodium cyanide and formaldehyde to each mole of amine. The yields obtained in this way were very low. The reaction conditions were then varied by using a low temperature and by bubbling air through the reaction mixture to remove the ammonia as soon as it was produced. Under these conditions 25% of the theoretical amount of product was isolated. It was felt that the yield of the tetraacetic acid was much higher than 25%, however, since the by-products of the reaction formed a syrupy solution which seemed to inhibit crystallization of the amino acid.

Probably one of the chief difficulties with this reaction, when carried out in this manner, is the side reaction of ammonia with sodium cyanide and formaldehyde to form mono-, di-, and tri-glycine. This is due to carboxymethylation of the ammonia formed by hydrolysis of intermediate nitrile groups according to the following reaction:



Repetition of this step, together with hydrolysis, leads to the formation of sodium salts of glycine, diglycine, and triglycine. The formation of diglycine and triglycine from glyconitrile was demonstrated by Polstorf and Meyer (8). This suggested the possibility that the ammonia was not being removed fast enough and that a lower temperature should be used to hinder the rate of hydrolysis of the nitrile intermediate. It was also decided to add the reagents very slowly in order to keep the concentration of the nitrile as low as possible. The reaction, when carried out in this way, showed somewhat improved yields. When the evolution of ammonia was further increased by carrying on a slow distillation

under reduced pressure during the course of the reaction and by raising the pH of the solution through the addition of sodium hydroxide, the yield was finally raised to about 75% of the theoretical amount. No further modification of the conditions seemed to result in any further increase.

The detection of an appreciable amount of hexamethylenetetramine as by-product and the competing carboxymethylation of ammonia suggested the possibility that not enough formaldehyde and cyanide were present to completely substitute all the hydrogens of ethylenediamine. After carrying out the reaction as described above and the subsequent isolation of the desired tetraacetic acid, the mother liquor was treated with a fresh portion of sodium cyanide and formaldehyde under the same conditions. The isolation of an additional batch of ethylenediamine tetraacetic acid, indicated that some of the ethylenediamine was incompletely substituted. Accordingly, it was decided to run the reaction with an excess of sodium cyanide and formaldehyde under the optimum conditions described above. This resulted in yields of the desired product which amounted to as much as 96% of the theoretical amount. It is believed therefore that the problem of preparing this material has been adequately solved.

The industrial method outlined in the Bersworth patents (5) employs very high temperatures, is much more rapid and also gives high yields. It is not adapted to small-scale preparations, however, and when carried out in the laboratory often results in the formation of colored by-products.

The detailed procedure for the reaction is given under two sets of conditions in the experimental part. The identity and purity of the product was established by Dumas nitrogen determination, analysis of the dibarium salt, and pH titration curve.

The carboxymethylation reaction has been thoroughly investigated in this laboratory and extended to a large number of amines. These reactions will be reported in subsequent publications.

EXPERIMENTAL

Ethylenediamine tetraacetic acid. I. A one-liter, three-necked flask was equipped with a dropping-funnel, distilling-arm and condenser, and a glass stirrer fitted through a vacuum-tight rubber stopper. The stirrer was operated by an eccentric attached to a stirring motor directly above the rubber stopper, the under portion of which was hollowed out to allow free motion of the stirrer. In the reaction flask was placed 33.3 g. (0.333 mole) of a 60% solution of ethylenediamine, and to this was added a solution containing 81.5 g. (1.66 mole) of sodium cyanide and 7.0 g. of sodium hydroxide. The mixture was diluted to about 200 cc. and was brought to a temperature of 10° by means of a water-bath. 27.8 g. of a 36% solution (0.333 mole) of formaldehyde was diluted to 200 cc. and added continuously over a period of twenty hours. During this time the reaction mixture was stirred and maintained at a temperature of 10°. When all the formaldehyde had been added, the temperature was brought to 60° and the solution was distilled under vacuum until 200 cc. of distillate had been collected. The reaction mixture was then brought to a temperature of 16° while 100 cc. of a solution containing 0.166 mole of formaldehyde was added continuously over a period of eight hours. The mixture was vacuum distilled as before until 100 cc. of distillate had been collected. The process was continued with further additions of formaldehyde followed by distillation until 1.66 mole of formaldehyde had been added. The amounts added, time allowed for reaction, temperature, and quantity of water removed by distillation are shown in Table I.

The resulting product was a clear and nearly colorless solution. It was diluted to one liter and brought to a pH of 1.2 by the slow addition of 6 *M* sulfuric acid over a period of two hours with stirring. Stirring was then continued for two hours more to insure complete precipitation. The white crystalline product was filtered and washed twice with a little water. After it was dried in an oven at 110° for two hours, 93.3 g. (96% of the theoretical amount) of ethylenediamine tetraacetic acid was obtained.

Anal. Calc'd for $C_{10}H_{16}N_2O_8$: N, 9.58. Found: N, 9.50.

II. The following method is shorter and more convenient but results in somewhat reduced yields:

The reaction was carried out exactly as described above with the same concentrations of reagents but with the modifications in the conditions indicated below in Table II.

TABLE I
PREPARATION OF ETHYLENEDIAMINE TETRAACETIC ACID

REACTION TIME, HRS.	MOLES CH_2O ADDED	TEMP., °C	WATER REMOVED CC. DISTILLATE
20	0.333	10	200
8	.166	16	100
8	.166	16	100
6	.166	25	100
6	.166	25	100
4	.166	30	100
4	.166	30	100
4	.166	30	100
4	.166	30	200

TABLE II
PREPARATION OF ETHYLENEDIAMINE TETRAACETIC ACID BY SHORT METHOD

REACTION TIME, HRS.	MOLES CH_2O ADDED	TEMP., °C	WATER REMOVED CC. DISTILLATE
6	0.333	20	100
4	.333	25	100
4	.333	30	100
3	.333	35	100
3	.333	35	400

From the light reddish-brown solution obtained, the acid was isolated just as is described above. 76.5 g. (79% of the theoretical amount), of the amino acid was obtained.

Dibarium salt. Six g. of ethylenediamine tetraacetic acid was dissolved in 60 cc. of 0.40 *M* barium hydroxide to give a nearly clear solution. The solution was clarified by filtration and was mixed with 90 cc. more of the barium hydroxide solution. When the resulting solution was heated a heavy white mass of crystals of the dibarium salt precipitated. The crystals were decanted and washed several times by decantation, and then dried for three hours at 160°. The product was analyzed gravimetrically for barium by the sulfate method.

Anal. Calc'd for $C_{10}H_{12}Ba_2N_2O_8$: Ba, 48.90. Found: Ba, 48.81.

Glyconitrile. Seventy-three and one-half g. of sodium cyanide (1.5 mole) was dissolved in 200 cc. of water and was treated with 47.5 g. (1.58 moles) of formaldehyde while the solution was cooled by direct addition of ice. After it was allowed to stand for five minutes the solution was slowly made acid with 200 cc. of 7.82 *N* sulfuric acid. The temperature was kept down during this process by further additions of ice. 10 g. of dry sodium carbonate

was then added to bring the pH of the solution to approximately 6.0. A sample of this solution was extracted with ether several times. Evaporation of the ether extracts resulted in the formation of a colorless liquid. Various tests performed on this material showed that it behaved like glyconitrile as described in the literature.

Action of sodium salt of glyconitrile on ethylenediamine. In a 1000-cc. three-necked flask equipped as described for the preparation of ethylenediamine tetraacetic acid was placed 500 cc. of a solution containing 20.0 g. (0.33 mole) of ethylenediamine and 0.40 moles of sodium hydroxide. To this was added, continuously and with stirring, an aqueous solution containing 0.333 mole of the nitrile. The addition was made over a period of 10 hours while the reaction mixture was kept at room temperature. At the end of this time, the mixture was distilled under vacuum until the volume of the remaining liquid was about 500 cc. After further addition of 0.33 moles of sodium hydroxide the reaction mixture was treated as before with one-third mole of glyconitrile and again distilled until the volume had been reduced to 500 cc. The process was repeated twice more in the same manner. The resulting, nearly colorless, reaction mixture was treated with acid as described previously for the isolation of ethylenediamine tetraacetic acid.

The weight of the dried crystals was 41.2 g. representing 42% of the theoretical amount. The nature of the product was established as above with nitrogen analysis and preparation and analysis of the dibarium salt.

Hexamethylenetetramine. The presence of hexamethylenetetramine in the unacidified reaction solution was determined as follows:

A sample of the alkaline solution obtained as a reaction product in the carboxymethylation reaction of ethylenediamine described above and containing 0.0327 mole of the tetrasodium salt, was treated with 0.179 moles of dilute sulfuric acid and steam-distilled until 400 cc. of distillate had been collected. The distillate was diluted to 1000 cc. and several 25-cc. samples were analyzed for formaldehyde by the peroxide titration method. The results showed that 0.0477 mole of hexamethylenetetramine had been formed per mole of ethylenediamine used in the reaction; assuming all the formaldehyde was formed from hexamethylenetetramine.

Ammonia in the form of ammonium salts was determined by a Van Slyke analysis of the acidified reaction product obtained as in the formaldehyde determination. The results also indicated the formation of 0.048 mole of hexamethylenetetramine per mole of ethylenediamine used.

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SUMMARY

A detailed procedure is presented for the carboxymethylation of amines, with ethylenediamine as an example. A shorter and more convenient procedure which results in somewhat reduced yields is described. This is a general reaction for the replacement of the hydrogens of aliphatic amines with acetic acid groups. The reaction may be carried out with an alkali cyanide and formaldehyde, or with an alkali hydroxide and glyconitrile.

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