[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CLARK UNIVERSITY AND THE BERSWORTH LABORATORIES]

THE CARBOXYMETHYLATION OF AMINES. II. TRIGLYCINE

A. E. MARTELL[1] AND F. C. BERSWORTH[2]

Received June 15, 1949

It is the purpose of this paper to report a new and improved method for the preparation of triglycine through the use of sodium cyanide and formaldehyde. This reaction represents an extension of the carboxymethylation reaction previously described (1).

In 1912 Polstorff (2), investigating the reaction between equimolar parts of sodium cyanide and formaldehyde, isolated a little triglycine and a smaller amount of diglycine. From the filtrate glycollic acid was obtained as the zinc salt. The combined products accounted for only about 42% of the cyanide used in the reaction. As a synthesis of triglycine, the method of Polstorff is unsatisfactory, since a large proportion of other products, such as glycollic acid, diglycine, and no doubt glycine itself, is formed. Under conditions which were substantially the same as those used by Polstorff, Kohn (3) found the main products to be hexamethylenetetramine and glycollic acid. He stated that glyconitrile, though probably formed as an intermediate, was too unstable to exist more than momentarily, and that it hydrolyzed to glycollic acid and ammonia, which in turn combined with formaldehyde to produce hexamethylenetetramine. Kohn attempted to isolate the nitrile, as prepared from calcium cyanide and formaldehyde, but was not successful. On the other hand, Romijin (4) suggested that the reaction between an alkali cyanide and formaldehyde takes place quantitatively with the formation of the alcoholate salt of glyconitrile. Although Polstorff assumed that the alcoholate salt of the nitrile was formed, the existence of this substance was not considered certain. As recently as 1944, Mowry (5) stated "In aqueous solution, a mixture of formaldehyde and sodium cyanide reacts like glyconitrile and sodium hydroxide."

Mutschin (6) found that at room temperature very little decomposition of the nitrile took place after 50 hours, and that in the presence of sodium hydroxide, it remained unchanged up to one hundred hours. The work done in this laboratory shows that the nitrile, though fairly stable at room temperature, decomposes rapidly above 30°. The authors have succeeded in isolating both the free nitrile and its alcoholate salts, and its use in the carboxymethylation reaction has already been reported.

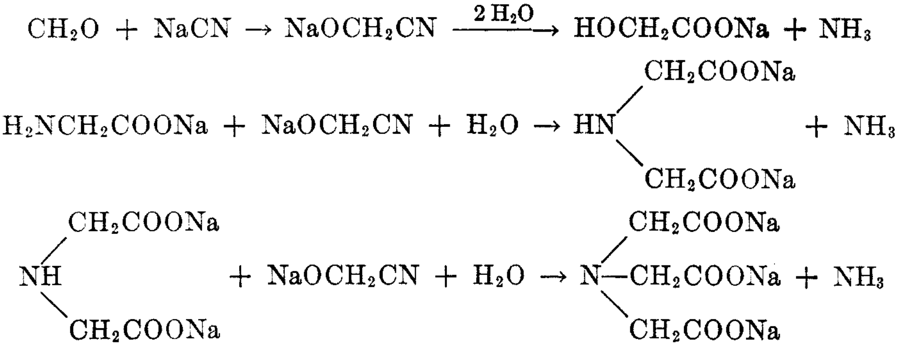
Polstorff assumed that the formation of triglycine took place through hydrolysis of the alcoholate salt of glyconitrile, with the formation of ammonia and glycollic acid, subsequent reaction of ammonia with free glyconitrile to form by dehydration the nitriles of glycine, diglycine, and triglycine and formation of triglycine itself in the same reaction mixture by final hydrolysis of the corresponding trinitrile. It is to be noted that he postulated dehydration and hydrolysis as taking place in the same solution at the same time.

---

[1] Department of Chemistry, Clark University, Worcester, Mass.

[2] Bersworth Laboratories, Framingham, Mass.

In a previous publication (1) we have shown that the carboxymethylation reaction does not take place with the formation of an intermediate nitrile, but goes directly to the amino acid salt. The formation of triglycine, therefore, probably did not take place by the method indicated by Polstorff but rather by the following steps:



$$CH_2O + NaCN \rightarrow NaOCH_2CN \xrightarrow{2\,H_2O} HOCH_2COONa + NH_3$$

$$H_2NCH_2COONa + NaOCH_2CN + H_2O \rightarrow HN(CH_2COONa)_2 + NH_3$$

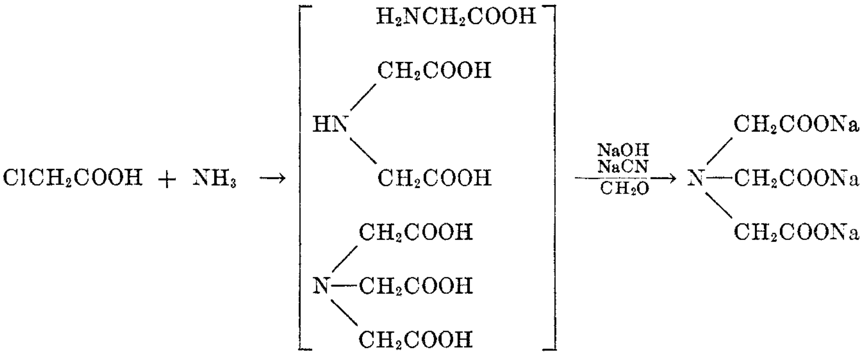
$$NH(CH_2COONa)_2 + NaOCH_2CN + H_2O \rightarrow N(CH_2COONa)_3 + NH_3$$

We have previously shown that high alkalinity favors the carboxymethylation reaction, partly because ammonia is more rapidly evolved and partly because the reaction is speeded up. The conditions used by Polstorff are not satisfactory for maintaining high alkalinity. This leads to a lowered rate of evolution of ammonia and gives an opportunity for the reaction of ammonia with the alcoholate of glyconitrile to form triglycine. The yield of this product is very low, as would be expected under these conditions. We have found that attempts to improve the yield by increasing the alkalinity, however, are fruitless, since this increases the tendency of ammonia to escape from the reaction mixture before it has an opportunity to react.

On further study of these reactions, we found two methods of getting around these difficulties. The first involves the use of a non-volatile amine, glycine itself, as a starting material. The use of glycine in the carboxymethylation reaction according to the method already outlined, however, is unsatisfactory since here again the alkalinity is not sufficiently great to remove the ammonia as soon as it is formed. One might be inclined to suppose that the evolution of ammonia is not a serious matter since the reaction of ammonia with sodium cyanide and formaldehyde would lead to the production of triglycine. We have found, however, that this leads to the formation of considerable amounts of glycine and diglycine as well and makes it impossible to obtain a high yield of the desired product. Also, under these conditions, a large proportion of colored by-products is formed. On the other hand, when the sodium salt of glycine was treated by the carboxymethylation reaction at high temperature, and in the presence of excess alkali, it was found that the reaction rapidly went to completion and that the triglycine obtained was not contaminated with by-products.

The disadvantage of this process lies in its restriction to glycine as a starting material, since it is not readily obtained in the pure state from the ammonolysis of a halogenated acid. In this connection it is interesting to note the findings of Robertson (7) who reported the recovery of 63% of the theoretical amount of glycine when one mole of chloroacetic acid was treated with four liters of concentrated ammonia, but obtained a very low yield when the ammonia was cut down to about a liter or less. The use of a small amount of ammonia with a relatively large amount of chloroacetic acid, however, does not produce a satisfactory yield of triglycine. Usually a sirupy mixture is produced from which it is impossible to separate any products in appreciable amounts. This was mentioned by Robertson and by many other workers.

It is to be noted that the same end-product of the carboxymethylation reaction is obtained whether the starting material is glycine or a mixture of glycine, diglycine, and triglycine. It was therefore decided to investigate the carboxymethylation of the reaction mixture formed on the ammonolysis of chloroacetic acid. However, if the amount of sodium cyanide and formaldehyde needed is to be determined, it is necessary to know roughly how far the ammonolysis has proceeded toward the formation of secondary and tertiary amines. Conversely, since the conversion of glycine and diglycine to triglycine by the carboxymethylation reaction is practically quantitative, it is possible to determine roughly the extent of ammonolysis of the halogen acid from the yield of triglycine based on the halogen acid when an excess of sodium cyanide and formaldehyde is employed. The higher the proportion of glycine as the result of ammonolysis of the halo acid, the greater will be the amounts of sodium cyanide and formaldehyde required, and the greater will be the yield of the desired product. The overall synthesis can be represented schematically by the following reactions:



Two sets of reaction conditions were employed. First, the recommendations of Robertson for obtaining the highest possible yield of glycine were followed. Four liters of concentrated ammonia were used at room temperature per mole of chloroacetic acid. Under these conditions he isolated only 63% of the theoretical amount of glycine but estimated that 86% was present in the reaction mixture. The sodium cyanide and formaldehyde used in the subsequent step was based on the production of the maximum amount (one mole) of glycine, and 0.83 mole of triglycine was isolated. The large amount formed indicates not only that more than 60% of glycine was formed by ammonolysis of chloroacetic acid, but also, that a considerable amount of the by-products must have been in the form of diglycine which was subsequently converted to triglycine *via* carboxymethylation. It is also possible that a small amount of triglycine was formed directly from the chloroacetic acid and ammonia.

A second procedure was worked out using one liter of concentrated ammonia water per mole of chloroacetic acid, under conditions which Robertson found resulted in a negligible yield of glycine. The amount of sodium cyanide and formaldehyde needed for carboxymethylation of the reaction mixture was calculated assuming 50% conversion of chloroacetic acid to glycine and 50% to diglycine. As a result of the over-all process, 0.63 mole of triglycine was obtained, a surprisingly large amount in view of the low yields resulting from ammonolysis under the conditions employed in the first part of the synthesis. The method shows considerable promise as a convenient and inexpensive process for the preparation of triglycine.

Although it is not possible to calculate the theoretical yield, it is obviously considerably higher than 83% in the first case and very much higher than 63% in the second. It is probable that the sodium cyanide and formaldehyde were in considerable excess in both cases and may be cut down appreciably without notably decreasing the yield. A further advantage of this method is that the starting materials are ammonia and a halo acid and that the desired substance is obtained in high yield free from by-products without isolation or separation of the intermediates.

The laboratory procedure employed and the isolation and identification of the product are described below.

EXPERIMENTAL PART

Triglycine from glycine. Glycine (75 g.) was dissolved in 200 cc. of a solution containing 43 g. of sodium hydroxide and was treated with 300 cc. of a solution containing 103 g. of sodium cyanide and 3 g. of sodium hydroxide, and with 180 cc. of 37% formaldehyde solution, in a 2-liter reaction flask equipped with a dropping-funnel, reflux condenser, and mercury-seal stirrer. About 60% of the sodium cyanide solution was added at once and the reaction mixture heated to 80° with a water-bath and stirred vigorously. One-half of the formaldehyde was then added slowly from the dropping-funnel over a period of four hours. The remainder of the sodium cyanide was then added through the reflux condenser and the remaining formaldehyde was added over an additional four-hour period. The evolution of ammonia began almost immediately after addition of the formaldehyde was begun, and became more rapid as the reaction proceeded. After it had stood overnight, the reaction mixture was heated on the water-bath to 100° and stirred for a two-hour period. The resulting pale yellow solution was then cooled and brought to *p*H 1.2 with sulfuric acid. Crystallization took place immediately but the product was allowed to stand overnight to insure complete precipitation. It was then filtered, washed thoroughly with water, and recrystallized from boiling water, giving 89 g. of pure *triglycine;* yield 93%. The product was identified by carbon, hydrogen, and nitrogen analyses.

Triglycine from chloroacetic acid. A. Chloroacetic acid (47.5 g.) was dissolved in two liters of concentrated (28%) aqueous ammonia and allowed to stand for 24 hours at about 30°. Water and ammonia were then distilled off until the volume of the residue was about 250 cc. Sodium hydroxide (44 g.) was then added (with cooling) and the volume of the resulting solution was reduced to 200 cc. by further distillation. A solution containing 54 g. of sodium cyanide and 1 g. of NaOH was prepared and 60 cc. of this solution was added to the reaction mixture, which was then heated to 80° (water-bath) and stirred vigorously while 50 cc. of 37% formaldehyde solution (containing 0.575 mole) was added dropwise over a period of four hours. The remainder of the sodium cyanide solution was then added, and another 50 cc. of formaldehyde solution was added in the same manner. After the pale yellow product had stood overnight, it was heated by a boiling-water bath for two hours and stirred vigorously to remove ammonia. The solution was adjusted to *p*H 1.2 with sulfuric acid and the white precipitate which formed was allowed to stand for an hour before filtering The colorless crystalline triglycine, after filtration and thorough washing with water, weighed 78 g. (0.83 mole) and was analytically pure, based on C, H, and N.

B. Chloroacetic acid (95 g.) was dissolved in 1 liter of concentrated (28%) aqueous ammonia, and allowed to stand for 24 hours at about 30°. The excess ammonia was removed by distillation of the solution to a volume of about 250 cc.; 44 g. of sodium hydroxide was then added with cooling and an additional 50 cc. of water was distilled off. The pale yellow solution was then placed in a 2-liter round-bottom flask equipped with a reflux condenser, dropping-funnel, and mercury-seal stirrer. A solution containing 75 g. of sodium cyanide and 2 g. of sodium hydroxide was added and the reaction mixture was heated to 80° (water-bath) and stirred vigorously while a 37% formaldehyde solution containing 1.58 moles of formaldehyde was added dropwise over a period of seven hours. After the product had stood overnight, it was heated and stirred on a boiling-water bath for two hours, cooled, and acidified to *p*H 1.2 with sulfuric acid. A white precipitate of triglycine formed immediately. It was allowed to stand for one hour, and was filtered and washed thoroughly with water giving 124 g. (0.63 mole) of triglycine which was sufficiently pure to give a correct elementary analysis.

WORCESTER, MASS.
FRAMINGHAM, MASS.

BIBLIOGRAPHY

(1) SMITH, BULLOCK, BERSWORTH, AND MARTELL, *J. Org. Chem.*, **14**, 355 (1949).
(2) POLSTORF AND MEYER, *Ber.*, **45**, 1905 (1912).
(3) KOHN, *Monatsh.*, **20**, 903 (1896).
(4) ROMIJIN, *Z. anal. Chem.*, **36**, 18 (1897).
(5) MOWRY, *J. Am. Chem. Soc.*, **66**, 371 (1944).
(6) MUTSCHEN, *Z. anal. Chem.*, **99**, 355 (1934).
(7) ROBERTSON, *J. Am. Chem. Soc.*, **49**, 2889 (1927).