

*Anal.* Calcd. for  $C_{10}H_8ONClS$ : N, 6.21; mol. wt., 225.5. Found: N, 6.06, 6.18; mol. wt. 231.5 (Rast method).

When 2-phenyl-4-hydroxymethyl-5-chlorothiazole was boiled with dilute alkaline potassium permanganate, however, there was isolated by conventional methods a 29.2% yield of benzoic acid which was identified by the mixed melting point method.

**2-Phenyl-4-acetoxymethyl-5-chlorothiazole (II, R = OCOCH<sub>3</sub>).**—This was obtained in 98% yield from the alcohol by refluxing with acetic anhydride. From petroleum ether (b. p. 35–60°) it formed long pale yellow rods, m. p. 63.3–64.1° cor.

*Anal.* Calcd. for  $C_{12}H_{10}O_2NCIS$ : N, 5.23; mol. wt., 267.7. Found: N, 5.40, 5.50; mol. wt. (Rast method), 259.

Although this material gave a strong Beilstein test for halogen, the latter could not be removed by boiling alcoholic silver nitrate.

**2-Phenyl-4-(3,5-dinitrobenzoyl)-methyl-5-chlorothiazole (II, R = OCOC<sub>6</sub>H<sub>3</sub>(NO<sub>2</sub>)<sub>2</sub>).**—This ester was obtained in 93% yield from the alcohol with 3,5-dinitrobenzoyl chloride and pyridine. Recrystallization from acetone or from benzene/ligroin (2:1) gave rosetts of very fine pale yellow needles, m. p. 155.1–155.3° cor.

*Anal.* Calcd. for  $C_{17}H_{10}O_6N_3ClS$ : N, 10.0. Found: N, 10.2, 10.2.

**2-Phenyl-5-chloromethylthiazole-4-carboxylic Acid (IV).**—A mixture of 2-phenyl-4-hydroxymethyl-5-chlorothiazole (0.45 g. = 0.002 mole) and chromic acid/sulfuric acid reagent (8 ml. of a solution of 10 g. of chromium trioxide dissolved in a mixture of 8 ml. of concd. sulfuric acid in 60 ml. of water) was worked with a stirring rod for ten minutes. The mixture was then heated on a steam-bath for twenty minutes, cooled, filtered with suction and the residue washed with water. Extraction of this residue with dilute sodium carbonate followed by filtration and acidification of the filtrate with dilute hydrochloric acid

gave a yield of 0.20 g. (41.6% theoretical) of 2-phenyl-5-chlorothiazole-4-carboxylic acid, m. p. 194.4–195.5° cor. Further purification from benzene and subsequently from acetone yielded fern-like clusters of very fine lustrous needles, m. p. 198.8–199.3° cor. with evolution of gas.

*Anal.* Calcd. for  $C_{10}H_8O_2NCIS$ : N, 5.84; neut. eq., 239.7. Found: N, 5.97, 6.05; neut. eq., 239.3.

This same 2-phenyl-5-chlorothiazole-4-carboxylic acid was also obtained in 21% yield from 2-phenylthiazole-4-carboxylic acid chloride (0.22 g. = 0.001 mole) upon refluxing for one hour with a solution of concd. nitric acid (1.0 ml.) in water (2.4 ml.). The product which separated on cooling was recrystallized from a mixture of ligroin (b. p. 90–100°) and benzene, giving 0.05 g. of material which melted at 184.2–189.2° cor. However, it gave a positive Beilstein test and failed to depress the melting point of an authentic sample of 2-phenyl-5-chlorothiazole-4-carboxylic acid prepared with chromic acid from 2-phenyl-4-hydroxymethyl-5-chlorothiazole. From the nitric acid filtrate there was obtained a 54% yield of 2-phenylthiazole-4-carboxylic acid, resulting from simultaneous hydrolysis of the original acid chloride.

### Summary

1. Treatment of 2-phenyl-4-chloromethylthiazole and of 2-phenylthiazole-4-carboxylic acid chloride with hot dilute nitric acid has been found to yield 2-phenyl-4-hydroxymethyl-5-chlorothiazole and 2-phenyl-5-chlorothiazole-4-carboxylic acid, respectively. This surprising change in the position of the halogen does not appear previously to have been observed in the thiazole series.

2. A number of 4-substituted derivatives of 2-phenylthiazole have been characterized.

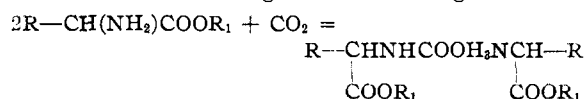
CAMBRIDGE, MASSACHUSETTS RECEIVED APRIL 10, 1943

[CONTRIBUTION FROM THE LABORATORY OF HIGH MOLECULAR CHEMISTRY, THE HEBREW UNIVERSITY]

## Derivatives of N-Carboxy- $\alpha$ -amino Acid Esters

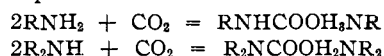
BY MAX FRANKEL AND EPHRAIM KATCHALSKI

In a preliminary note<sup>1</sup> it was reported that new compounds were obtained from  $\alpha$ -amino acid esters and carbon dioxide for which the constitution of N-carboxy- $\alpha$ -amino acid esters was proposed. Subsequently it was found that the new compounds isolated were salts of one molecule of the free N-carboxy- $\alpha$ -amino acid ester with one molecule of the corresponding  $\alpha$ -amino acid ester formed according to the following scheme



The present paper gives details of the preparation of these new compounds and offers proof of their constitution.

It is further known that carbon dioxide is able to react with ammonia, primary and secondary amines. With ammonia the ammonium salt of carbamic acid  $H_2NCOOH_4N^2$  is formed. With primary or secondary amines the following reaction takes place<sup>3</sup>



(2) Cf. Meyer and Jacobson, "Lehrbuch der organischen Chemie," Vol. I, part 2, 1370 (1913).

(3) Fichter and Becker, *Ber.*, **44**, 3481 (1911).

(1) Frankel, Neufeld and Katchalski, *Nature*, **144**, 832 (1939).

The compounds described here are the corresponding derivatives of  $\alpha$ -amino acid esters. The ability of carbon dioxide under certain conditions to combine with the amino group of  $\alpha$ -amino acids to form N-carboxy derivatives was described by Siegfried.<sup>4</sup> He obtained salts of the general formula 
$$\begin{array}{c} \text{R}-\text{CH}-\text{COO} \\ | \\ \text{NH}-\text{COO} \end{array} \text{Ba}(\text{Ca})$$
 by passing a stream of carbon dioxide through aqueous solutions of  $\alpha$ -amino acids in the presence of barium hydroxide or calcium hydroxide.

The physical chemistry of the carbamino reaction was exhaustively studied by Faurholt,<sup>5</sup> in the case of ammonia, the methylamines and glycine. In the course of this work he proved that it is only the more basic form of the amines, *viz.*,  $\text{NH}_3$ ,  $\text{CH}_3\text{NH}_2$ ,  $(\text{CH}_3)_2\text{NH}$  and  $\text{H}_2\text{NCH}_2\text{COO}^-$ , that is able to combine with carbon dioxide. That the anionic form of amino acids,  $\text{H}_2\text{NCH}(\text{R})\text{COO}^-$ , is alone reactive, and not the zwitterionic form, was further confirmed by Meldrum and Roughton,<sup>6</sup> and especially by Stadie and O'Brien.<sup>7</sup>

Henriques<sup>8</sup> suggested that hemoglobin may partially combine with carbon dioxide under physiological conditions to form carbamino compounds, but this was not firmly established until the work of Roughton and his collaborators.<sup>9</sup> The evidence collected by Roughton indicates that these compounds, owing to their lability, play an important part in the transport of carbon dioxide by the blood.

As mentioned above the salts of N-carboxy- $\alpha$ -amino acid esters with the corresponding  $\alpha$ -amino acid esters are obtained by passing a stream of dry carbon dioxide for several hours through a solution of amino acid esters in water-free ether. The reaction was carried out below  $0^\circ$  with the rigorous exclusion of water.

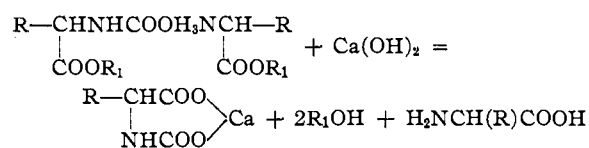
The new derivatives of N-carboxy- $\alpha$ -amino acid esters are stable at low temperatures (below  $0^\circ$ ) and retain their crystalline form in absence of moisture. In an atmosphere of dry carbon dioxide gas they remain undecomposed, for a certain time at least, even at room temperature. On exposure to air at room or at elevated tempera-

tures, they liquefy owing to their hygroscopic nature, and decompose with visible evolution of carbon dioxide. Carbon dioxide is also evolved on dissolving the compounds in water at room or at elevated temperatures; no evolution of gas was observed on dissolving the compounds in water at  $0^\circ$ . On heating the salts of N-carboxy- $\alpha$ -amino acid esters with the corresponding  $\alpha$ -amino acid esters in vacuum, decomposition into carbon dioxide and the corresponding  $\alpha$ -amino acid esters occurs.

The splitting off of carbon dioxide from the N-carboxyl group becomes quantitative in the presence of strong acids. Thus it is possible to determine the carboxyl group bound to the nitrogen in the form of carbon dioxide (by Van Slyke's procedure<sup>10</sup>) and also the  $\text{NH}_2$  group regenerated by the evolution of carbon dioxide (Linderstrøm-Lang titration<sup>11</sup>).

In order to prove the constitution of these new compounds and in particular to show the presence of the carboxyl group attached to the nitrogen of the amino group, we carried out the following reactions:

(a) On treating the salt of N-carboxyglycine methyl ester and glycine methyl ester with calcium hydroxide under the conditions described in the experimental part, a calcium salt was obtained, which proved to be identical with Siegfried's calcium salt of carbaminoacetic acid. Thus the following relationship between Siegfried's calcium salts and our compounds was established



(b) The substance obtained by the action of carbon dioxide on glycine ethyl ester for which formula (I) is proposed, was allowed to react with diazomethane. This yielded compound (II) and glycine ethyl ester in about equimolar amounts. (II) proved to be identical with N-carbomethoxyglycine ethyl ester obtained by Leuchs<sup>12</sup> by the reaction of methyl chlorocarbonate on glycine ethyl ester. This is proof that the product of interaction of glycine ethyl ester and

(4) Siegfried, *Z. physiol. Chem.*, **44**, 85 (1905); **46**, 401 (1905); Siegfried and Schutt, *ibid.*, **81**, 260 (1912).

(5) Faurholt, *J. chim. phys.*, **22**, 1 (1925).

(6) Meldrum and Roughton, *J. Physiol.*, **80**, 143 (1933).

(7) Stadie and O'Brien, *J. Biol. Chem.*, **112**, 723 (1936); **117**, 439 (1937).

(8) Henriques, *Biochem. Z.*, **200**, 1 (1928).

(9) Ferguson and Roughton, *J. Physiol.*, **83**, 68, 87 (1934); Roughton, *Physiol. Rev.*, **15**, 241 (1935).

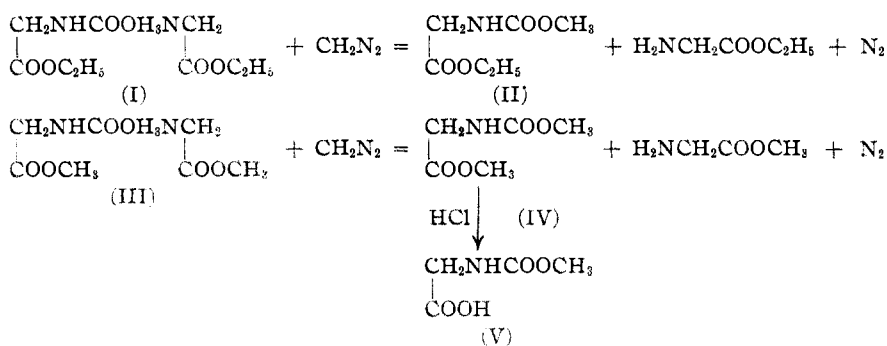
(10) Van Slyke and Neill, *J. Biol. Chem.*, **61**, 523 (1924); Peters and Van Slyke, "Quantitative Clinical Chemistry," vol. II, 283 (1932).

(11) Linderstrøm-Lang, *Z. physiol. Chem.*, **173**, 32 (1928).

(12) Leuchs, *Ber.*, **39**, 857 (1906).

carbon dioxide contains a carboxyl group attached to the nitrogen.

Similarly from the salt of N-carboxyglycine methyl ester with glycine methyl ester (III) the hitherto unknown N-carbomethoxyglycine methyl ester (IV) was prepared. On hydrolysis of this di-ester by hydrochloric acid, the alcoholic group bound to the original carboxyl group of the amino acid is removed, while that of the N-carboxyl group remains unaffected.<sup>13</sup> Thus from (IV), (V) was obtained. (V) is identical with carbomethoxyglycine, obtained by Leuchs<sup>12</sup> N-hydrolysis of N-carbomethoxyglycine ethyl ester.



The literature does not seem to contain any references to the reaction of diazomethane on ammonium salts or substituted ammonium salts of organic acids. As the new compounds described here belong to this class, we also investigated the action of diazomethane on the ammonium salts of two organic acids.

On allowing diazomethane to react with ammonium benzoate under the conditions specified in the experimental part, evolution of nitrogen and ammonia occurred, and methyl benzoate was obtained in practically quantitative yield. Under similar conditions methyl propionate was obtained from ammonium propionate. It appears, therefore, that diazomethane is able to react with ammonium salts of organic acids with the formation of esters.

Owing to their instability the N-carboxy  $\alpha$ -amino acid ester derivatives described here are of interest because they readily give rise to polycondensation products. In a following paper we shall deal with this point.

### Experimental

**Preparation.**—The salts of N-carboxy- $\alpha$ -amino acid esters with their corresponding  $\alpha$ -amino acid esters were obtained by passing dry carbon dioxide through a solution

(13) Cf. Hantzsch and Metcalf, *Ber.*, **29**, 1680 (1896).

of one part freshly distilled  $\alpha$ -amino acid ester in about fifteen parts of water-free ether. The solution was cooled by an ice-salt mixture, water being rigorously excluded. In the course of a few hours the new compounds either crystallize in well developed crystals or are deposited as oils which, on standing overnight in the ice box, become crystalline. In some cases, owing to their solubility in ether, the N-carboxy salts could be obtained in solid form only by evaporating the ether in a rapid stream of dry carbon dioxide.

**Analysis.**—In view of the lability and hygroscopic nature of the salts of N-carboxy- $\alpha$ -amino acid esters with the corresponding  $\alpha$ -amino acid esters, special precautions had to be taken in preparing the substances for analysis and in carrying out the determinations.

The crystalline substances obtained as described above

were separated from the ether by decantation, washed twice with small amounts of dry ether and decanted. The remaining ether was removed by passing a stream of dry carbon dioxide for several hours through the vessel containing the substance. All weighing operations were carried out in closed vessels.

### Determination of Carbon Dioxide Split Off from the

#### N-Carboxyl Group on Addition of Hydrochloric Acid.

—The carbon dioxide determinations were carried out according to Van Slyke and Neill.<sup>10</sup> A weighed sample (about 50 mg.) was dissolved in 25 ml. of 0.1 *N* sodium hydroxide solution free of air and carbon dioxide. One ml. of this solution was introduced into the Van Slyke manometric apparatus and the carbon dioxide liberated by means of 3.5 ml. of carbon dioxide-free 0.1 *N* hydrochloric acid. The carbon dioxide liberated was afterward absorbed by means of 0.3 ml. of 5 *N* sodium hydroxide solution and the pressure read before and after absorption. A blank experiment was carried out, and the pressure difference measured. This difference (amounting to approximately 10 mm. of mercury) was subtracted from the pressure difference obtained in the estimation in order to calculate the amount of carbon dioxide evolved.

All other determinations including the Linderström-Lang titration were carried out in the usual way.

**Salt of N-Carboxyglycine Ethyl Ester with Glycine Ethyl Ester.**—Prepared as above from glycine ethyl ester; yield about 95% of the theoretical; crystallizes in short needles, arranged in roset-like form. It is scarcely soluble in ether, soluble in water, pyridine, alcohol and other organic solvents. At room temperature these solutions evolve carbon dioxide.

*Anal.* Calcd. for  $\text{C}_9\text{H}_{16}\text{O}_6\text{N}_2$ : C, 43.17; H, 7.25; N, 11.19;  $\text{CO}_2$ , 17.59. Found: C, 43.13; H, 7.38; N, 11.04;  $\text{CO}_2$ , 17.76.

**Salt of N-Carboxyglycine Methyl Ester with Glycine Methyl Ester.**—Prepared similarly to the corresponding ethyl ester which it resembles closely in properties and crystalline form; yield about 90% of the theoretical.

*Anal.* Calcd. for  $C_7H_{14}O_6N_2$ : C, 37.82; H, 6.35; N, 12.61;  $CO_2$ , 19.81. Found: C, 37.77; H, 6.15; N, 12.37;  $CO_2$ , 19.57.

**Salt of N-Carboxyalanine Ethyl Ester with Alanine Ethyl Ester.**—Prepared as above from alanine ethyl ester; yield 90% of the theoretical. Crystalline, resembles in its properties the above mentioned compounds.

*Anal.* Calcd. for  $C_{11}H_{22}O_6N_2$ : C, 47.44; H, 7.99; N, 10.07;  $CO_2$ , 15.82. Found: C, 47.24; H, 7.81; N, 10.04;  $CO_2$ , 16.10.

**Salt of N-Carboxy-C-phenylglycine Ethyl Ester with C-Phenylglycine Ethyl Ester.**—Prepared as above. As this N-carboxyamino acid ester derivative is soluble in ether it was obtained only after removal of the ether in a rapid stream of carbon dioxide, yield about 95% of the theoretical. Crystalline, readily soluble in ether, alcohol and acetone but only slightly soluble in water.

*Anal.* Calcd. for  $C_{21}H_{28}O_6N_2$ : C, 62.65; H, 6.51; N, 6.96;  $CO_2$ , 10.94. Found: C, 62.53; H, 6.63; N, 6.85;  $CO_2$ , 10.71.

**Salt of N-Carboxyleucine Ethyl Ester with Leucine Ethyl Ester.**—Prepared as above. Obtained in crystalline form from its solution in ether only after evaporation of the ether in a stream of carbon dioxide; yield 90% of the theoretical. Soluble in ether, alcohol, pyridine and water; insoluble in acetone.

*Anal.* Calcd. for  $C_{17}H_{34}O_6N_2$ : C, 56.31; H, 9.46; N, 7.73;  $CO_2$ , 12.14. Found: C, 55.98; H, 9.59; N, 7.89;  $CO_2$ , 12.32.

**Preparation of the Calcium Salt of N-Carboxyglycine from the Salt of N-Carboxyglycine Methyl Ester with Glycine Methyl Ester.**—1.35 g. of the salt of N-carboxyglycine methyl ester with glycine methyl ester was dissolved in 16 ml. of water at 0°. A thick suspension of calcium hydroxide in water (30 ml.) was added and the mixture kept overnight at 0° in order to complete the hydrolysis of the ester group. The suspension was filtered and an equal volume of absolute alcohol was added to the clear filtrate. A white precipitate formed at once. It was filtered, washed with alcohol and ether and dried *in vacuo* at 60° (0.912 g.). The yield was 96% of the theoretical. On analysis it proved to be the calcium salt of N-carboxyglycine previously obtained by Siegfried.<sup>4</sup>

*Anal.* Calcd. for  $CaC_2H_3O_4N$ : Ca, 25.51. Found: Ca, 25.04.

**N-Carbomethoxyglycine Methyl Ester.**—To 5 g. of the salt of N-carboxyglycine methyl ester with glycine methyl ester, 100 ml. of a solution of diazomethane in ether (= 1.3 g. of  $CH_2N_2$ ) was added. The temperature was kept at 0°. The initially slow evolution of gas increased considerably during the reaction. The N-carboxyglycine methyl ester salt dissolved gradually and the yellow color of the solution became fainter. After standing overnight in the ice box, the now almost colorless solution was filtered and dried over sodium sulfate. The ether was distilled off at normal pressure and the remaining liquid fractionally distilled. 1.5 g. of glycine methyl ester distilled over between 45–60° at 20 mm. Between 125–135° (20 mm.) a second fraction amounting to 1.8 g. was obtained from which, on redistillation, a fraction distilling at 130° was obtained (1.6 g.). This latter after drying

*in vacuo* showed a neutral reaction and was stable (in contrast to the first fraction which underwent condensation). Analysis shows that it is N-carbomethoxyglycine methyl ester.

*Anal.* Calcd. for  $C_5H_9O_4N$ : N, 9.52;  $CH_3O$ , 42.17. Found: N, 9.61;  $CH_3O$ , 42.00.<sup>14</sup>

**N-Carbomethoxyglycine from N-Carbomethoxyglycine Methyl Ester.**—0.5 g. of the latter was dissolved in 2 ml. of concentrated hydrochloric acid and kept in a vacuum desiccator over sulfuric acid and sodium hydroxide for a week. Well developed monoclinic crystals were formed in large amounts and were then separated from the mother liquid. They were purified by recrystallization from ether; yield 85% of the theoretical. Melting point 95°; Leuchs<sup>12</sup> gives m. p. 95°. It dissolves in water and shows a strong acid reaction.

*Anal.* Calcd. for  $C_6H_9O_4N$ : N, 10.52;  $CH_3O$ , 23.30. Found: N, 10.21;  $CH_3O$ , 22.86.<sup>14</sup>

**N-Carbomethoxyglycine Ethyl Ester.**—Prepared according to the method used for N-carbomethoxyglycine methyl ester described above. 5 g. of the salt of N-carboxyglycine ethyl ester with glycine ethyl ester yielded 1.4 g. glycine ethyl ester and 2.0 g. of N-carbomethoxyglycine ethyl ester. The latter distilled at 127–129° at a pressure of 13 mm. The analysis and boiling point prove that this product is identical with that obtained by Leuchs<sup>12</sup> by another reaction.

*Anal.* Calcd. for  $C_8H_{11}O_4N$ : N, 8.69. Found: N, 8.50.

**Action of Diazomethane on Ammonium Benzoate.**—A solution containing about 0.7 g. diazomethane in 70 ml. ether was added to 0.95 g. of ammonium benzoate and the mixture left overnight at room temperature. Bubbles of gas were evolved and the ammonium benzoate dissolved. The ethereal solution was filtered and the ether distilled off. The liquid residue distilled between 185–190° at 690 mm.; yield 0.90 g.

The methyl benzoate thus obtained was hydrolyzed by boiling in alcoholic sodium hydroxide, and from the alkaline solution benzoic acid was precipitated by the addition of hydrochloric acid. The melting point after filtering and washing is 121°.

**Action of Diazomethane on Ammonium Propionate.**—A solution of diazomethane in ether was added (in slight excess) to ammonium propionate and the reaction mixture treated as above. An 80% yield of methyl propionate was obtained; boiling point 76° at 690 mm.

### Summary

On passing carbon dioxide through  $\alpha$ -amino acid esters N-carboxy- $\alpha$ -amino acid esters in the form of their salts with the corresponding  $\alpha$ -amino acid esters, were obtained. These have the general formula



The following compounds were obtained in crystalline form, salts of: N-carboxyglycine methyl ester with glycine methyl ester; N-

(14) On long heating with hydriodic acid, *d* 1.96.

carboxyglycine ethyl ester with glycine ethyl ester; N-carboxyalanine ethyl ester with alanine ethyl ester; N-carboxyleucine ethyl ester with leucine ethyl ester; N-carboxy-C-phenylglycine ethyl ester with C-phenylglycine ethyl ester.

On treating the salt of N-carboxyglycine methyl ester and glycine methyl ester with calcium hydroxide, Siegfried's calcium salt of carbaminoacetic acid was obtained.

Diazomethane reacted with the salt of N-carboxyglycine methyl ester with glycine methyl ester yielding N-carbomethoxyglycine methyl ester

and free glycine methyl ester in about equimolar amounts. Similarly N-carbomethoxyglycine ethyl ester and glycine ethyl ester were obtained in about equimolar amounts from the salt of N-carboxyglycine ethyl ester with glycine ethyl ester.

By hydrolyzing N-carbomethoxyglycine methyl ester, carbomethoxyglycine was obtained.

Diazomethane reacted with ammonium benzoate and ammonium propionate giving methyl benzoate and methyl propionate, respectively.

JERUSALEM, PALESTINE

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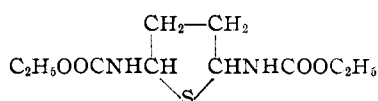
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

## The Synthesis of a Tetrahydrothiophene with Substituted Amino Groups in the 2- and 5-Positions

BY GEORGE BOSWORTH BROWN AND GLEN W. KILMER

In the original discussion<sup>1</sup> of possible formulas for biotin, several structures came under consideration which involved the presence of nitrogen attached to one or both  $\alpha$ -carbons of a tetrahydrothiophene. These possibilities were dismissed with the argument that "the remarkable stability of the diaminocarboxylic acid toward hydrolytic agents renders unlikely structures with both sulfur and nitrogen attached to a single carbon atom." While no compounds of the type  $RSCH(NH_2)R'$  have come to our attention, cyclic compounds of the thiazolidine type with sulfur and nitrogen linked to the same carbon atom are known. For example, thiazolidine-4-carboxylic acid which has been studied by Ratner and Clarke<sup>2</sup> is decomposed slowly by boiling 1 *N* hydrochloric acid. This instability, however, is in contrast to the stability of the diaminocarboxylic acid derived from biotin (DAC).

To support or refute the argument with regard to the stability of structures involving the presence of sulfur and nitrogen attached to the same carbon atom, we decided to test the stability of a tetrahydrothiophene with nitrogen in the  $\alpha$ -positions. 2,5-bis-(Carbomethoxyamino)-tetrahydrothio-



phene was prepared but on hydrolysis the urethan decomposed to yield ammonia, hydrogen sulfide and succinaldehyde. This instability toward hydrolytic agents of the 2,5-diaminotetrahydrothiophene substantiated the postulation quoted above and contrasted markedly with the stability of DAC<sup>3</sup> and with that of 3,4-diaminotetrahydrothiophene<sup>4,5</sup> which has subsequently been shown to be related to biotin.<sup>6,7,8</sup>

The synthesis of 2,5-bis-(carbomethoxyamino)-tetrahydrothiophene was accomplished by treatment of the ester of 2,5-dicarboxytetrahydrothiophene with hydrazine hydrate to give the dihydrazide. This hydrazide was smoothly converted to the azide and then to the corresponding urethan, 2,5-bis-(carbomethoxyamino)-tetrahydrothiophene in 53% yield. Hydrolysis of the diurethan with boiling 1 *N* hydrochloric acid resulted in decomposition of the urethan with copious liberation of hydrogen sulfide, while hydrolysis with boiling 5% barium or sodium hydroxide resulted in liberation of 80% of the nitrogen as ammonia within thirty minutes. When the hydrolysis was car-

(3) K. Hofmann, D. B. Melville and V. du Vigneaud, *J. Biol. Chem.*, **141**, 207 (1941).

(4) G. W. Kilmer, M. D. Armstrong, G. B. Brown, and V. du Vigneaud, *ibid.*, **145**, 495 (1942).

(5) K. Hofmann, G. W. Kilmer, D. B. Melville, V. du Vigneaud and H. H. Darby, *ibid.*, **145**, 503 (1942).

(6) V. du Vigneaud, *Science*, **96**, 455 (1942).

(7) V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy and S. A. Harris, *J. Biol. Chem.*, **146**, 475 (1942).

(8) D. B. Melville, A. W. Moyer, K. Hofmann and V. du Vigneaud, *ibid.*, **146**, 487 (1942).

(1) V. du Vigneaud, K. Hofmann and D. B. Melville, *This Journal*, **64**, 188 (1942).

(2) S. Ratner and H. T. Clarke, *ibid.*, **59**, 200 (1937).